Summary

Lifestyle modification is the main treatment for obesity. An understanding of the mechanisms of appetite control, nutrient metabolism, and energy expenditure has greatly enhanced drug development for disorders, such as diabetes, cancer, obesity, and inflammation. *Peucedanum japonicum* Thunb (PJT), also known as “Chomeiso” (長命草), is a traditional herb that has been used to provide nutrition; the herb has a broad spectrum of physiological and biological properties. In our previous studies, we found that PJT has antiobesity effects, more specifically, the ethanol extract of PJT reduces obesity and diabetes symptoms in high-fat diet–fed mice. Furthermore, in one of our recent studies, we isolated the active component of PJT with antiobesity properties called pteryxin, a previously known coumarin. However, its biological properties against obesity were unknown. We determined that pteryxin played a role in adipogenesis suppression mechanisms, both in vitro and in vivo. This study details the current understanding of antiobesity effects of PJT extracts, its active compound pteryxin, and the distinctive properties of pteryxin compared with those of other natural compounds against obesity. This review will
help in understanding the molecular mechanisms underlying pteryxin’s effects for
development of pharmaceutical drugs and nutritional products.

Key words: Obesity, *Peucedanum japonicum* Thunb, pteryxin

1. Background

Obesity is one of the most concerning health dilemmas worldwide and needs to
be addressed. It is characterized by excess accumulation of lipid and triglycerides
(TG) in the adipose tissue, which plays an essential role in the regulation of lipid and
energy homeostasis. Exercise has been prescribed as the mainstream treatment for
obesity and several other metabolic diseases. However, a majority of the individuals
do not respond to life style therapies and understanding of nutrient metabolism
may be a possible treatment option for these individuals. For centuries, plants have
been used as potential therapeutics for many diseases and metabolic syndromes,
especially in countries such as India and China. Flavonoids, a large group of
naturally occurring polyphenolic compounds, found in fruits, vegetables, nuts, plant-
derived wines, and tea, exert protective roles against cancers, coronary heart
diseases, and atherosclerosis. Specific flavonols, such as quercetin, gallic acid, and
caffeic acid have been shown to have antioxidant, antibacterial, antiinflammatory,
anticarcinogenic, antiviral, and antiobesity effects. Furthermore, anthocyanins,
which are naturally occurring polyphenol pigments in black soybean and blood
oranges, have been shown to decrease weight and insulin resistance in mice fed
a high-fat diet (HFD). (-)-Epigallocatechin-3-gallate (EGCG) is a major polyphenol
catechin found in green tea and has been shown to exert many beneficial effects
against cancer, obesity, diabetes, and cardiovascular diseases. It is also considered
to be a powerful inhibitor of lipid absorption. Resveratrol, a naturally occurring
polyphenol found in grapes, berries, and peanuts is also reported to have various
biological and pharmacological properties, such as attenuation of lipid accumulation,
anticancer, antiinflammatory, and antioxidant activity. Moreover, berberine, an
alkaloid in the roots and bark of *Coptis chinensis*, a Chinese herb that has been used
to treat diabetes for thousands of years in China, is also an over-the-counter drug
used for the treatment of gastrointestinal infections in China and has been shown
to decrease body weight and improve glucose metabolism in db/db mice. In addition to the above-mentioned natural constituents, curcumin, a low-molecular weight polyphenol derived from turmeric, which is a popular dietary spice in Asia, has also been reported to have antiinflammatory, anticancer, antiplatelet, and antiobesity effects as well as decrease blood glucose levels in type 2 diabetic KK-Ay mouse model. Taken together, these studies provide an overview of the highly abundant natural polyphenolic compounds that are used to treat health problems worldwide. Although further research is warranted, these compounds show good potential for use as pharmaceutical drugs.

