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Review

A comprehensive review on *Pueraria*: Insights on its chemistry and medicinal value

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ABSTRACT

Pueraria species are listed in the Chinese Pharmacopeia and are being used for the treatment of various health ailments and in protecting health. Puerarin, a chemotaxonomic marker of *Pueraria*, received investigational drug status for the treatment of alcohol abuse and listed in DRUGBANK database. The purpose of this review is to provide insights on health benefits of *Pueraria*, its bioactive constituents and molecular mechanisms. The information is retrieved from various databases. The most investigated plant part is tuber and the major bioactive constituents are isoflavones. The *Pueraria* is reported to possess a lots of health benefits on brain, liver, heart, kidney, bone, stomach, muscle, skin, and reproductive system. *Pueraria* also shown beneficial effects in postmenopausal women. In this review, the scientific information on *Pueraria* reported until May 2020 were analysed and summarized logically to appreciate its health benefits and to identify research gaps.

1. Introduction

The genus *Pueraria* (family: Leguminsoae, sub-family: Pioideae) consisting of 26 species, is mostly distributed in Asia, North America, and South America. It presents a taxonomic challenge in the identification of correct species as many synonyms are reported for a single species. For example, there are 96 plant names under the genus '*Pueraria*' recorded in the plant database www.theplantlist.org, out of which 26 are accepted names, 65 are synonym names, 4 are unresolved names and 4 are misapplied names. The *Pueraria* plants are well known for their health and cosmetic benefits in addition to being used in agriculture as a weed plant for preventing soil erosion. They are being used in various traditional systems for treating menopausal syndrome-related ailments. The most widely used part of the plant in traditional medicine is tubers. The medicinally important plants of this genus are commonly known as kudzu. The predominant phytochemical constituents are isoflavones, also known as phytoestrogens. The formulations containing *Pueraria* species are being sold in different parts of the world as a health supplement. Scientific studies have been carried out on the genus *Pueraria* to prepare phytoestrogen rich extracts, to develop analytical methods for authentic identification of the species & quality control of formulations, to validate the traditional claims & identify the molecular mechanisms, to determine the pharmacokinetic profile of phytoestrogens, to optimize the formulations for improving the bioavailability of phytoestrogens, to characterize the chemical compounds, to explore the beneficial interactions with other herbs & drugs and to evaluate the efficacy in humans. In the scientific literature, the findings on the following P-*Pueraria* species were recorded: *Pueraria peduncularis*, *P. candollei* var. *mirifica* (Synonym: *P. mirifica*), *P. montana var. lobata* (Synonyms: *P. thunbergiana* Benth., *P. lobata* (Willd.) ohwi) *P. tuberosa* (Willd.) DC and *P. candollei* Grah. ex Benth.

There is only one review article published on *Pueraria montana var lobata* [1]. Due to the fact that many *Pueraria* species are being used for health benefits, the aim of the review to provide insights on their traditional uses, bioactivities & molecular mechanisms, chemical constituents, analytical methods used for authenticity, formulation development and quality control studies, synergistic studies and clinical studies as well as to identify the research gaps for future research.

1.1. Purearia in Chinese Pharmacopoeia - preparation and therapeutic indications

Pueraria peduncularis (Benth.) Benth (Chinse name: ku ge, 苦葛). Ten grams of roast dried powder of *P. peduncularis* is mixed with five grams

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of pepper and taken orally twice for the treatment of haemorrhoids. Twenty-five grams of it is boiling with water and taken orally for the treatment of alcoholism and poisoning. *Pueraria lobata* (Willd.) ohwi (Synonym: *Pueraria thunbergiana* Benth, Chinese name: ye ge, 野葛) and *Pueraria thomsonii* Benth (Chinese name: gan ge teng, 甘葛藤) are collected during winter or autumn. They are made into small slices and dried. A decoction is prepared by boiling 10–15 gram of dried slices in water. The decoction is used for the treatment of fever, alcoholism, rashes, measles, diarrhoea, headache, backache, dysentery, dizziness, headache, chest pain and hemiplegia stroke.

The literature on *Pueraria* was collected from electronic databases; Scopus, PubMed, and Web of Science until May 2020. The literature was retrieved using the key term 'Pueraria'. The articles published in English were reviewed. The authors screened the titles and abstracts for choosing the relevant articles. The chemical structures of the compounds isolated from *Pueraria* were obtained either from the research article or PubChem.

1.2. Compounds and metabolites isolated from Pueraria sp

The chemical structures of the isolated compounds are shown in Table 1. The compounds include Isoflavones (**1-49**), Flavone (**50**), Flavonols (**51-54**), Coumestrols (**55-60**), Triterpenoid saponins (**61-72**), Xanthones (**73-74**), Puerariafurans (**75-76**), Lignans (**77-80**), Sterols (**81-87**), and miscellaneous compounds (**88-103**). Puerarin, an isoflavone, is a chemotaxonomic maker of the genus *Pueraria*. Many of the bioactivities of the genus *Pueraria* is attributed to isoflavone glycosides, mainly puerarin. Among the sterols, Miroestrol is widely investigated for its biological activities.

2. Biological activities

2.1. Alcoholism

It was historically documented that *Pueraria mirifica* plant was traditionally used in China for the treatment of alcoholism. This has led researchers to investigate the protective effect of *P. mirifica* as well as its mechanism of action. Tectoridin (**43**) is an isoflavone glycoside from the *P. mirifica* plant and is believed to be responsible in the attenuation of alcoholism. Yu Xiong *et. al.* performed this study in male C57BL/6 mice where the mice were orally given ethanol (5 g/kg) every 12 h and Tectoridin (**43**) was given 1 h after the final dose of ethanol. The results revealed that Tectoridin (**43**) significantly and dose-dependently decreased the levels of the serum ALT, AST and TG and reversed the alcoholic steatosis. Microscopic examination of H&E and Oil Red O stained section of liver revealed that it reversed the decreased levels of PPAR-α and the related target genes medium-chain acyl-CoA dehydrogenase, acyl-CoA oxidase, cytochrome P450 4A. In addition, it also increased the fatty acid oxidation. [2] The molecular mechanisms are shown in Fig. 1.

Another factor that contributes to alcohol-induced neuroinflammation is microglial activation. Dan Yuan *et. al.* reported the presence of 6-hydroxygenistein-6,7-di-O-glucoside (**3**), 6-hydroxygenistein-7-O-glucoside (**22**), Gehuain (**31**), Genistein (**32**), Genistin (**33**), Irisolidone (**34**), Kakkalide (**35**), Tectoridin (**43**), Tectorigenin (**44**) and Tectorigenin-7-O-xylosyl-glucoside (**45**) in ethanol extract of *P. thomsonii* Benth. flowers. The authors investigated the effect of these compounds on lipopolysaccharide (LPS)-induced neuroinflammation in primary rat microglia cells. The most potent compounds were Genistein (**32**; IC_{50}: 1.3 μM), Tectorigenin (**44**; IC_{50}: 9.3 μM) and Irisolidone (**34**; IC_{50}: 2.3 μM). From the structure-activity relationship (SAR) studies, it was postulated that glycosylation at the C-7 hydroxyl group of these compounds results in decreased activity, while methoxylation at the 6-position improved the activity. [3]

R.C. Lin *et. al.* performed an experiment to investigate the anti-dipsotropic effect of Daidzein (**25**), Daidzin (**29**) and Puerarin (**40**) from *P. lobata* using an alcohol-preferring-selectively-bred P line of rats as the animal model. The results revealed that all the three Isoflavones dosed at 100 mg/kg/day showed its effect in the suppression of voluntary alcohol consumption by the P rats. The intervention showed that Daidzein (**25**) was the most potent and it decreased the alcohol consumption by 75 % followed by Daidzin (**29**; 50 %) and Puerarin (**40**; 40 %). The suppression was evidently observed after one day of oral administration; and discontinuation of treatment resulted in the rats returning to the original preference in alcohol consumption. These findings suggested that the Isoflavones extracted from *P. lobata* were effective in the suppression of alcohol preference. [4]

Puerarin received an investigational drug status (https://www.drugbank.ca/drugs/DB12290) for the treatment of alcohol abuse. However, it is interesting to note that it's mechanism is not investigated as extensively as that for Puerarin. Thus, further research should be carried on puerarin to investigate its mechanism of action in the treatment of alcoholism, *in vitro* and *in vivo* drug metabolism & pharmacokinetic (DMPK), toxicity, adverse effects, and interactions with drugs & food.

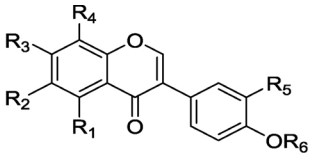
2.2. Antioxidant activity

P. thunbergiana Benth. leaves, stems, sprouts and roots extracts (70 % ethanol) were investigated for their antioxidant activity [5]. The evaluation of antioxidant activity was performed by measuring free radical scavenging (FRS) activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) systems, and xanthine oxidase inhibition activity assay. The FRS_{50} values of the leaves, stems, sprouts, and roots were found to be 436, 1135, 755 and 16.4 μg/mL in DPPH system and 121, 455, 342 and 135 μg/mL in ABTS system, respectively. Extract (200 μg/mL) of the leaves, stems, sprouts, and roots were found to cause 37, 4.6, 4 and 52 % xanthine oxidase inhibition, respectively. The total phenolic content (TPC) in leaves, stems, sprouts, and roots was found to be 58, 25, 38 and 63 mg/g respectively. The major compounds found in the extracts were Isoflavones; Daidzein (**25**), Daidzin (**29**), Genistein (**32**), Genistin (**33**), Ononin (**38**), Puerarin (**40**) and Schaftoside (**103**). The total Isoflavones content in leaves, sprouts and roots were found to be 66, 2.3 and 73 mg/g respectively while the Isoflavones are not detected in stems. Overall the antioxidant activity is in the order of roots > leaves > sprouts > stems which is correlated with relative TPC and total isoflavone content. [5]

Reactive oxygen species (ROS) cause apoptosis of vascular endothelial cells and it is one of the causes for the pathogenesis of cardiovascular diseases. Gao *et. al.* [6] reported the protective and therapeutic effect of ethanol extract of *P. lobata* root on human umbilical vein endothelial cells (HUVECs). The roots were extracted with 70 % ethanol and purified over AB-8 resin column to prepare the isoflavone enriched extract. The concentration of isoflavones were found to be Puerarin (**40**; 69.33 %), Daidzin (**29**; 13.70 %) and Daidzein (**25**; 1.31 %). Rotenone was used as toxicant; the protective effect of the extract was assessed by adding the extract before adding the rotenone while therapeutic effect was assessed by adding the extract together with rotenone. The extract was found to promote the HUVECs proliferation, attenuates the ROS levels, apoptosis and prevents the loss of mitochondrial membrane potential. These results are suggesting the extract has a potential cardioprotective effect.

The different extracts (petroleum ether, chloroform, acetone, methanol, and water) of *P. tuberosa* roots were investigated for antioxidant activity. The acetone extract was discovered to contain highest total phenol content and total tannin content while methanol extract was discovered to be rich in total flavonoid content. All the extracts [7] were reported to exhibit varied Phosphomolybdenum, ferric reducing antioxidant potential (FRAP), ABTS + metal ion radical and DPPH scavenging activities. These results suggesting that *P. tuberosa* is a good source for natural antioxidants and has a potential to be used in many diseases.

Table 1
Chemical structures of isolated compounds from *Pueraria*.