*Peucedanum* spp. belongs to the family Apiaceae (earlier known as Umbelliferae) and originates mainly from Taiwan, Philippines, southern Japan, and China. There are different species of *Peucedanum*, such as *P. officinale*, *P. japonicum*, *P. ruthenicum*, *P. glaucopruinosum*, *P. knappi*, *P. translucens*, and *P. oreoselinum*. There are several studies on *P. japonicum*, especially on *P. japonicum* Thunb [1] and *P. japonicum* Formosan [2], and this species has been found to possess important pharmacological properties. In terms of geographical distribution, *Peucedanum japonicum* Thunb (PJT) is widely found in the sub-tropical regions of Asia (Fig. 1). PJT is consumed as a leafy vegetable and is used as a garnish for raw fish in southern Japan. In the Okinawan Islands in Japan, the PJT plant grows on the coastal and cliff areas, and its leaves have been used to treat cold, cough, and as an antifebrile. Both its roots and aerial parts have been used as a folk medicine for the treatment of colds and neuralgia in Taiwan, and as a folk medicine for the treatment of cough, headaches, and anodyne in Korea. Increasing evidence shows the health benefits of PJT, including effects on cardiovascular diseases and its antioxidant, antiplatelet, and antidiabetic effects. It can also serve as a treatment for rheumatic disorder. Moreover, it was recently found that PJT has potent chemopreventive properties against breast cancer metastasis. PJT is a rich source of different coumarins that possess many of the above-mentioned benefits. The coumarin-backboned acetylangeloylkhellactone, acetyltigloylkhellactone, and praeruptorin A, have been shown to have strong antagonistic effects on histamine- and (Ca$^{2+}$)-induced contractions in smooth muscle cells, and the khellactones cause increased...
arterial blood flow in a dose-dependent manner. Strong inhibition of platelet aggregation was observed owing to psoralen, eugenin, (−)-selinidin, (+)-pteryxin, and (+)-peucedanocoumarin, which possess a coumarin structure [2]. It was also found that rutin and caffeoylquinic acid isomers, isolated from the leaves of the PJT plant, to be major antioxidant constituents [1]. However, properties of this plant against obesity were unknown. Thus, we investigated the antiobesity properties of PJT plant and to the best of our knowledge, we were the first group to report the significant characteristics of PJT that allow adipogenesis suppression, both in vivo and in vitro.