Compound	R_1	R_2	R3	R_4	R_5	R_6
5,6,7,4'-tetrahydroxyisoflavone-6,7-di-O-glucoside [**1**]	OH	O-Glc	O-Glc	H	H	H
5,6,7-trihydroxy-4'-methoxyisoflavone-6,7-di-O-glucoside [**2**]	OH	O-Glc	O-Glc	H	H	Me
6-hydroxygenistein-6,7-di-O-glucoside [**3**]	OH	O-Glc	O-Glc	H	H	Me
8-C-glucosyl-4',7-dihydroxy-3'-methoxyisoflavone 4'-O-glucoside [**4**]	H	H	OH	-Glc	OMe	Glc
3'-Hydroxypuerarin [**5**]	H	H	OH	-Glc	OH	H
3'Hydroxypuerarin-4'-O-deoxyhexoside [**6**]	H	H	OH	-Glc	OH	Deoxyhexose
3'-methoxydaidzein 8-C-apiosyl-glucoside [**7**]	H	H	OH	-Glc-Api	OMe	H
3'-Methoxy-6"-O-xylosyl-puerarin [**8**]	H	H	OH	-Glc-Xyl	OMe	H
3'-Methoxydaidzein [**9**]	H	H	OH	H	OMe	H
3'Methoxydaidzein-7-O-methyl ether [**10**]	H	H	OMe	H	OMe	H
3'Methoxydaidzin [**11**]	H	H	O-Glc	H	OMe	H
3'-Methoxyformononetin [**12**]	H	H	OH	H	OMe	Me
3'Methoxypuerarin [**13**]	H	H	OH	-Glc	OMe	H
4',6-dihydroxy-7-methylisoflavone [**14**]	H	OH	Me	H	H	H
4,7-dihydroxy- 3-methoxyisoflavone 8-C-glucosyl-glucoside [**15**]	H	H	OH	-Glc-Glc	OMe	H
4',7-dihydroxy-3'-methoxyisoflavone 8-C-apiosyl-glucoside [**16**]	H	H	OH	-Glc-Api	OMe	H
4'-hydroxy-6-methoxy-7-methylisoflavone [**17**]	H	OMe	Me	H	H	H
4'-hydroxy-7-hydroxymethyl-6-methoxyisoflavone [**18**]	H	OMe	CH_2OH	H	H	H
5-hydroxypuerarin [**19**]	OH	H	OH	-Glc	H	H
6"-O-Xylosylpuerarin [**20**]	H	H	OH	-Glc-Xyl	H	H
6-hydroxygenistein-6,7-di-O-glucoside [**21**]	OH	O-Glc	O-Glc	H	H	H
6-hydroxygenistein-7-O-glucoside [**22**]	OH	OH	O-Glc	H	H	H
7,8,4'-trihydroxyisoflavone [**23**]	H	H	OH	OH	H	H
Biochanin A [**24**]	OH	OH	OH	H	H	Me
Daidzein [**25**]	H	H	OH	H	H	H
Daidzein4',7-diglucoside [**26**]	H	H	O-Glc	OH	H	Glc
Daidzein-7-O-methyl ether [**27**]	H	H	OMe	H	H	H
Daidzein-8-C-apiosyl-glycoside [**28**]	H	H	OH	-Glc-Api	H	Cyclohexane
Daidzin [**29**]	H	H	O-Glc	H	H	H
Formononetin [**30**]	H	H	OH	H	H	Me
Gehuain [**31**]	H	OMe	O-Glc-Xyl	H	H	Me
Genistein [**32**]	OH	OH	OH	H	H	H
Genistin [**33**]	H	H	O-Glc	OH	H	H
Irisolidone [**34**]	OH	OMe	OH	H	H	Me
Kakkalide [**35**]	OH	OMe	O-Glc-Xyl	H	H	Me
Neopuerarin A [**36**]	H	H	OH	-Glc	H	H
Neopuerarin B [**37**]	H	H	OH	-Glc	H	H
Ononin [**38**]	H	H	O-Glc	H	Me	Me
Phormononetin [**39**]	H	H	OH	H	H	H
Puerarin [**40**]	H	H	OH	-Glc	H	H
Puerarin-4'O-glucoside [**41**]	H	H	OH	-Glc	H	Glc
Sissotrin [**42**]	OH	H	O-Glc-Xyl	H	H	Me
Tectoridin [**43**]	OH	OMe	O-Glc	H	H	H
Tectorigenin [**44**]	OH	OMe	OH	H	H	H
Tectorigenin-7-O-xylosyl-glucoside [**45**]	OH	OMe	O-Glc-Xyl	H	H	H
Calycosin [**46**]	H	H	OH	H	OH	Me
Glycitein [**47**]	H	OMe	OH	H	H	H
Glycitin [**48**]	H	OMe	O-Glc	H	H	H

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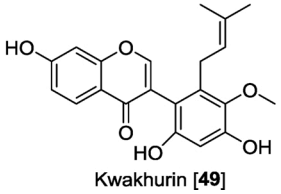
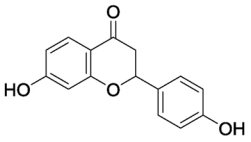


Kwakhurin [**49**]

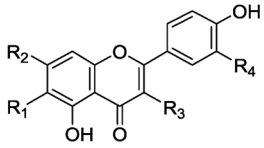
Table 1 (*continued*)

Flavones



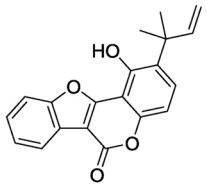
Liguiritigenin [**50**]

Flavonols

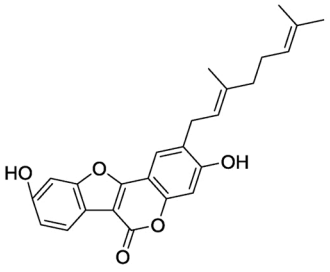


Compound	R_1	R_2	R_3	R_4
Isoorientin [**51**]	Glc	OH	H	OH
Nicotiflorin [**52**]	H	OH	O-Glc-Rha	H
Robinin [**53**]	H	O-Rha	O-Gal-Rha	H
Rutin [**54**]	H	OH	O-Glc-Rha	OH

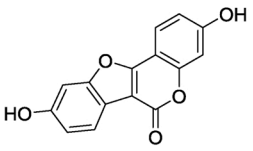
Coumestrols



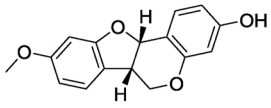
2-(α,α-dimethylallyl)coumestol [**55**]



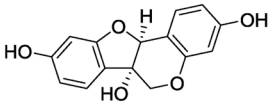
Puerarol [**56**]



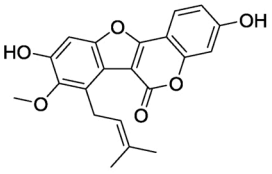
Coumestrol [**57**]



(-)-Medicarpin [**58**]

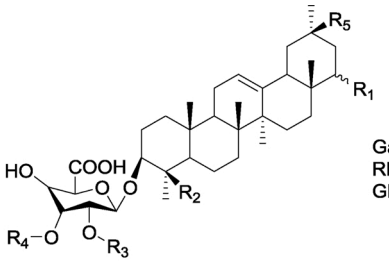


(-)-Glycinol [**59**]



Mirificoumestan [**60**]

Triterpenoid saponins



Gal = Galactose
Rha = Rhamnose
Glc = Glucose

Compound	R_1	R_2	R_3	R_4	R_5
Kakkasaponin I [**61**]	β-OH	CH_3	-Ara-Rha	H	H
Kakkasaponin II [**62**]	keto	CH_3	Gal	H	H
Kakkasaponin III [**63**]	keto	CH_3	-Xyl-Rha	H	H
Pedunsaponin A [**64**]	H	CH_2OH	H	Glc	CH_3
Pedunsaponin C[**65**]	H	H	H	Glc	CH_3
Pedunsaponin D [**66**]	keto	CH_2OH	H	Glc	H
Pedunsaponin E [**67**]	keto	CH_2	-Glc-Xyl	Glc	COOH
Phaseoside IV [**68**]	keto	CH_3	-Gal-Rha	H	H
Sophoradiol monoglucuronide [**69**]	β-OH	CH_3	H	H	H
Soyasaponin I [**70**]	β-OH	CH_2OH	-Gal-Rha	H	H
Soyasaponin III [**71**]	β-OH	CH_2OH	Gal	H	H
Soyasaponin IV [**72**]	β-OH	CH_2OH	Ara	H	H

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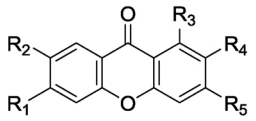
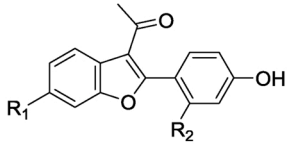
Table 1 (*continued*)

Xanthones



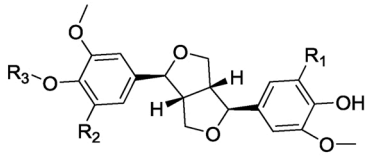
Compound	R_1	R_2	R_3	R_4	R_5
Mangiferin [**73**]	OH	OH	OH	Glc	OH
Xanthone [**74**]	H	H	H	H	H

Puerariafurans

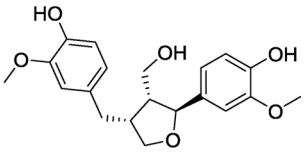


Compound	R_1	R_2
3′-de-O-methylpuerariafuran [**75**]	OH	OH
Puerariafuran [**76**]	H	OMe

Lignans

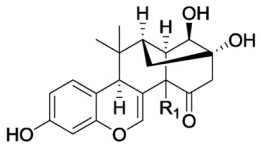


Compound	R_1	R_2	R_3
(+)-Pinoresinol [**77**]	H	H	H
(+)-Syringaresinol [**78**]	OCH_3	OCH_3	H
(+)-Syringaresinol-O-glucoside [**79**]	OCH_3	OCH_3	Glc

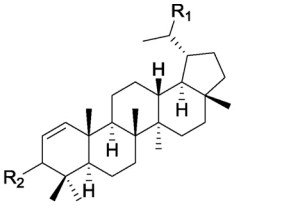


(+)-Lariciresinol [**80**]

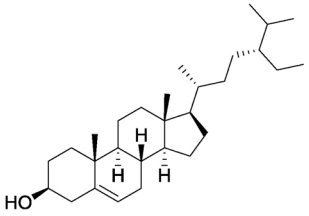
Sterols



Compound	R_1
Miroestrol [**81**]	OH
Deoxymiroesterol [**82**]	H



Compound	R_1	R_2
Lupenone [**83**]	CH_3	=O
Lupeol [**84**]	$=CH_2$	OH



β-Sitosterol [**85**]

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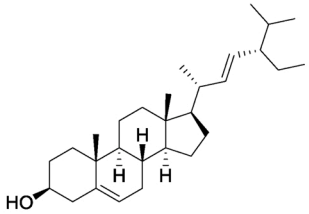
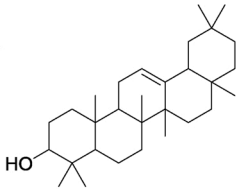
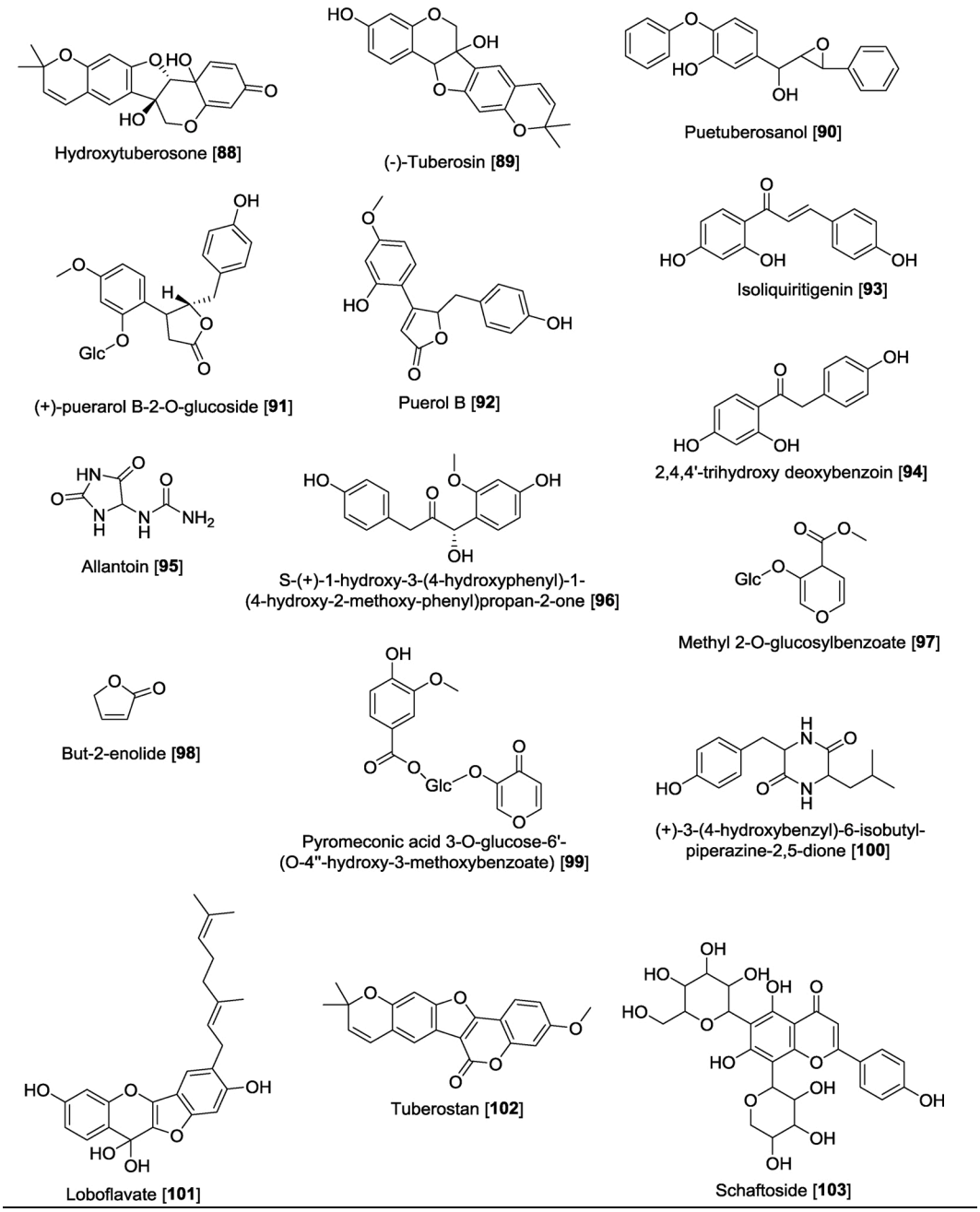


Stigmasterol [**86**]



β-Amyrin [**87**]

Table 1 (*continued*)



Compound Miroestrol **(81)**, a bioactive molecule found in the roots of *P. mirifica* improved antioxidant status in procarcinogen-exposed mice model induced by β-naphthoflavone (BNF). The compound, Miroestrol **(81),** administered at 5 mg/kg/day in mice, enhanced the antioxidation activity *via* the increased superoxide dismutase (SOD), and catalase activity while it reduced the levels of malondialdehyde in liver. [8]

In a study conducted by Chatuphonprasert *et. al.*, crude extract of *P. mirifica* (25 mg/kg per day) and its active phytoestrogen, Miroestrol **(81**; 1 mg/kg per day), were given to ovariectomized female ICR mice. The extract and Miroestrol **(81)** have shown to significantly increase the levels antioxidant enzymes, glutathione, reduced glutathione and GSH/GSSG $_{ratio}$ in liver and uterus; regulated the glutathione peroxidase, SOD and CAT to normal levels. Chatuphonprasert *et. al.* concluded that *P. mirifica* is believed to be a promising alternative hormone replacement candidate of estradiol. [9]

Chen *et. al.* provided insights through their investigation on the isoflavononid content and the antioxidant activity levels exerted by different segments of the *P. lobata* roots. In their work, they reported that the root outer bark contained the significantly higher isoflavonoid content, determined using HPLC, than that of kudzu root and whole root. The DPPH and ABTS free radicals scavenging activity revealed that the root outer bark of *P. lobata* showed the highest antioxidant activity. In their analysis, the isoflavonoids; Daidzein (**25**), Daidzin (**29**), Genistein (**32**), Genistin (**33**) and Puerarin (**40**); were found in all the sections of roots and highest concentration was found in the root outer bark. Thus, root outer bark has shown the highest antioxidant activity [10].

Seong Eun Jin *et. al.* isolated known compounds from the ethylacetate fraction: Daidzein (**25**), Genistein (**32**), Puerarin (**40**) and (+)-puerarol B-2-O-glucoside (**91**); n-hexane fraction: Puerarol (**56**), Coumestrol (**57**), Lupenone (**83**), Lupeol (**84**); and butanol fraction: 3'-Hydroxypuerarin (**5**), 3'Methoxypuerarin (**13**), Daidzein-8-C-apiosyl-

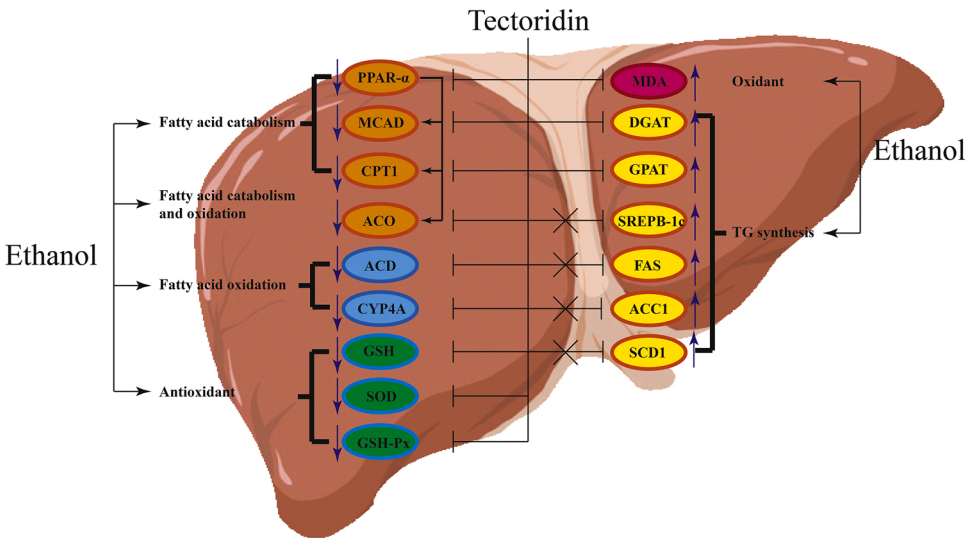


Fig. 1. Molecular mechanisms of Tectoridin (**40**) in alcoholism.
Legend: PPAR-α: Peroxisome proliferator-activated receptor - alpha, MCAD: Medium-chain acyl-CoA dehydrogenase, CPT1: Carnitine palmitoyl transferase 1, ACO: Acyl-CoA-oxidase; ACD: Acyl-CoA-dehydrogenase, CYP4A: Cytochrome P 450 4A, GSH: Glutathione, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, DGAT: Diacylglycerol acyltransferase, GPAT: Glycerol-3-phosphate acyltransferase, SREBP-1c: Sterol regulatory element-binding protein-1c (SREBP-1c), FAS: Fatty acid synthase, ACC1: Acetyl-CoA-Carboxylase-1, SCD1: Steraroly-CoA-desaturase-1.

glycoside (**28**), Daidzin (**29**), Genistin (**33**), Puerarin (**40**) and Allantoin (**95**). Their anti-inflammatory and antioxidant activity were investigated using *in vitro* model; LPS induced inflammation in murine macrophages, RAW 264.7 cells. They reported that compounds Lupenone (**83**) and Lupeol (**84**) reduced NO production and iNOS and COX-2 protein levels. Lupeol (**84**) has emerged as a compound that inhibited intracellular ROS generation significantly. [11]

Sucontphunt *et. al.* evaluated the extract and compounds; Daidzein (**25**) and Genistein (**32**); obtained from *P. mirifica* for their antioxidant activities. The DPPH radical scavenging assay revealed that the EC_{50} of *P. mirifica* extract was 0.192 mg/mL, Genistein (**32**) was 14.259 mg/mL and Daidzein (**25**) was 7.56 mg/mL. These results suggest that the combination of bioactive molecules found in the *P. mirifica* extract resulted in higher antioxidant activity [12].

In another study performed by Lidiya Bebrevska *et. al.*, the *in vivo* antioxidant activity of *P. lobata* (Willd.) Ohwi (Fabaceae) root extract (from 50 % EtOH) in streptozotocin (STZ)-induced diabetes accompanying oxidative stress in male Wistar Hannover rats was determined. The main bioactive constituent, Puerarin (**40**), was quantified in the root extract using HPLC and its concentration was found to be 10.42 ± 0.15 %. Small amounts of other isoflavones; 3'-Hydroxypuerarin (**5**), 3'Methoxypuerarin (**13**), 6"-O-Xylosylpuerarin (**20**), Daidzein (**25**), Daidzin (**29**), Genistein (**32**) and Genistin (**33**); were detected. The rats were administered with root extract (500 mg/kg) daily for three weeks. This dose corresponds to 50 mg/kg of the compound Puerarin (**40**). The findings from this study revealed that there was a decrease in malondialdehyde in plasma. [13]

Antioxidant effect results in a cascade of beneficial activities, one of which is to prevent the damage of auditory cells. H.-H. Yu *et. al.* reported that *P. thunbergiana* ethanol extract (5−100 μg/mL) exhibited dose-dependent protective effect against cisplatin-induced damage in conditionally immortalized House Ear Institute-Organ of Corti 1 (HEI−OC1). The authors also reported that the plant extract exhibited antioxidant activity in numerous scavenging assays of hydrogen peroxidase, SOD, hydroxyl radical and DPPH and the effect was found to be dose dependent. It is concluded that the protective effect of *P. thunbergiana* extract can be connected to antioxidant activities. [14]

Through DPPH scavenging assay, W. Cherdshewasart *et. al.* reported that tuber extracts of *P. mirifica* collected from provinces of Thailand and *P. lobata* collected from China exhibited weaker antioxidant activity than that of α-tocopherol. However, further analysis revealed that the compounds; Puerarin (**40**) and Daidzein (**25**); are equipotent to that of α-tocopherol in their antioxidant activity. [15] It has been reported that the aqueous root extract of *P. lobata* exhibited higher antioxidant activity than that of *P. thomsonii* and it is believed that *P. lobata* contains higher isoflavones content. J.-W. Jiang *et. al.* investigated the antioxidant activity of compounds using free-radicals-mediated damage in red blood cells. Six isolated compounds were investigated and out of which, Puerarin (**40**; IC_{50}: 756.2 ± 5.9 μM) showed equipotent antioxidant activity to that of ascorbic acid 720.5 ± 3.6 μM; while Daidzein (**25**) and Daidzin (**29**) (IC_{50}: 1000 μM) were less potent. [16] The molecular mechanism is concisely shown in Fig. 2.

Despite *Pueraria* extracts and compounds are being extensively investigated for their antioxidant properties, further research must be carried out to determine their antioxidant efficacy in physiologically relevant oxidative stress models.

2.3. Hepatoprotective activity

Puerarin (**40**) isolated from *P. lobata* (Willd.) has demonstrated to reverse liver fibrotic process in dimethyl nitrosamine (DMN)-induced liver fibrosis in rats. This was evidently shown as Puerarin (**40**) was found to regulate TGF-β1/Smad pathway and significantly reduced serum levels of hyaluronic acid (HA), laminin (LN), alanine amino-transferase (ALT), aspartate amino-transferase (AST), type III pre-collagen (PCIII) and type IV collagen(CIV) [17].

T. Arao *et. al.* firstly reported their findings on crude saponin extract and pure saponin, Soyasaponin I (**70**), from the roots of *P. lobata* inhibited ALT activity at 90 μg/mL. It presents a stronger protective effect compared to that of the positive control, glycyrrhizin in immunological liver injury model using primary cultured rat hepatocytes [18]. The same team later investigated the protective activity of individual saponins, and their SAR is established. The ALT was determined from the medium after 40 min. The authors presented that through SAR analysis, sapogenol with hydroxy group at C-21 enhanced the hepatoprotective activity [19].