2. PJT powder and its crude extracts for obesity treatment

In our laboratory, Okabe et al. examined the antiobesity activity of PJT in C57BL/6 mice. The HFD was supplemented with different concentrations of
powdered leaves and stems of PJT, varying from 0.5 to 20%, and the animals were fed for 4 weeks. The composition of experimental diets was prepared according to the AIN-76 formulation and for the dietary groups of PJT, carbohydrate, protein, fat, and fiber derived from PJT were compensated at the expense of the sucrose, β-corn starch, casein, corn oil and cellulose, respectively. Treatment with 10% and 20% PJT showed the highest suppression in body weight (Fig. 2A) and abdominal (Fig. 2B) and subcutaneous fat (Fig. 2C). However, the energy intake (kcal/day) remained unchanged both in control and the PJT 0.5–20% treatment groups [3]. A significant reduction in serum leptin levels after 10% and 20% PJT powder treatments (Fig. 2D) is mainly attributed to decreased adipocyte volume in the animal body. Leptin is an adipocyte-derived hormone, which is important in regulating body weight and energy expenditure. Leptin mRNA levels in both subcutaneous and omental fat depots have been found to have a positive correlation with the body mass index and adipocyte volume. In contrast, comparable adipocyte volumes in subcutaneous and omental fat depots with a higher level of leptin secretion in subcutaneous adipocytes in obese human subjects indicate that the adipocyte size may not be the main determinant of leptin secretion. In this regard, we found that PJT intake elevated the proportion of n-3 fatty acids, possibly increasing fatty acid oxidation via peroxisome proliferator-activated receptor (PPAR) alpha activation and decreased levels of sterol regulatory binding protein-1 (SREBP1) in the liver. Furthermore, reduction in TG and total cholesterol (TC) levels in the liver and serum was observed with no changes in the daily fecal weight after 4 weeks of treatment. On the other hand, persimmon leaf powder reduced TC and TG levels in the liver and plasma with increased daily fecal weight and fecal excretion of TC, TG, and acidic sterols in male Sprague-Dawley rats, suggesting that the phenolic compounds and high fiber content in persimmon leaf powder may be responsible for the effect. However, in our study, the fiber concentration of all experimental diets were adjusted to the same level at the expense of cellulose, suggesting that the polyphenol content may be a probable indicator for antiobesity activity. In this context, our group further [4] investigated the activity of 10% PJT powder in the C57BL/6 mice and obesity-related gene modulation pattern in vivo. In concordance
Figure 2
C57BL/6 mice were fed with a high-fat diet supplemented with PJT powder varying from 0 – 20% for 4 weeks. The final body weight was measured (A). Abdominal (B) and subcutaneous fat weight (C) were calculated based on the tissue weight (g per 100 g of body weight). The serum leptin levels were measured (D). C57BL/6 mice were fed with a high-fat diet supplemented with PJT powder 0% (control; closed column) or 10% (open column) for 4 weeks. The final body weight was measured and abdominal fat weight was calculated based on the tissue weight (E). Further, the serum triglyceride and serum leptin levels were measured (F) along with liver triglyceride and total cholesterol levels (G). Values are expressed as mean ± S.E. of five mice. Significant difference compared with the control (0%) group by Dunnett’s test, *p < 0.05; **p < 0.01.
with the previous study [3], 10% PJT powder significantly decreased the final body weight, abdominal fat weight (Fig. 2E), serum TG and leptin levels (Fig. 2F) after a 4-week treatment period. Moreover, the liver TG level was repressed by the diet supplemented with 10% PJT powder (Fig. 2G) [4]. Furthermore, we also used different PJT extracts, such as water, 50% ethanol, and ethanol, to determine the properties of compounds related to antiobesity in C57BL/6 mice [5]. As observed in earlier studies, there was a significant reduction in the final body weight of mice fed 10% and 20% PJT powder and the ethanol extract of PJT powder, indicating that PJT soluble in ethanol may be possess antiobesity properties (Fig. 3). These hydrophobic properties of the active compound(s) led to further fractionation of the ethanol extract in hydrophobic organic solvents to obtain partially purified compound(s) for suppression of obesity [6]. We also showed increased fecal excretion of TC and TG on administration of 10% PJT powder and ethanol extract treatments in vivo (Table 1). The white adipose tissue weight and liver TG levels were significantly reduced due to ethanol extract administration, and the ethanol extract possessing high lipase inhibition activity partially explains the increased fecal excretion of TG. However, bile acid (BA) in feces was significantly low, thereby suggesting increased return flow of BAs from the small intestine to the liver, which regulates lipid homeostasis in the entire body. Alternatively, increased fecal BA was shown to have reduced fecal cholesterol, thereby suggesting increased microbial breakdown of cholesterol in the large intestine. The discrepancy between the two studies may be due to the difference in the mechanism of a specific diet and the animal type. The farnesoid X receptor (FXR) α is mainly expressed in the liver, intestines, and to a lesser extent, in adipose tissue. BAs are governed by FXRa and play an integral role in solubilization and absorption of lipids. BAs can also catabolize cholesterols in the body. In our studies, FXRa expression was increased in both adipose tissue and liver, suggesting of the ability of 10% PJT powder [4] and ethanol extract [5] to activate BA secretion. In the adipose tissue, PPARγ is the key regulator of adipogenesis and was significantly upregulated. Thiazolidinediones (TZDs), which are synthetic PPARγ ligands, have been shown to stimulate adipocyte differentiation and increase insulin sensitivity. However,
Figure 3
C57BL/6 mice were fed with a high-fat diet supplemented with no PJT powder (control), PJT powder 10 or 20%, water extract (0.8%), 50% ethanol extract (0.8%) or ethanol extract (0.8%) for 4 weeks. The final body weight was measured (A). Abdominal (B) and subcutaneous fat weight (C) were calculated based on the tissue weight (g per 100 g of body weight). Values are expressed as mean ± S.E. of six mice. Significant difference compared with the control group by Dunnett’s test, *p < 0.05; **p < 0.01.
clinical use of TZDs do not cause any significant weight gain in humans. *Magnolia officinalis*, which is a Chinese herb, was shown to stimulate adipocyte differentiation and increase glucose uptake. The ethanol extract of leaves of *Ilex paraguariensis* induced decreased PPARγ expression in 3T3-L1 cells and attenuated adipogenesis, due to its polyphenolic content. Studies on the relative roles of PPARγ1 and PPARγ2 isoforms in adipogenesis have shown contradicting activities in adipogenesis. Some studies have shown both these isoforms are important for differentiation activation in PPARγ1−/− fibroblasts. Alternatively, the PPARγ2-knockout mice model developed insulin resistance but showed normal adipose tissue mass and morphology. Although the differences for these deviations are not clear, PPARγ2 showed that it is not an absolute requirement in adipocyte development in vivo. Taken together, these results suggest that the PJT ingredient is a weak PPARγ ligand with high oxidative capacity and energy expenditure. Further, in our studies, the hexane phase was found to have increased PPARγ expression [6] similar to that of melatonin, thereby improving adipocyte differentiation along with acceleration of mitochondrial biogenesis in 3T3-L1 preadipocytes and thus suppressing obesity.