The protective effects of *P. tuberosa* DC. butanol extract against carbon tetrachloride (CCl_4) induced hepatotoxicity were investigated by S. Shukla *et. al.* The study was performed using adult male rats of the Sprague-Dawley induced with CCl_4 (0.1 m/kg/day: intraperitoneal) for seven days as the experimental model. The treatment with butanol extract of *P. tuberosa* (150 mg/kg; *p.o.*) for 7 days resulted in significant protection and reversed the increased levels of lipid peroxidation, hepatic acid phosphatase and total protein caused by CCl_4. [20]

P. lobata and *P. tuberosa* has shown promising hepatoprotective activity. However, their efficacy was evaluated in very limited experimental models. Thus, further research is warranted to evaluate their hepatoprotective activity in drug-induced hepatotoxic models.

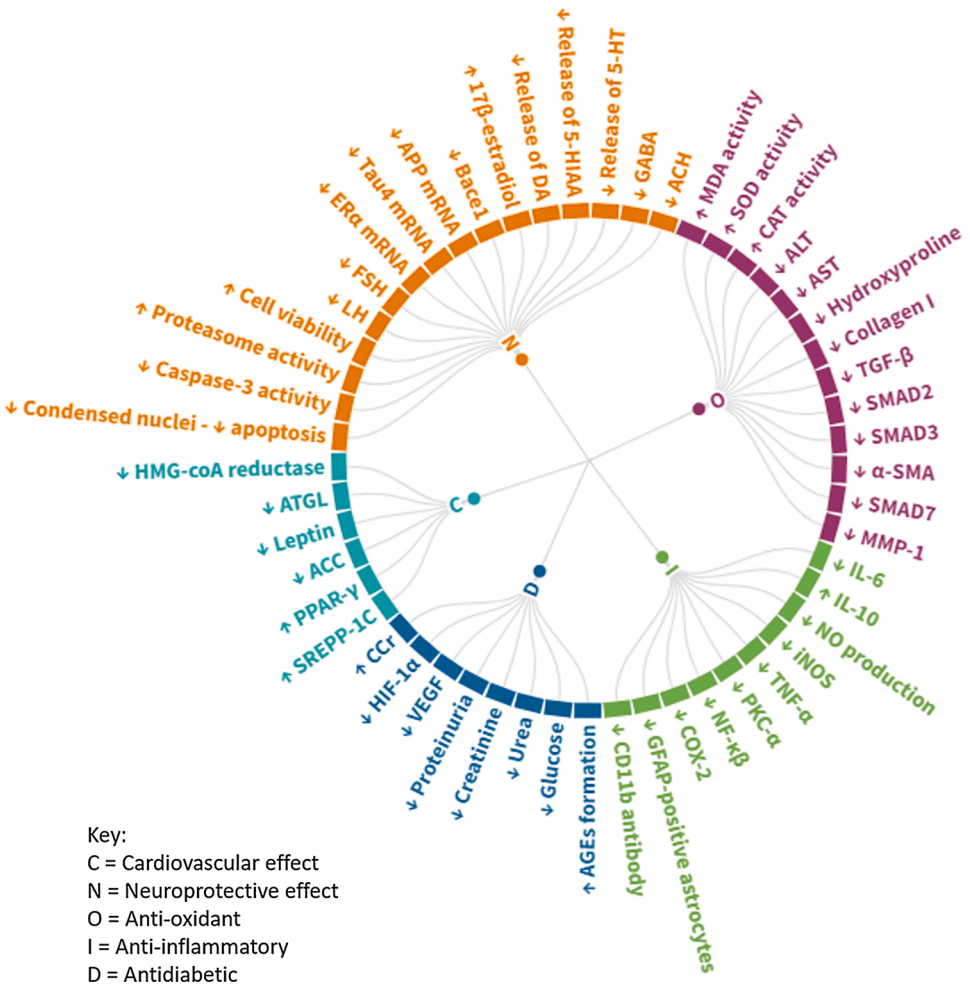


Fig. 2. Molecular mechanisms involved in the biological activities of *Pueraria*.
Legend: HMG-CoA: 3-hdyroxy-3-methylglutaryl coenzyme-A, ATGL: Adipose triglyceride lipase, ACC: Acetyl-CoA carboxylase, PPAR-: Peroxisome proliferator-activated receptor – gamma, SREPP-1C: Sterol regulatory element-binding protein – 1c, CCr: Cinnamoyl CoA reductase, HIF-1α: Hypoxia-inducible factor 1-alpha, VEGF: Vascular endothelial growth factor, AGEs: Advanced glycation end products, CD11b: Integrin alpha-M, GFAP: Glial fibrillary acidic protein, COX-2: Cyclooxygenase–2,NF-κB: Nuclear factor kappa-light-chain-enhanced of activated B cells, PKC-α: Protein kinase C alpha, TNF-α: Tumour necrosis factor-alpha, iNOS: Inducible nitric oxide synthase, NO: Nitric oxide, IL-10: Interleukin-10, IL-6: Interleking-6, MMP-1: Matrix metalloproteinase-1, SMAD7: Mothers against decapentaplegic homolog 7, α-SMA: Alpha smooth muscle actin, SMAD3: Mothers against decapentaplegic homolog 3, TGF-β: Transforming growth factor beta, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde, ACH: Acetylcholine, GABA: Gamma aminobutyric acid, 5-HT: 5-Hydroxytryptamine, 5-HIAA: 5-Hydroxyindoleacetic acid, DA: Dopamine, Bace1: Beta-secretase 1, APP: gene encoded for amyloid beta precursor protein, Tau4: Gene encoding for E2 ubiquitin conjugating enzyme, Erα: Gene encoded for estrogen receptor, FSH: Follicle stimulating hormone, LH: Luteinizing hormone.

2.4. Antidiabetic activity

Diabetes is mainly of two types: Type 1 and Type 2. It is well documented that Type 1 diabetes is an insulin-dependent diabetes, whereas Type 2 diabetes is known as diabetes mellitus or insulin-independent diabetes; and is the most prevalent form of diabetes. Chronic diabetes is associated with various comorbidities. The current clinically used antidiabetic agents are not good enough to fully control the diabetes and thus a lot of studies have been carried out to find other alternatives especially from plants. The ethanol (75 %) extract of *P. lobata* roots has shown antidiabetic activity [25] through inhibition of the activity of diabetes related proteins, increased glucose uptake and glucose tolerance. The IC_{50} values of the extract are 0.046 mg/mL against protein tyrosine phosphatase-1B (PTP1B), 0.088 mg/mL against protein tyrosine phosphatase-2 (SHP-2) and 0.28 mg/mL dipeptidyl peptidase-4 (DPP-4). The extract was proven to improve the glucose uptake in insulin resistant HepG2 cells relative to the control and the effect was dose-dependent (0.0014 – 0.0115 mg/L). The extract has shown to improve the glucose tolerance in HFD/STZ-diabetic C57BL/6 mice in the dose range of (0.25–2 g/kg).

The compounds showing α-glucosidase inhibitory activity [26] with IC_{50} reported Daidzein (**25**; 23.01 μM), Puerarin (**40**; 524.08 μM), Daidzin (**29**; 253.78 μM), Ononin (**38**; 422.89 μM), (-)-Tuberosin (**89**; 378.89 μM) and Loboflavate (**101**; 1.79 μM). All the isoflavones were more potent than acarbose, a standard α-glucosidase inhibitor. The reaction kinetic studies revealed that Loboflavate (**101**) was irreversible whereas isoflavone Puerarin (**40**) was a reversible and non-competitive α-glucosidase inhibitor.

Water decoction of *P. tuberosa* tubers [27] has shown to reverse streptozotocin induced diabetic nephropathy in rats. Diabetic nephropathy was induced by injection of streptozotocin (55 mg/Kg, i.p.) and maintaining the diabetic condition for 60 days. The *P. tuberosa* decoction treatment (5 & 10 mg/kg, p.o. for 20 days) reversed the elevated levels of blood glucose, urine protein, serum urea and creatine; and decreased creatinine clearance in diabetic rats. The diabetic rats have shown the increased expression of vascular endothelial growth factor (VEGF) and hypoxia inducible factor (HIF)-1α up on treatment with *P. tuberosa* water decoction. Liquid Chromatography-Mass spectroscopy analysis suggested that *P. tuberosa* tubers water decoction contains Daidzein (**25**), Genistin (**33**), Robinin (**53**), Puerarin (**40**), (-)-Tuberosin (**89**), Hydroxytubersone (**88**), Puetuberosanol (**90**) and Tuberostan (**102**). The elevated expression of HIF-1α and VEGF are reported to be closely associated with aetiology of diabetic nephropathy thus *P. tuberosa* has a potential in the treatment of diabetic nephropathy. Diabetic nephropathic condition causes a progressive increase in urine protein expression, which is mediated through malfunctioning of podocytes (epithelial lining cells of Bowman's capsule). Nephrin is a protein and localized in podocytes. Malfunctioning of podocytes in diabetic nephropathy condition reduces the nephrin expression. The treatment of *P. tuberosa* reversed the decreased levels of nephrin in streptozotocin induced diabetic rats suggesting that it has beneficial effect in rejuvenating the podocytes.

The anti-diabetic potential of the roots of *P.lobata* and its constituents *via* Protein tyrosine phosphatase 1B (PTP1B) and α-glucosidase inhibition were further investigated [28]. The roots were extracted with 70 % ethanol and the extract was subsequently fractionated with organic

solvents: hexane, dichloromethane, ethylacetate and n-butanol. The compounds; Puerasol (**56**), Coumestrol (**57**), Lupenone (**83**), Lupeol (**84**), and (-)-Tuberosin (**89**); from hexane fraction; Calycosin (**46**) from dichloromethane fraction; Daidzein (**25**), Genistin (**32**), Puerarin (**40**) and (+)puerarol B-2-O-glucoside (**91**) from EtOAc fraction; and 3′Methoxypuerarin (**13**), 6-hydroxygenistein-7-O-glucoside (**22**), Daidzein-8-C-apiosyl-glycoside (**28**), Daidzin (**29**), Puerarin **(40)** and Allantoin (**95**) from n-butanol fraction were isolated. The IC_{50} values of ethanol extract, hexane fraction, dichloromethane fraction, ethylacetate fraction and n-butanol fraction in inhibiting PTP1B were 384.21, 12.43, 85.68, 9.97 & 118.20 μg/mL and in inhibiting α-glucosidase were 531.24, 37.35, 1.96, 40.39 & 596.92 μg/mL respectively. The ethylacetate fraction was the most potent PTP1B inhibitor and dichloromethane fraction was the most potent α-glucosidase inhibitor. The IC_{50} values of the isolated compounds; Daidzein (**25**; 145.15 μM), Genistein (**32**; 207.00 μM), Puerarin (**40**; 115.81 μM), (+)-puerarol B-2-O-glucoside (**91**; 115.81 μM), Allantoin (**95**; 258.15 μM), 3'-Hydroxypuerarin (**5**; 1078.04 μM), Daidzein-8-C-apiosyl-glycoside (**28**; 573.38 μM), 3'Methoxypuerarin (**13**; 222.73 μM), Daidzin (**29**; 175.09 μM), Genistin (**33**; 207.68 μM), Lupeol (**84**; 157.30 μM), Lupenone (**83**; 38.89 μM), Puerarol (**56**; 15.11 μM), Coumestrol (**57**; 110.65 μM), (-)-Tuberosin (**89**; 415.52 μM) and Calycosin (**46**; 183.95 μM) against PTP1B. Overall the compound Lupenone (**83**) was found to be the most potent PTP1B inhibitor while genistein is the most potent α-glucosidase inhibitor. [28]

The hypoglycaemic efficacy of the aqueous extract of *P. tuberosa* roots [29] is reported to inhibit DPP-IV in an *in vitro* model and the effect was dose-dependent in the dose range of 10–20 mg/mL. The hypoglycaemic efficacy of the extract was also investigated in normoglycemic rats. The extract at a dose of 50 mg/100 g bw, decreased the plasma DPP-IV activity, decreased plasma glucose concentration after glucose load, and increased plasma glucagon-like peptide 1 (GLP-1) concentration in rats.

Compound Puerarin (**40**; 20, 40 and 80 mg/kg) has shown to significantly reduce glycemia and increased in serum insulin in STZ (150 mg/kg, i.p.)-induced diabetic male BALB/c mice in dose-dependent manner. In histopathological examination, it was observed that Puerarin **(40)** has alle*via*ted STZ-lesioned pancreas tissue. The results suggested that Puerarin **(40)** exhibited a higher antidiabetic activity compared to the reference drug, Metformin (40 mg/kg). Ka Wu *et. al.* reported that Puerarin **(40)** demonstrated antidiabetic effect *via* hypoglycaemic and hypolipidemic activity. [30]

In a different study, Prasain *et. al.* aimed to study the effects of *P. lobata* extract in the amelioration of impaired glucose and lipid metabolism in male type 2 diabetes mellitus (C57BL/6J-ob/ob) obese mice. It was reported that kudzu root extract administration for eight months of up to 0.2 % w/w in diet achieved a reduction of fasting plasma glucose baseline and improvement in glucose and insulin tolerance. [31]

The advanced glycation end products (AGEs) formation inhibition properties of compounds; Daidzin (**29**), Genistin (**33**), Puerarin (**40**), (-)-Medicarpin (**58**), (-)-Glycinol (**59**), (-)-Tuberosin (**89**), Puerol B (**92**) and But-2-enolide (**98**); from methanol extract of the roots of *P. Iobata* were also investigated. It was reported that Puerarin (**40**; IC_{50}: 8.7 μg/mL) and Puerol B (**92**; IC_{50}: 28.6 μg/mL) were found to be the most potent than positive control, aminoguanidine ($IC_{50:}$ 71.1 μg/mL). Thus, the authors suggested that Puerarin (**40**) can be considered as a therapeutic agent for the treatment of diabetes related complications. [32] D.-S. Jang also reported the potent AGEs inhibitors compared to positive control aminoguanidine (35.0 μg/mL); Puerariafuran (**76**; IC_{50} : 0.15 μg/mL), Coumestrol (**57**; IC_{50} : 0.05 μg/mL), Daidzein (**25**; IC_{50} : 12.0 μg/mL), and Genistein (**32**; IC_{50} : 70.1 μg/mL); from *P. lobata* roots. [33]. The inhibitory effect upon aldose reductase (AR), antioxidant content and enzyme activities in opacification of lenses were investigated *in vitro* using lenses of 8-weeks old Sprague-Dawley (SD) rats. Nan Hee Kim *et. al.* reported that Puerariafuran (**76**) inhibited AR activity and xylose-induced lenses opacity in dose dependent manner. Puerariafuran (**76**) also significantly increased the SOD and CAT activities. Thus, with this results from this study, it is believed that Puerariafuran (**76**) has the potential of exhibiting clinical benefit in the prevention and care of diabetic cataracts. [34] The molecular mechanism is concisely shown in Fig. 2.

P. lobata, *P. tuberosa* and their chemical compounds have shown promising *in vitro* and *in vivo* antidiabetic activity. The mode of antidiabetic activity of the extracts and compounds were reasonably elucidated. Because of these promising results, future research can be carried out using genetic models: Biobreeding (BB) rats, Lewis insulin dependent diabetes mellitus (LEW 1AR1/-iddm) rats, nonobese diabetic mice and Akita mice for type 1 diabetes mellitus; Zucker diabetic fat (ZDF) rats and Goto-Kakizaki rats for type 2 diabetes mellitus.

2.5. Neuroprotective activity

Neurodegeneration is the primary cause for many central nervous system (CNS) disorders. Many research organisations have been carrying out the research for the identification new chemicals, including from plant sources, for the treatment of neurodegeneration. Puerarin (**40**) is a major isoflavone isolated from the roots of *P. lobata*. It was shown to exhibit neuroprotective activity [35] through preventing 1-methyl-4-phenylpyridinium (MPP+) induced SH-SY5Y cell death *via* regeneration of ubiquitin-proteasome system (UPS) function and inhibition of neuronal apoptosis [36]. Puerarin (**40**) (0.12 mg/kg/day; i.p. for 10 days) in rats has shown protective effect on dopaminergic neurons of substantia nigra against 6-hydroxydopamine induced neuronal cell death and reduces the tyrosine hydroxylase cell count. Mechanism studies revealed that it prevented the 6-hydroxydopamine induced apoptosis through reducing the BAX expression. In addition, Puerarin (**40**) restored the levels of dopamine and its metabolites, dihydroxyphenylacetic acid and L-dihydroxyphenylalaine, and increased the expression levels of intrastriatal glial cell line-derived neurotrophic factor (GDNF) in the striatum.

Oxidative stress is one of the main causative factors for the aetiology of neurodegenerative disorders such as Alzheimer's Disease (AD). Thus, many researchers have been investigating the role of plant antioxidants in mitigating neurodegenerative disorders. In this study, two major compounds; Coumestrol (**57**) and Puerarol (**56**) from *P. lobata* roots were investigated for their potential in AD treatment. The antioxidant activity of these two compounds were evaluated using DPPH and ONOO − Scavenging Potentials. Both compounds; Coumestrol (**57**) and Puerarol (**56**) respectively showed potent antioxidant activity with IC_{50} values 53.98 and 82.55 μM in DPPH assay and 1.17 and 6.99 μM in peroxynitrite assay [37]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) regulates the metabolism of neurotransmitter, acetylcholine; thus, they are associated with Alzheimer's disease progression. The AChE/BChE inhibitors were reported to possess therapeutic potential in the treatment of AD. In this study, the IC_{50} values of Coumestrol (**57**) and Puerarol (**56**) in AChE inhibition were 42.33 and 144.88 μM respectively while their IC_{50} values in BChE inhibition were 24.64 and > 200 μM, respectively. The presence of β-amyloid peptides in brain is the hallmark of AD and β-site amyloid precursor protein cleaving enzyme 1 (BACE 1) plays a responsible role in the generation of these peptides in the neurons. Therefore, BACE-1 inhibitors have a role in the treatment of AD. In this study, both compounds, Coumestrol (**57**; $IC_{50:}$ 51.04 μM) and Puerarol (**56**; $IC_{50:}$ 28.17 μM) showed potent inhibition. Thus, these two compounds have a potential application in AD treatment and from these results it is concluded that Coumestrol (**57**) is more potent than Puerarol (**56**). In addition, Lupeol (**84**; $IC_{50:}$ 5.12 mM) and Lupenone (**83**; $IC_{50:}$ 62.98 mM)) isolated from *P. lobata* roots exhibited BACE1 inhibitory activity [38].

The *P. lobata* roots were macerated with water [39] and water decoction was eluted with 70 % ethanol over type AB-8 resin to obtain isoflavones enriched extract. the test material, which is used to determine the neuroprotective activity. The amounts of isoflavones; 3'-Hydroxypuerarin (**5**), 3'Methoxypuerarin (**13**), Daidzein (**25**), Daidzein-8-C-apiosyl-glycoside

(**28**) and Puerarin (**40**) were quantified. The pharmacokinetics of the extract was determined by quantifying these Isoflavones in microdialysate from striatal extracellular fluid of the rats treated with 80 and 160 mg/Kg extract intravenously. At a dose of 80 mg/kg extract, the concentrations of Puerarin (**40**), 3'Methoxypuerarin (**13**), Hydroxypuerarin (**5**), and Daidzein-8-C-apiosyl-glycoside (**28**) in extracellular fluid were increased gradually and reached the peak concentration at about 0.95 h indicating that these four Isoflavones can penetrate blood–brain barrier (BBB) quickly. Their half-life in ECF was short ranging from 0.29 h to 0.99 h, with 3'Methoxypuerarin (**13**) being having the longer half-life and Daidzein-8-C-apiosyl-glycoside (**28**) has shorter half-life. The dose of 160 mg/kg showed a similar trend and the levels of these Isoflavones in ECF were found to be dose dependent. The levels of compound Daidzein (**25**) were undetectable in extracellular fluid. The neuroprotective effect of the extracts was evaluated by measuring the levels of neurotransmitters; glutamic acid (GLU), γ-aminobutyricacid (GABA), acetylcholine (Ach), dopamine (DA), 5-hydroxytryptamine (HT) and 5-hydroxyindole acetic acid (HIAA). A dose of 80 mg/kg PLF dose was reported to promote DA metabolism, inhibit 5-HT metabolism, decline the GABA levels at first, followed by incline then further decline and cause no change in glutamic acid (GLU) levels. Another dose of 160 mg/Kg PLF was reported to enhance DA & 5-HT metabolism, lower GLU levels without changing GABA levels. The results confirm that extract has a potent neurochemical modulation effects in neurodegenerative diseases.

Anukulthanakorn *et. al.* [40] reported the neurotherapeutic effects of *P. mirifica* roots extract and Puerarin (**40**) in early- and late-stage cognitive impaired rats. The extract was prepared by extracting the roots with 70 % ethanol and concentrated to dryness. Early- and late-stage cognitive impairment were induced by Ovariectomizing (OVX) rats and kept for 2 and 4 months, respectively. The rats were treated with 100 mg/kg of extract and 7 mg/kg of Puerarin (**40**) for 4 months. It was observed that the treatment with extract abrogated oestrogen deficiency symptoms in OVX rats, however Puerarin (**40**) does not show any effect. The early- and late-stage cognitive impairment models results in upregulated amyloid production associated genes (App and Bace1) and hyperphosphorylated Tau (Tau4). The extract and Puerarin (**40**) have shown to reverse the App, Bace1 and Tau 4 levels in both early- and late-stage cognitive impairment models. The *P. mirifica* roots extract and Puerarin (**40**) decreased the levels of App and Bace1 while Puerarin (**40**) only decreased Tau4 levels. The activity was found to be more in early-stage model suggesting the extract and Puerarin (**40**) elicited neurotherapeutic effects in different pathways.

P. lobata (Willd.) Ohwi was reported to demonstrate amelioration of lipid and bone metabolism *in vivo* model of ovariectomized (OVX) mice. OVX mice treated with *P. lobata* (500 mg/day/kg body weight) for a duration of four weeks showed significantly higher 17β-estradiol concentration while plasma triglycerides were lower. It was reported that there was a reduction in abdominal adipose tissue weight significantly inhibited the decrease of femur bone mineral density. [41]

Another factor that contributes to neuroprotective effect is protection against Aβ toxicity. Y.-H. Choi *et. al.* successfully isolated four known active compounds using bioassay-guided fractionation from the ethyl acetate-soluble extract of *P. lobata*. The authors investigated the protective effects of these isolates using PC12 rat pheochromocytoma cells (ATCC) against Aβ-induced neurotoxicity. They reported that two of the isolated compounds exhibited potent to mildly potent neuroprotective effects were Genistein (**32**; ED_{50} : 33.70 μM), Puerol B (**92**; ED_{50}: 56.0), Biochanin A (**24**; ED_{50}: 27.80 μM), Sissotrin (**42**; ED_{50} : 36.3) [42]. The molecular mechanism is concisely shown in Fig. 2.

There is a sufficient evidence for the potential use of *Pueraria* in neurodegenerative diseases. Thus, their efficacy in other models of neuroprotection such as in vitro and animal models of cerebral ischemia should be investigated to further confirm their potential use as a neuroprotective agent.

2.6. Cardiovascular protective activity

Lee *et. al.* [43] investigated the lipid accumulation inhibitory effect of *P. lobata* root ethanol extract (PLREE) during 3T3-L1 differentiation to adipocytes. The total phenol and flavonoid content inn PLREE were found to be 47 and 29 mg/g respectively. The concentration of Daidzein (**25**), Genistein (**32**) and Puerarin (**40**) in PLREE was 1.77, 0.26 and 148.44 mg/g respectively. At 1 mg/mL PLREE, its electron donating capacity was 48.8 %. Eight days' treatment at doses of 100, 250 and 500 μg/mL the lipid content was reduced, and the effect was discovered to be dose dependent. The extract upregulated mRNA expression of adipogenic genes (SREBP-1c and PPARγ) however, it did not affect the C/EBPβ and C/EBPα at transcriptional levels. PLREE downregulated mRNA expression of lipogenic genes (ACC and leptin) but did not show effect on FAS mRNA expression. PLREE upregulated mRNA expression of ATGL, a lipolytic gene but did not affect HSL mRNA expression. The molecular mechanism is concisely shown in Fig. 2.