In addition, the upregulation of adipose triglyceride lipase (ATGL) in the in vivo study performed using 10% PJT powder [4] and hexane phase [7] indicated increased triglyceride catabolism. Improvement in fatty acid metabolism is further confirmed by the downregulation of paternally expressed gene 1/mesoderm specific transcript (MEST), the adipocyte size marker gene, whereas the elevated levels of RAR-related orphan receptor C (RORC) contributes to the reduction of TG levels via fatty acidβ-oxidation. Thus, our studies demonstrated the partial mechanism of action of lipogenic gene modulation due to PJT powder in adipose and liver tissues. However, energy mobilization activities in the muscle tissue due to 10% PJT were not investigated in our earlier study [4], therefore we could not conclusively elucidate the role of PJT activity in accelerating energy expenditure. In this context, we further found antiobesity phytochemicals in the ethanol extract bypassing the TG from the small intestine before absorption and excretion via feces, as one of its main roles, while the liver, adipose, and muscle tissue gene modulation alters lipid metabolism partially contributing to obesity attenuation.
In the muscle tissue, carnitine palmitoyltransferase 1 alpha (CPT1α), crucial for fatty acid β-oxidation, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), which is the key regulator of mitochondrial biogenesis leading to increased energy expenditure, was upregulated owing to both 10% PJT powder and ethanol extract, suggesting increased energy production. Moreover, the increased levels of uncoupling protein (UCP) 3, may increase fatty acid oxidation and glucose metabolism in the muscle tissue. In addition, UCP3 overexpression using adenovirus gene transfer in L6 myotubes increased glucose transporter 4 (GLUT4) translocation and glucose uptake. This prompted us to further examine the effects of PJT ethanol extract for antidiabetic activity in obese diabetes animal model ob/ob mice. Our group found that increased expressions in CPT1α, UCP2/3, and GLUT4 in the soleus muscle tissue [8] confirmed previous results of high fatty acid β-oxidation and energy production [5] caused by the PJT ethanol extract. The serum glucose level also showed a reduction tendency, with a significant suppression in the homeostasis model assessment of insulin resistance (HOMA-IR) index, which is the hallmark of insulin sensitivity. However, it remained unexplored whether the phytochemicals in the ethanol extract alter lipid metabolism to attenuate adiposity and body weight gain in HFD–fed mice. Thus, we investigated the inhibitory effects of partially purified ethanol extract on obesity in vitro for further identification of molecular mechanisms related to antiobesity.