The anti-atherosclerotic effect of *Pueraria* compounds was investigated by S.-W. Min *et. al* using two established hyperlipidaemic male ICR mice *in vivo* models induced by Triton WR-1339 and high fat diet, respectively. The authors investigated the effect compounds, Kakkalide (**35**) and Irisolidone (**34**), at a dose of 25 mg/kg from the flowers of *P. thunbergiana* exerted potent hypolipidemic effects when administered orally. It is believed that the mode of action was by inhibiting the rate-limiting enzyme responsible for the biosynthesis of cholesterol from acetate, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. The compounds were found to significantly reduce triglycerides and total cholesterol. [44]

The *Pueraria* species has shown significant activities on cardiovascular system and thus their efficacy should be further investigated in physiologically relevant models such as spontaneously hypertensive rats (SHR), high-fat diet induced atherosclerotic hamsters, apolipoprotein E deficient ($apoE^{-/-}$), low density lipoprotein receptor deficient ($LDLR^{-/-}$), ApoE/LDL receptor double-knockout, ApoE3-Leiden and pro-protein convertase subtilisin/kexin type 9 (PCSK9)-adeno associated virus (AAS) mouse models.

2.7. Nephroprotective activity

Nephrotoxicity issues are usually a result of a side effect from chronic treatments, especially cancer. It was primarily believed that *P. tuberosa* DC. tuber gives nephroprotective effect. Yadav et al. [45] investigated the protective effect of *P. tuberosa* root methanol extract against 35 % glycerol induced acute kidney injury (AKI). The serum urea and creatinine levels along with catalase activity, SOD activity and lipid peroxidation levels in kidney homogenates were measured. The glycerol causes an increase in serum urea, creatinine and lipid peroxidation levels and a decrease in the catalase & superoxide dismutase (SOD) activities in kidney homogenates. *P. tuberosa* treatment reversed the urea level, creatinine level, lipid peroxidation level, catalase activity and SOD activity. Histological examination revealed relatively lower accumulation of hyaline casts in the treatment group. These results suggesting that *P. tuberosa* roots have a potential in the treatment for rhabdomyolysis induced AKI.

Tripathi *et. al.* reported in their study, the protective effect of polar fraction of *P. tuberosa* roots against cisplatin-induced nephrotoxicity in Swiss albino mice. The mice were fed with *P. tuberosa* in the form of biscuits containing 1, 2, and 4 g of it for 10 days. On 7th day the rats were injected with cisplatin at a dose of 8 mg/kg bw intraperitoneally. Cisplatin administration caused an increase in serum urea and creatine levels and *P. tuberosa* biscuits containing 4 g of powder reversed these elevated levels. The authors concluded that *P. tuberosa* is able to prevent cisplatin induced kidney damage and thus suggested it could be used as a supplement. [46]

Because *P. tuberosa* has shown encouraging results in drug induced nephrotoxicity, further studies can be carried out to investigate its

potential in diabetes and hypertension induced nephrotoxicity.

2.8. Anti-inflammatory activity

P. thunbergiana Benth. leaves, stems, sprouts and roots extracts (70 % ethanol) were investigated for their anti-inflammatory activity [5] using LPS induced inflammation in mouse macrophages (RAW 264.7 cells) model. The IC_{50} values of the extracts (leaves, stems, sprouts, and roots) in inhibiting nitric oxide (NO) production were found to be 107, 202, 293 and 354 μg/mL. It is noted that the anti-inflammatory potency of the extracts was not following the order to either TPC or total isoflavone content. The anti-inflammatory activity of the extracts was found to mediated through inhibition of cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) protein expression. Compounds Puerarin (**40**), Isoorientin (**51**), Mangiferin (**73**) isolated from roots of *P. tuberosa* DC showed promising COX-1 and COX-2 and 5-lipoxygenase (5-LOX) inhibitory activities[47]. At 100 μM concentration; Puerarin (**40**) inhibited COX-1 and 5-LOX activity by 1.5 and 10 % respectively, isoorientin inhibited COX-2 activity by 64 % and 5-LOX activity by 14 %, and Mangiferin (**73**) inhibited COX-1, COX-2 and LOX activities by 79.4, 45.9 and 5% respectively.

Inflammation is responsible in the pathogenesis of diabetic nephropathy (DN). Therefore, the efficacy of *P. tuberosa* roots extract was tested for its anti-inflammatory role in DN rat model [48]. The levels of iNOS, IL-6, TNF-α, PKC-α and NF-kB levels were increased in DN rats' kidney tissues. The *P. tubersoa* extract was prepared by boiling with water followed by fractionating with hexane to remove fats. The defatted water decoction was lyophilized. The treatment of DN rats with lyophilized extract (50 and 100 mg/100 g of rat weight) reversed the elevated levels and the anti-inflammation effect was found to be dose dependent.

The anti-inflammatory effects of total isoflavones from *P. lobata* was investigated. In a study performed by Dong et al., isolated total isoflavones from 70 % ethanol was prepared and analytically characterised. The authors used Male Sprague-Dawley (SD) rats undergoing middle cerebral artery occlusion (MCAo) as their *in vivo* stroke animal model. It was reported that the total isoflavones (100 mg/kg; p.o.) treated SD rats demonstrated a decrease in brain infract volume and mitigated the upregulation of ischemia-induced COX-2; decreased glial fibrillary acid protein and CD11b antibody after MCAo. [49]

Puerarin was studied by Sheng Quan *et. al.* on its protective mechanism on systemic inflammatory response syndrome (SIRS) in zymosan-A (500−1000 mg/kg body weight; i.p.) induced SIRS in Sprague Dawley rats. The treatment group of SD rats received a Puerarin (**40**; 62.5 mg/kg body weight) administration through caudal vein immediately after zymosan-A. Their results revealed that SIRS rats treated with puerarin resulted in a 9:3 (survival:death ratio) compared to the untreated group which resulted in a 2:10 (survival:death ratio). The protective mechanism was experimentally determined using quantitative tumour necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-10 ELISA assays. The results consolidated that zymosan-A resulted in elevated pro-inflammatory cytokines but reduces anti-inflammatory IL-10; however, with the treatment of Puerarin (**40**), the levels of TNF-α and IL-6 significantly ($P < 0.01$) reduced whereas IL-10 was elevated. Thus, Puerarin (**40**) is believed to confer protective effects on the experimental SIRS *in vivo* model though reversing the upregulated TNF-α and IL-6 and resulting in up-regulation of IL-10. [50]

The isoflavones and Biochanin A (**24**) isolated from *Pueraria* radix were evaluated for their anti-inflammatory activity using mouse ear oedema as the *in vivo* model. ICR mice ear oedema was induced following the arachidonic acid and croton oil described by Kim *et. al.*, 1993. Mouse administered with Daidzein (**25**) and Puerarin (**40**) at 2 mg/mouse showed the highest potency in anti-inflammation. [51] The molecular mechanism is concisely shown in Fig. 2.

Pueraria species have shown significant anti-inflammatory activity in both *in vitro* and *in vivo* models. However, their mechanism studies are confined only to the regulation of cytokines. Thus, further studies should be carried out to identify the upstream regulators of inflammation and to evaluate their anti-inflammatory efficacy in bacteria and virus challenged rodent models. The clinical trails on Puerarin for its potential use in the treatment of rheumatoid arthritis is still in progress. So, there is an opportunity to further investigate its' activity, efficacy, pharmacokinetics, safety and interactions with clinical drugs.

2.9. Antipyretic, analgesic and muscle relaxant activity

T. Yasuda *et. al.* evaluated *Pueraria* isoflavones and their metabolites in mice for antipyretic, analgesic and muscle relaxant activities. The results showed that Daidzin (**29**) and Genistin (**33**) showed significant antipyretic activity in mice with an elevated body temperature after the administration of LPS (50 mg/kg) *via* subcutaneous injection. The compound Daidzein (**25**) have exhibited analgesic activity in the acetic acid-induced writhing test, while a positive control, equol exhibited muscle relaxant activity in the rotarod and horizontal wire test. These results are indicative of these isoflavones have been playing a major role in the therapeutic activity of *Pueraria*. [52]Very little information is available on antipyretic, analgesic and muscle relaxant activities of *Pueraria*. So, further detailed studies can be carried out to further explore the usefulness of *Pueraria* in the maintenance of fever and pain.

2.10. Mutagenic activity

The mutagenic activity of *P. mirifica* and *P. lobata* were evaluated using Ames tests. The study revealed that both extracts exhibited mild cytotoxic effect of $IC_{50} > 1000$ μg/mL at 20 mg/plate against *Salmonella typhimurium* strains. The extracts were further evaluated for micronucleus assay using male Wistar rats dosed with the plant extracts at 300 mg/kg BW; sacrificed at 24,48 and 72 h after administration. The results revealed that the plants exhibited antimutagenic properties as no significant micronucleus formation in male Wistar rats were observed. The authors concluded that results evidently presents that *P. mirifica* and *P. lobata* extracts are mostly non-mutagenic and anti-mutagenic. [53]

In a different study conducted by K–Y Park *et. al.*, *P. thunbergiana* (Leguminosae) flowers extracts were evaluated for their antimutagenic activity using a modified experiment incorporation reported by Matsushima et al., 1980. The study reported that ethylacetate fraction at 1 mg/plate) significantly reduced the number of revertants of *Salmonella typhymurium* TA100 against aflatoxin B1 (AFB) by 95 %. Out of the isoflavones isolated, it was revealed that the most potent compound was Kakkasaponin III (**63**) reductions by 99 % against AFB; 75 % against *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG)) followed by Tectorigenin (**44**; reduction by 90 % against AFB; 76 % against MNNG). The authors suggested that Kakkasaponin III (**63**) may be able to prevent metabolic activation of AFB. [54]

2.11. Immunomodulatory activity

P. tuberosa tuber extract at 100−200 mg/kg and compound Genistein (**32**) at 25−50 mg/kg administered orally demonstrated dose-dependent immunomodulatory potential in swiss albino rats by increasing the total leukocyte cell count, monocyte and lymphocyte counts, nitroblue-tetrazolium reduction, haemagglutinin titre and phagocytic index, while suppressed the delayed type hypersensitivity response in their specified concentration range in rats immunised with IgG Anti-Sheep Red Blood Cells (SRBC). [55] The molecular mechanism involved in the immunomodulatory activity of *P. tuberosa* extracts should be elucidated in future studies.

2.12. Immunohemolysis activity

S.-R. *et. al.* investigated the anticomplementary properties of several compounds such as Kakkasaponin III (**63**), Soyasaponin I (**70**),

Kakkasaponin I (**61**) and Soyasaponin III (**71**), isolated from *P. lobata*. The immunohemolysis activity was investigated using the modified version of complement titration method reported by Kabat and Mayer for the classical pathway and Platts Mills and Ishizara for the alternative pathway. The authors identified two most potent compounds showing anticomplementary activity *via* the CP method were Kakkasaponin I (**61**; 13 ± 0.7 μM) and Soyasaponin III (**71**; 28 ± 1.7 μM). It was concluded that saponins of *P. lobata* showing anticomplementary activity could be the constituents responsible for the hepatoprotective activity. These saponins have a potential as anti-inflammatory agents. [56]

2.13. Antihelicobacter pylori activity

E.A. Bae *et. al.* investigated the effects of *P. thunbergiana* (Leguminosae) isoflavone isolates in the inhibition of *Helicobacter pylori* (HP) growth *in vitro*. Of the investigated isolates, it was concluded that isoflavone glycosides did not exhibit any inhibitory activity; whereas isoflavones, especially Irisolidone (**34**), exhibited inhibition with an MIC of 12.5 μg/mL against numerous HP bacterial strains: HP ATCC43504 (ATCC); NCTC11637 and NCTC11638 (NCTC); and HP82516, HP82548 and HP4 (clinical isolates). [57] The potential of Irisolidone (34) in the treatment of ulcers caused by *H. pylori* should be further investigated

2.14. Anticancer activity

The four compounds; Daidzein (**25**), Daidzin (**29**), Genistein (**32**) and Puerarin (**40**); were investigated for their inhibitory activity [58] against four human carbonic anhydrase forms (hCA I, II, IX and XII). hCA IX and XII were found to regulate pH in tumour microenvironment thus play a role in characterizing primary and metastatic cancer cell lines [59]. One of the key areas of anticancer drug discovery programs is by the development of selective hCA IX and XII inhibitors. All the four isolates were found to selectively inhibit hCA IX and XII forms over hCA I and II. Compound Puerarin (**40**) was found to be the most potent inhibitor of hCA IX & XII followed by Daidzein (**25**), Daidzin (**29**), Genistein (**32**) respectively. From these results it was concluded that the presence of sugar moiety (as in Puerarin (**40**) and Daidzein (**25**) improves the hCA IX & XII inhibitory activity.

It was reported [60] that methods of extraction (soxhlet, microwave and maceration) has an influence on *in-vitro* cytotoxicity of *P. tuberosa* roots in brine shrimp lethality assay and the microwave assisted extract is the most potent.

Treatment of ER + human mammary adenocarcinoma, MCF-7 cells with *P. lobata* resulted in no proliferation and a mild anti-proliferation effect. However, MCF-7 cells treated with ethanolic extract of *P. mirifica* resulted in proliferation at 1 μg/mL and anti-proliferative effect. The experiments record an ED_{50} value of 642.83 μg/mL. The study showed that *P. mirifica* exhibited estrogenic effect on MCF-7 cell growth and a distinct antagonistic effect with estradiol at high concentration. The authors concluded that *P. mirifica* shown stronger estrogenic activity than *P. lobata* and bear the possibility for phytoestrogen replacement therapy and breast cancer prevention. [61]

K.-T. Lee *et. al.* examined the cytotoxic effects of *P. thunbergiana* BENTH flower isoflavones, Tectoridin (**43**), Tectorigenin (**44**), Glycitein (**47**) and Glycitin (**48**). The experimental model used were *in vitro* MTT assays using HL-60, U-937, HepG2 and SNU-C5 cells. The most potent isoflavones were found to be Tectorigenin (**44**; IC_{50}: 22.3 (HL-60), 28 (U-937), 84 (HepG2), 62.7 (SNUC-5) μM) and Genistein (**32**; IC_{50}: 86.4 (HL-60), 103.5 (U-937), 136.5 (HepG2), 165.8 (SNUC-5) μM). The authors reported that Tectorigenin (**44**) resulted in apoptotic changes of DNA in the cells and autophosphorylation of EGF receptor. Thus, Tectorigenin (**44**) may emerge as a therapeutic agent for leukaemia. [62]

A quite number of compounds isolated from *Pueraria* has shown promising anticancer activity against several cancer cells. However, the molecular mechanisms involved in their anticancer activity are not yet elucidated.

2.15. Estrogenic activity

The methanol extract of *P. lobata* root was reported to significantly increase the estrogen receptor positive (ER +) MCF-7 cells proliferation [63]. The methanol extract was further fractionated using four different solvents: hexane, dichloromethane, ethylacetate and n-butanol (n-BuOH). The highest estrogenic activity was found to be lie in n-butanol fraction. Phytochemical analysis of the n-butanol fraction revealed the presence of ten isoflavones. Of the isoflavones, Genistein (**32**) had shown significant estrogen-like activity and it has synergistic action with 17β-estradiol. The estrogenic activity of roots was found to be mediated through decreased expression of estrogen receptor (ER)α and phospho-ERα in MCF-7 cells. It is interesting to note that genistein also has opposing effect, cytotoxicity against MCF-7 cells with an IC_{50} value of 53.66 μM. The cytotoxicity of Genistein (**32**) was found to be mediated through extrinsic apoptotic signalling pathway (caspase 8) and intrinsic apoptotic signalling pathway (Bcl-2/Bax). Thus, Genistein (**32**), a component of *P. lobata* roots has dual effect on MCF-7 cells; estrogen-like effects mediated through ER pathway activation and cytotoxic effect *via* apoptosis pathway. [63]

P. mirifica roots are being used to promote men and women youthfulness. Kakehashi *et. al.*[64] investigated the effects of *P. mirifica* on mammary and endometrial carcinogenesis in female ovariectomized and normal Donryu rats. *P. mirifica* root powder, produced by Seiko Yakuhin Kogyo K.K., Japan; containing Miroestrol (**81**; 0.00053 %), Deoxymiroestrol (**82**; 0.00063 %), Puerarin (**40**; 0.00217 %), Daidzin (**29**; 0.00129 %), Genistin (**33**; 0.00087 %), Daidzein (**25**; 0.00482 %), Genistein (**32**; 0.00255 %) and Kwakhurin (**49**; 0.00035 %) was used in this study. The test diets were prepared by mixing *P. mirifica* root powder (the percentage – 0.03, 0.3, 1 and 3% with NIH-07PLD diet). Three different groups of rats; ovariectomized, normal and post pubertal, were fed with test diets. The first group was fed with test diets containing 0.03 %, 0.3 %, and 3% *Pueraria mirifica* root for 2 weeks. The second group was fed with test diet containing 3% *P. mirifica* root for 4 weeks after 7, 12-dimethylbenz[a]anthracene (DMBA) initiation. The DMBA causes mammary carcinogenesis. The third group was fed with test diet containing 0.3 % and 1% *P. mirifica* roots at a daily intake 200 mg/kg body weight for 36 weeks after DMBA and *N*-ethyl-N'-nitro-*N*-nitroguanidine (ENNG). In this group, mammary carcinogenesis was initiated using DMBA and endometrial carcinogenesis was initiated using ENNG. In first group, the rats have shown significant uterus weight gain. In second group, the increased mammary gland cell proliferation was observed. In third group, an increase of mammary adenocarcinoma incidence and endometrial atypical hyperplasia multiplicity was observed. In addition, 3rd group rats fed with 1% test diet induced dilatation, haemorrhage, and inflammation of the uterine wall. These finding suggest that feeding rats with test diet containing *P. mirifica* roots has shown estrogenic effects in the mammary gland and uterus.

To investigate the osteogenic mechanism of phytoestrogen-containing *P. mirifica* demonstrated in ovariectomized rats, Tiyasatkulkovit *et. al.* performed a series of analysis to uncover the effects of isoflavones on cellular level using rat osteoblast-like UMR106 cells. In this study, it was reported that the Puerarin (**40**) increased the mRNA expression of alkaline phosphatase (ALP) and osteoprotegerin; but not Runx2, osterix or osteocalcin; decreased an osteoclastogenic factor which is the receptor activator of nuclear factor-κB ligand. These findings suggested that Puerarin (**40**) enhanced bone formation *via* the promotion of osteoblast differentiation, thus it is believed that *P. mirifica* extract and Puerarin (**40**) may have potential benefits in the preventing and treatment of postmenopausal osteoporosis. [65]

The modulatory effects of Miroestrol (**81**) on two bone-specific genes – receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) mRNAs were reported by Udomsuk *et. al.* In their study, ovariectomized female ICR mice were used as an *in vivo* model that exhibits suppressed expression of OPG and RANKL. They reported that Miroestrol (**81**) subcutaneously administered at 0.1 or 1.0 mg/kg/

day for 60 days significantly increased the expression of OPG mRNA while lowering the RANKL mRNA than that of estradiol benzoate (0.1 mg/kg). This combination resulted in an increased OPG/RANKL ratio thus, attenuating the progress of osteoporosis. This study suggested that Miroestrol (**81**) has the potential on bone loss prevention and can be used as an alternative hormone replacement therapy of the current estradiol benzoate [66].

Tanapan Siangcham *et. al.* evaluated the estrogenic activity of *P. mirifica*. The estrogen bioassay of *P. mirifica* aqueous extract (0.1 and 0.2 g/mL) was carried out in immature ovariectomized mice which could acclimate for seven days. Results revealed that *P. mirifica* significantly increased the uterine weight and the height of luminal epithelium and proliferation of glandular epithelium in a dose-dependent manner.

N. Sookvanichsilp *et. al.* investigated the estrogenic activity of *P. mirifica* dichloromethane, ethanolic and water extracts using uterotropic and MCF-7 cell proliferation models. Immature female Wistar rats were used as the uterotropic model where the animals were dosed with plant extracts at 1–10, 10 to 100 and 0.1 to 1 mg/kg/day, for a total of three days. The results showed that dichloromethane extract was the most potent estrogenic activity followed by the ethanolic extract [67]. The molecular mechanism is concisely shown in Fig. 3.

2.16. Antioesteroporosis activity

Osteoporosis, characterized by low bone mass, is a common disorder during older age. The estrogen replacement therapy is being used for the prevention and cure for bone loss. Since synthetic estrogens exhibit various side effects the role of plant phytoestrogens for their anti-osteoporosis effects are being investigated. Suthon *et. al.*[68] reported the antiosteoporosis effect of *P. candollei* var. mirifica (PCM) and Puerarin (**40**) in ovariectomy-induced estrogen-deficient female Sprague-dawley rats. The rats were treated for 12 weeks with oral doses (5, 25 and 50 mg/kg body weight) of PCM powder; and Puerarin (**40**) at 7 mg/Kg body weight (subcutaneous) for 12 weeks. The trabecular bone material density (BMD) at tibia metaphysis and 4th lumbar vertebra was determined to investigate the effect of treatment. The tibia and vertebra BMD values were decreased in a disease model. Up on treatment with test compounds the BMD values were increased, however the significant activity was found only after 8th of week of administration. Further studies should be carried out to explore the molecular mechanisms involved in antiosteoporosis activity of puerarin.

3. Cosmetic properties of *Pueraria*

Tyrosinase plays a key role in mammalian melanogenesis. Tyrosinase inhibitors are being researched for their possible applications in dermatological treatments and as cosmetic ingredients. The *P. lobata* stem was extracted with methanol followed by successive partitioning with hexane, chloroform, ethylacetate and n-butanol. All the extracts except n-hexane extract have shown tyrosinase inhibitory activity [69], n-butanol extract is being the most potent. Four compounds; (+)-Lariciresinol (**80**), Kwakhurin (**49**), Isoliquiritigenin (**93**) and Daidzein (**25**) were reported to inhibit tyrosinase enzyme with IC_{50} mean values of 21.49, 25.24, 4.85 and 17.5 μM respectively.

Kim *et. al.* reported the skin regeneration effects of *P. thunbergiana* Benth (PTBF) flowers in human epidermal keratinocytes (HaCats). The

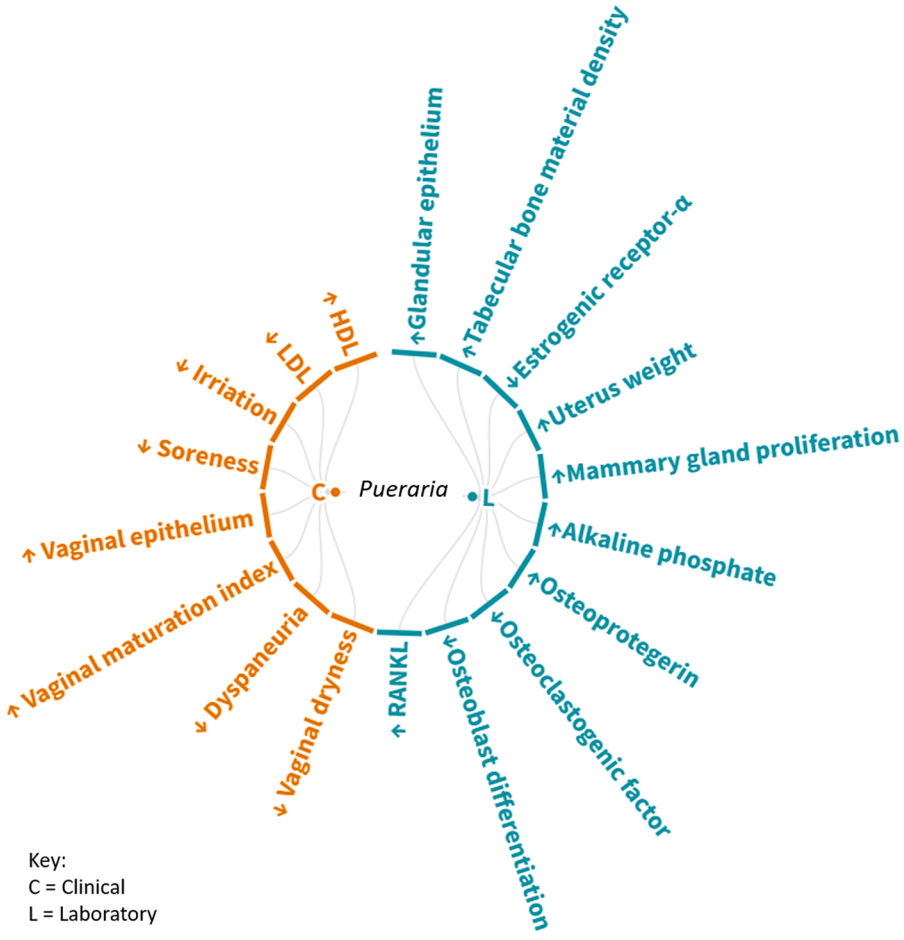


Fig. 3. Estrogenic effects of *Pueraria* in lab and clinic.
Legend: HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, RANKL: Receptor activator of nuclear factor-kappa B ligand.