In this context, we showed the inhibitory effects of ethanol extract, and its fractions, the hexane phase and residual water phase, on obesity in 3T3-L1 adipocytes, HepG2 hepatocytes, and C2C12 myocytes [6]. The PJT powder was extracted in ethanol to obtain the ethanol extract (11.0%) followed by the fractionation of hexane phase (3.3%) and water phase (7.0%). All extracts were dried, measured the dried weight, re-dissolved in ethanol, and stored at −20°C until further use. For experiments, 1 mL of each extract was dried, measured dried weight and dissolved in DMSO to adjust the concentration of each extract to 50 mg/mL. When adding to the cell, the concentration of each extract was further diluted in the respective cell culture medium to 50 µg/mL for ethanol extract, 11 µg/mL for hexane phase, and 37 µg/mL for water phase to ascertain relative antiobesity activities of hexane and
water phases depending on the mixing ratio of each phase in the ethanol extract. A significant inhibition in the TG accumulation was observed in both the ethanol extract and hexane phase treatments with highest amelioration in the hexane phase indicating the hydrophobicity of the active constituent of PJT (Fig. 4). We also showed phenolic compounds of ethanol extract with related peak intensities eluted by an ODS column; low polarity compounds with a higher retention time were speculated to be the active component(s). Therefore, we further fractionated the ethanol extract and its fractions via HPLC ODS column and elucidated that the latter portion of the compounds are solubilized in the hexane phase and were responsible for the antiobesity activity. Contrary, piperine, a major alkaloid-amine compound and pungent ingredient in black pepper, which is more hydrophilic, has shown strong attenuation of adipogenesis in 3T3-L1 cells. On the other hand, 70% ethanol extract of the roots of Platycodon grandiflorum and Scutellaria baicalensis showed antiobesity activity in HFD–fed C57BL/6 mice. In general, there are two ways to diminish adipose tissue mass: by reduction in the adipocyte volume, usually by increased lipolysis of the stored lipids, and by reduction of the adipocyte number. The PJT hexane phase seemed to follow the first pathway, which losses the lipid content by accelerated lipolysis. It is well known that a cascade of transcription factors play a key role in the control of the differentiation of preadipocytes into adipocytes [9]. After mitotic clonal expansion, the expressions of CCAAT/enhancer binding proteins (C/EBP) β and δ gradually declined followed by high expression of C/EBPα and PPARγ. This upregulation progress throughout the mid to late stage of adipogenesis allows the upregulation of lipogenic genes at the late stage of adipogenesis. In our studies, we identified increased PPARγ and C/EBPα expression in the hexane phase, indicating accelerated differentiation [6], as observed in vivo studies [4, 5, 8]. However, decreased adipocyte size and increased fatty acid oxidation due to the hexane phase confirmed the hydrophobicity of the partially purified active constituent in vitro, as suggested in our in vivo studies. Inhibition of lipogenesis of HepG2 cells in the hexane phase was controlled by SREBP1c, fatty acid synthase (FASN), and increased levels of lipolytic genes. The inhibitory activity was however decreased to a certain extent by addition of
insulin into the cell growth medium. A similar mechanism has been observed in berberine, with increased glucose consumption in HepG2 hepatocytes in an insulin-independent manner. In our in vitro study, the glucose consumption did not

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**Figure 4**

3T3-L1 preadipocytes (treatment period: day 2 to day 6; A) and HepG2 hepatocytes (treatment period: 12 h; B) were treated with ethanol extract (50 µg/mL), hexane phase (11 µg/mL) or water phase (37 µg/mL) and TG content was evaluated. Data are shown as mean ± S.E. of three independent experiments. Significant difference compared with the control group by Dunnett’s test, *p < 0.05. Pre: non-differentiated 3T3-L1 preadipocytes.
attain the level of significance probably due to lack of acceleration in glycolysis in the myotubes. Thus, it is a timely requirement for further in vitro studies using either the hexane phase or purified pteryxin [10] to address this issue in C2C12 cells. In an investigation using a HFD supplemented with the ethanol extract, hexane phase, and water phase, C57BL/6 mice showed significant reduction in body weight and epididymal fat content due to hexane phase treatment after 4 weeks of experimentation [7]. Despite the fact that the concentration of the hexane phase in the diet was low, the treatment was strong enough to reduce the body weight of mice. Quantification of the pteryxin in each fraction showed that the hexane phase had the highest level of pteryxin, which may have partially contributed to the antiobesity effect in vivo. It has been shown in a recent study that pteryxin was rapidly absorbed (in 2 hours), and distributed to liver and other peripheral tissues in mice, which may support above our proposal. In a human trial, increased levels of quercetin in plasma were observed following dietary quercetin supplementation. However, quercetin was intensely metabolized following absorption in the gastrointestinal tract, as the hydroxyl groups of the quercetin molecule leads to conjugation with other molecules such as glucuronic acid. On the other hand, flavangenol, a mixture of polyphenol, catechin and epicatechin showed uninterrupted circulation in rat serum making flavangenol an effective therapy in ROS-related skin diseases. Moreover, we found that the position of the methyl group attachment to the basic khellactone structure is an important factor of pteryxin to possess the antiobesity activity when compared with the other similar khellactones extracted from the PJT leaves (unpublished data), also suggesting that pteryxin is entered to the blood circulation in a non-conjugated form following administration in order to enhance its bioavailability at its desired target point.

3. Antiobesity effects of pteryxin isolated from the hexane phase of PJT

We further fractionated the hexane phase by open column chromatography, followed by HPLC using a silica gel column due to the high hydrophobicity of the compounds solubilized in the hexane phase [10]. Pteryxin, a coumarin, was isolated (Fig. 5A) as the antiobesity (Fig. 5B) compound in PJT, and the direct
gene modulation pattern related to the suppression of adipogenesis was studied. Although we earlier suggested less absorption of TG and TC in the small intestines and high excretion of these metabolites via feces as a main effect of PJT [4, 5], we found no significant change in the fecal excretion of TG and TC when the C57BL/6 mice were fed a HFD supplemented with the pteryxin-rich hexane phase [7], whereas direct interference of the lipogenic genes regulated the lipid metabolism [10].

**Figure 5**
The chemical structure of pteryxin in 2D and 3D (A) form. The 3T3-L1 preadipocytes were treated with pteryxin 10 or 20 µg/mL from day 2 to day 6. The TG content was measured on the day 6. Data are shown as mean ± S.E. of three independent experiments. Significant difference compared with the control group by Dunnett’s test, *p < 0.05.
and led to alterations in the molecular profile at a protein level due to pteryxin (either at 20 or 50 µg/mL) in 3T3-L1 adipocytes (unpublished data), which plays an active role in the suppression of obesity. Pteryxin (20 µg/mL) significantly downregulated key lipogenic genes such as SREBP1c, FASN, and acetyl-CoA carboxylase-1 (ACC1), with an increase in the lipolytic genes, such as hormone sensitive lipase (HSL) and PPARα, thereby accelerating lipolytic activity and reducing TG storage in both adipocytes and hepatocytes, as reported for the hexane phase of PJT. Pteryxin (20 µg/mL) upregulated the PPARγ mRNA level, however, the activity was lower than that in the hexane phase probably due to the purification. Further, as a consequence of increased PPARγ, adiponectin levels were high due to pteryxin. Adiponectin is an adipokine that is produced by the adipose tissue and induced by the activity of PPARγ. Unlike other adipokines, adiponectin levels increase with reduced obesity, even though one can argue that adiponectin is an adipose tissue-specific hormone. We observed that the effect of pteryxin was highest during the early stage of adipogenesis and was greater than chlorogenic acid, one of the polyphenols, already identified in PJT [5] in both 3T3-L1 and HepG2 cells. Apart from chlorogenic acid, rutin and neochlorogenic acids were also identified in the ethanol extract of PJT. The inhibition of adipogenesis in 3T3-L1 adipocytes using phenolic acids has identified rutin with the highest inhibition on intracellular TG and neochlorogenic acid to be an enhancer of hepatic carnitine palmitoyltransferase activity. Also, caffeine and chlorogenic acid possess a tendency to reduce visceral fat and body weight. On the other hand, a HFD supplemented with chlorogenic acid increased lipid content and steatosis in vivo. Although the reasons for these contradictions are unknown, the antiobesity activity of pteryxin was greater than that of chlorogenic acid. Furthermore, we discovered several other coumarins in the hexane phase of PJT, namely isosamidine and peucedanocoumarin III (unpublished data), which are already known coumarins in PJT, although their antiobesity effects are less than that of pteryxin. Thus, it will provide aid in the comparison of these compounds to elucidate the strongest coumarin or synchronized effect of several coumarins to be used as an antiobesity drug from the PJT plant.

It is noteworthy that we have examined the effect of pteryxin dose higher than
50% of Fr. 3 concentration in cells. In this context, we performed experiments using two concentrations of pteryxin 20 µg/mL and 50 µg/mL in 3T3-L1 preadipocytes (unpublished data). It was clear that pteryxin inhibited adipogenesis irrespective of the dose, with the highest inhibition observed at a dosage of 50 µg/mL. The gene modulation patterns were completely different in the two doses suggesting two pathways triggered by pteryxin. At 50 µg/mL, pteryxin abolished \( \text{PPAR}_\gamma \) and \( \text{C/EBP} \alpha \) expressions. Unlike the crude ethanol extract or hexane phase, pteryxin completely suppressed adipocyte differentiation at a higher dose. Further, it downregulated the key lipogenic genes, such as \( \text{SREBP1c}, \text{FASN}, \) and \( \text{ACC1} \), and suppressed fatty acid transportation by inhibiting the \textit{fatty acid binding protein 4 (FABP4)} and \textit{lipoprotein lipase (LPL)} gene expressions. A possible reason for the change in the pattern of adipogenesis suppressive effect could be that pteryxin at a higher doses of 50 µg/mL is crucial enough to target AMPK regulation, which is the central regulator of cellular metabolism for which it serves as a port of energy homeostasis, in-charge of glucose and lipid metabolism. It has also known that AMPK mediates major metabolic responses to exercise indicating the probable upregulation in energy expenditure due to higher pteryxin dose, as observed in our gene expression results (unpublished results).