PTBF contains β-amyrin (**87**), Lupenone (**83**), Kakkalide (**35**), Tectorigenin (**44**), and Glycitin (**48**). The PTBF treatment stimulated the migration and proliferation of HaCats, elevated the phosphorylation of serine/threonine-specific protein kinase and extracellular signal-regulated kinase1/2 in HaCats, induced type I and IV collagen synthesis and increased sprout growth in HaCats. These findings are indicative that PTBF has a potential to be used for skin rejuvenation. [70]

4. Pharmacokinetic studies on *Pueraria*

Xiao *et. al.* investigated [71] the brain penetration and pharmacokinetics profiles in the ventricular cerebrospinal fluid (CSF) and plasma of *P. mirifica* isoflavones. The experiment was carried out in SD rats after intravenous administration of *P. lobata* (Wild.) isoflavonoid enriched extract (PIE) at a dose of 80 mg/kg. The PIF was prepared by eluting the aqueous decoction of *P. lobata* root powder over macroporous resin (type AB-8) with 70 % ethanol. The concentrations of 3'-Hydroxypuerarin (**5**), 3'Methoxypuerarin (**13**), Daidzein (**25**), Daidzein-8-C-apiosyl-glycoside (**28**) and Puerarin (**40**) were measured using an ultra-fast liquid chromatography tandem mass spectrometry. After dosing, the average concentration of Puerarin (**40**), 3'Methoxypuerarin (**13**), Daidzein (**25**), Daidzein-8-C-apiosyl-glycoside (**28**) and 3'-Hydroxypuerarin (**5**) at zero time (C_0) in plasma were 6.53, 13.72, 1.54, 15.84 and 86.07 mg/mL. The maximum concentrations (C_{max}) of Puerarin (**40**), 3'Methoxypuerarin (**13**), Daidzein (**25**) and Daidzein-8-C-apiosyl-glycoside (**28**) were found to be 521.52, 415.00, 74.34 and 380.03 ng/mL in CSF at 0.5 – 0.8 h respectively. The concentration of 3'-Hydroxypuerarin (**5**) in CSF was undetectable. The elimination $t_{1/2}$ of Puerarin (**40**), Daidzein (**25**) and Daidzein-8-C-apiosyl-glycoside (**28**) in CSF and plasma were found to be same, while the $t_{1/2}$ of 3'Methoxypuerarin (**13**)in CSF was longer than that in plasma. The brain penetration index (AUC_{CSF}/AUC_{plasma}) for Puerarin (**40**), 3'Methoxypuerarin (**13**), Daidzein (**25**) and Daidzein-8-C-apiosyl-glycoside (**28**) were 9.29, 7.25, 11.96, and 4.21 % respectively. These results suggest that isoflavones can rapidly penetrate through the blood brain barrier (BBB) to the brain and elicit neuroprotective activities.

5. Safety issues with *Pueraria*

Nagwani Santosh *et. al.* investigated the hepatotoxicity of *P. tuberosa* Linn. tubers methanol extract (100−400 mg/100 g body weight, *p.o.*) in albino rats (male and female, 150–200 g). The study reported a LD_{50} of the extract is 227.5 mg. The following subchronic study on repeated dose (5−100 mg/100 g bw, p.o.) reported that methanol extract showed a significant and dose-dependent increase in sinusoidal congestion, disruption of central vein, inflammatory cell infiltration, hepatic enzymes in blood and hepatocellular necrosis in liver. The study also reported an increase in NO, iNOS and ROS levels. The results suggested that higher dose or long-term use of *P. tubersoa* do cause hepatotoxicity *via* the induction of oxidative stress. [21]

R.S. Gupta *et. al.* reported that *P. tuberosa* D.C. root extract administration (100 mg/day/rat for 60 days; p.o.) in male rats did not show any loss in body weight. However, the weights of testes, epididymides, seminal vesicles and ventral prostates were significantly reduced. The authors also observed that there was a significant reduction of spermatids, spermatocytes, Leydig cells and sperm motility. Thus, it was concluded that the treatment of *Pueraria* in male rats significantly reduced their fertility rate. [22]

S. Malaivijitnond *et. al.* evaluated the effect of *P. mirifica* on reproductive systems of male and female rats that were gonadectomized *in vivo* model. The rats were dosed with of *P. mirifica* suspended in water at 0, 10, 100, and 1,000 mg/kg BW per day, p.o. The authors reported that there was a significant elevation of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels within 1 week after gonadectomy, however, there was an attenuation in the elevation of LH levels in both male and females; and FSH levels in females within 1 week after the 1,000 mg/kg BW treatment. It was observed that the recovery of decreased gonadotropin levels was within one week in males and two weeks in females. These data revealed that *P. mirifica* can affect the reproductive functions in both sexes of rats, however the effects in females is greater than in males. [23]

It was observed that the biochemical and physiological alterations in the ovary and uterus of pregnant rats resulted after being administered with extract of *P. tuberosa* DC. The extract was administered up to five days post-coitally and this resulted in a significant increase in the wet weight, protein, and glycogen contents. Other observation on days 4 and 5 were the sharp [24] deplete in the activity of acid phosphatase and ATPase; and elevation of alkaline phosphatase activity. There were also an increased number of follicles in the ovaries of treated rats. These findings reported by S. Shukla correlates with anti-implantation action of the butanol extract.

6. Clinical studies

There were few clinical studies on individual species; *P.* mirifica, *P. lobata*, *P. thomsonii,* and *P. tuberosa*; and herbal formulations containing *Pueraria* species. The target population are mainly either postmenopausal women or patients with cardiovascular diseases. The details of the clinical studies are presented in Table 2. In general, the *Pueraria* has shown beneficial effects in reducing the postmenopausal symptoms in women and reduced the low-density lipoprotein (LDL) cholesterol. The clinical studies were mainly carried in Thailand, Japan, and Korea with a small number of subjects. In addition, these studies do not provide sufficient evidence of following good clinical research practice guidelines.

7. Conclusions

The compilation of data and published material suggests that *P. lobata* presents itself as a natural product comprising various biological activities, most notable antioxidant, protective effect against alcoholism and promotion of estrogenic activity. Researchers from different groups have carried out experiments to identify the bioactive constituents, prepare the enriched extracts, elucidate the molecular mechanisms, and pharmacokinetics in normal and few disease models. From the collective of results, it is apparent that the isolated compound, Puerarin (**40**) has shown to be possess multifaceted bioactivities with promising potency and received investigational drug status in DrugBank. Few clinical studies were carried out to determine health benefits of *Pueraria* in postmenopausal women. However, the results from al the clinical trials are inconclusive because of small population size. It is alarming to note that few studies reported the toxic effects of *Pueraria* on liver at higher doses and their antifertility activity.

Despite promising benefits of *Pueraria* in both lab and clinical studies, there are many gaps that are yet to be filled-in to further confirm its use as a safe plant medicine. These include the standard use of plant names, standardization of extraction methods, the influence of geolocation, weather and environment on the bioactive molecule content and bioactivity; more in-depth pharmacokinetics studies that will be required for dosage scaling and toxicity studies in human.

CRediT authorship contribution statement

Shengguang Wang: Conceptualization, Writing - original draft. **Shiming Zhang:** Methodology, Software, Resources, Data curation. **Shaoping Wang:** Validation, Formal analysis, Investigation, Visualization. **Peng Gao:** Conceptualization, Validation, Writing - review & editing, Project administration, Funding acquisition. **Long Dai:** Validation, Supervision.

Table 2
The details of clinical studies and observations on *Pueraria*.

Plant	Dose	Target population	Observations	Reference
P. mirifica	20, 30 & 50 mg for 24 weeks	PM women	↓ Mean vaginal dryness ↓ Dyspareunia ↑ Vaginal maturation index ∧ Signs of vaginal atrophy ≈ Atrophic vaginal epithelium ↑ Triglycerides ↓ Bone-specific AP ≡ Endometrial thickness ≡ Endometrial proliferation ≡ Breast tissue ≡ Blood count ≡ Liver function ≡ Renal function	[69,70]
P. mirifica	50 & 100 mg, QD, 24 weeks	PM women with hot flushes and night sweats	↓ MGCS ± Estradiol ≡ FSH	[71,72,73,74]
P. mirifica	Unknown dose for 2 months	PM women	↑ HDL-C ↑ apo A-1 ↓ LDL-C ↓ apo B ↓ LDL/HDL ratio	[75]
P. mirifica	0.5 g of gel applied intravaginally for 2 weeks followed by 3 times per week for another 10 weeks	PM women with at least one vulvovaginal symptom (dryness, soreness, irritation, dyspareunia, discharge)	≈ Vaginal epithelium ↓ Vulvovaginal symptom	[76]
P. thomsonii	200 & 300 mg, QD, 12 weeks	Obese Japanese male and females (BMI ≥ 25 kg/m^2)	↓ BMI ↓ Decreased visceral fat area	[77]
P. thomsonii	No dose mentioned	Alcoholics	↓ Flushing ↓ Palpitation ↓ Headache ↑ Blood acetaldehyde elimination	[78]
P. tuberosa	3 g in two divided doses for 12 weeks`	Patients with stage 1 hypertension	↓ Blood pressure ↓ Plasma fibrinogen ↑ Plasma fibrinolytic activity ↑ Serum antioxidant status	[79]
P. lobata	Extract equivalent to 100 mg isoflavone, QD, 3 months	PM women aged 50–65 years old	↓ LDL-C ≡ FSH ≡ LH	[80]
P. lobata S. miltiorrhiza	3 g daily for 24 weeks followed by 1.5 g daily for 6 more months	CHD patients	≡ Blood pressure, ≡ Haematological profile ≡ Biochemical profile ↓ LDL-C ∧ Brachial FMD ∧ carotid IMT ∧ Vascular function ∧ Vascular structure	[81]
Milk vetch root *Pueraria* root Ligustici *I. puhesceus A. caudatum*	10 g, TID, 4 weeks	CHD patients with LVDD	∧ Diastolic function	[82]
Soybean peptides Taurine *Pueraria* isoflavone Ginseng	2 g, 15 days	Male volunteers	∧ Exercise performance, ↓ Plasma lactate ↑ Non-esterified FA	[83]
S. baicalensis P. lobata	500 & 750 mg, TID, 12 weeks	Patients with acute IS	No results published	[84]

Legend:
↑: Increased.
↓: Decreased.
∧: Improved.
≈: Restored.
≡: No change.
±: Fluctuating.
TID: Thrice a day.
QD: Once a day.
IS: Ischemic stroke.
PM: Postmenopausal.
BIMT: Intima-media thickness.
FA: Fatty acid.
FMD: Flow-mediated dilation.
AP: Alkaline phosphatase.
FSH: Follicle stimulating hormone.

LH: Luteinizing hormone.
apo: Apolipoprotein.
LDL-C: Low density lipoprotein cholesterol.
HDL-C: High density lipoprotein cholesterol.
MGCS: Modified Greene Climacteric ScaleMI: Body mass index.
CHD: Coronary heart disease.
LVDD: Left ventricular diastolic dysfunction.
S. miltiorrhiza: *Salvia miltiorrhiza.*
S. baicalensis: *Scutellaria baicalensis.*
I. pubescens: *Ilex pubescens.*
A. caudatum: *Asarum caudatum.*

Declaration of Competing Interest

The authors report no declarations of interest.

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