4. Specificity of pteryxin against other natural antiobesity compounds and the proposed mechanism of action

The involvement of pteryxin in the suppression of adipocyte differentiation by different molecular pathways has made it a unique compound when compared to several other naturally existing pharmacological compounds. As shown in Figure 6, pteryxin has been compared with (1) wingless-type mouse mammary tumor virus integration site (Wnt)/\( \beta \)-catenin dependent curcumin, one of the most important ingredients in turmeric, EGCG from green tea and apelin, an adipokine secreted by adipose tissue, (2) Wnt/\( \beta \)-catenin independent berberine, an isoquinoline alkaloid from \textit{Coptis japonica} and quercetin from many vegetables and fruits such as guava, and (3) other compounds, where the activity against the Wnt-related pathway are currently unknown, such as ursolic acid, a natural pentacyclic triterpenoid in
many different plants, fruits and herbs, vitamin U in raw cabbage, capsaicin from chili peppers, apigenin from fruits like grape fruit, diallyl trisulfide from garlic and piperine from black pepper. This illustration summarizes the activity of pteryxin and its unique characteristics that lead to obesity suppression through Wnt5a non-canonical pathway (unpublished data). However, further investigations on Wnt signaling pathway are required to elucidate the activity of pteryxin through the stimulation of intracellular Ca$^{2+}$ or activation of phospholipase C and protein kinase C.
Figure 7

Proposed mechanism of action for pteryxin in the attenuation of obesity in adipose, liver and muscle tissue/cells. AMPK; AMP-kinase, PKA; protein kinase A, MAPK; mitogen-activated protein kinase, ERK; extracellular signal-regulated kinase, p-ACC; phosphorylated acetyl CoA carboxylase, MEST; paternally expressed gene 1 (Peg1)/mesoderm specific transcript (Mest), PPAR; peroxisome proliferator activated receptor, C/EBP; CCAAT/enhancer binding protein, SREBP; sterol regulatory element-binding protein, WNT; wingless-type mouse mammary tumor virus integration site, FASN; fatty acid synthase, FABP4; fatty acid binding protein 4, CPT; carnitine palmitoyltransferase, PGC1α; peroxisome proliferator activated receptor gamma coactivator 1-alpha, PGC1α-AC; acetylated peroxisome proliferator activated receptor gamma coactivator 1-alpha, FFA; free fatty acids, CAT; carnitine acyl transferase II, DAG; diacylglycerol, TAG; triacylglycerol, TCA; tricarboxylic acid cycle, RORC; RAR-related orphan receptor gamma C, UCP; uncoupling protein, SCD; stearoyl-CoA desaturase, ACC; acetyl CoA carboxylase, and h denotes human-related genes.
Based on the antiobesity data accumulated on pteryxin, we showed a proposed mechanism of action (Fig. 7) to express the activity of pteryxin to suppress adipogenesis. By taking into consideration the results of our previous and ongoing studies [4-7, 10], we were able to summarize the activity of pteryxin in both the adipose and liver, thereby reducing lipid accumulation and inducing the energy expenditure in the muscle.

5. Conclusion

Several research studies have clearly shown the antiobesity effect of pteryxin in rodent cell lines, rodents as in vivo models and human cell lines. Further research on the mechanisms related to pteryxin and clinical trials are warranted to confirm the clinical safety in humans. PJT has a great potential for use in the pharmaceutical industries due to the large number of coumarin compounds, with potential properties against obesity, thus, strategies of using this medically important and wild-grown plant should be implemented and much awareness of the values of this plant should be distributed among the general audience.

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