Therapeutic potential of genipin in central neurodegenerative diseases

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running title: *genipin in central neurodegenerative diseases*

accepted in CNS drugs

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Abstract

The central neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), have been one of the biggest health problems worldwide. Currently, there is no cure for these diseases. The Gardenia jasmenoides fruit is a common herbal medicine in Traditional Chinese Medicine (TCM), and there are a variety of preparations of Gardenia jasminoides fruits used as treatments for central nervous system (CNS) diseases. Pharmacokinetic studies suggest that genipin is one of the main effective ingredients of Gardenia jasmenoides fruit extract (GFE). Accumulated research data showed that genipin possesses a range of key pharmacological properties such as anti-inflammatory activity, neuroprotective and neurogenic action, antidepressant effects, and antidiabetic action. Based on this, genipin shows therapeutic potential for central neurodegenerative diseases. In the present review, we will review the pharmacological actions of genipin for the treatment of neurodegenerative diseases of the CNS, and additionally, potential mechanisms underlying its effects will also be described.

Keywords: central neurodegenerative diseases; Gardenia jasmenoides fruit; genipin; inflammatory reaction; neuroprotective; neuritogenic; depression; diabetes; insulin resistance

Key points:

- Neurodgenerative diseases pose a serious challenge to world health organisation
- novel findings suggest that active components from traditional Chinese medicine could help
- biomedical studies find convincing evidence for genipin to act as a neuroprotective drug

1. Introduction

As a result of aging populations in the industrialized nations, central neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), are one of the biggest health problems worldwide. At present, there is no cure or disease- modifying treatment for these diseases [1]. As a productive natural resource for drug discovery, Traditional Chinese Medicine (TCM) plays an important role in complementary and alternative medical systems, and has a great advantage for treatment of chronic diseases due to its long history. In oriental countries, TCM has been used to treat CNS diseases for thousands of years in clinical practice. However, the molecular mechanisms involved in TCM still remain unclear, and it can be challenging to systematically identify these using modern pharmacological and biochemical techniques [2]. Gardenia jasmenoides fruit is a classic herbal medicine in TCM which needs to be studied in more detail to uncover its molecular actions.

Gardenia jasminoides Ellis (Its Chinese herbal name is Zhi Zi) is an evergreen shrub that is mostly distributed in the southern regions of China. Gardenia jasmenoides fruit extract (GFE) has been used as an effective oral treatment for inflammation, jaundice and hepatic disorders in TCM [3]. It is one of the commonly used herbal medicines or functional food supplements in China and other oriental countries [4]. In TCM, there are a variety of preparations that contain extracts of Gardenia jasminoides fruits, such as Huang-Lian-Jie-Du-Tang [5], Tong-Luo-Jiu-Nao [6], or Xing-nao-jing [7], which have been proven to have good therapeutic effects on CNS diseases, including dementia, cerebral stroke, and depression [8]. However, the precise pharmacological mode of action of these TCM treatments is often unclear, and therefore it is not readily accepted by western modern medicine.

The pharmacokinetic studies suggested that genipin is the main active ingredient of GFE. Geniposide is a water-soluble iridoid glycoside component found in Gardenia jasmenoides fruit, but geniposide itself is not regarded as a main active ingredients of GFE. It was shown that geniposide is hydrolyzed by β-D-glucosidases into genipin in the intestine [9]. Genipin is liposoluble, and this feature makes it easy for it to permeate into intestinal mucosa and

facilitate absorption. Genipin itself is colorless but it reacts spontaneously with the amino groups of amino acids to form blue pigments which are widely used in the food industry. Genipin is also used as a crosslinking reagent for biological tissue fixation [10]. Importantly, genipin also possesses pharmacological properties such as anti-inflammatory effects [11], antiangiogenic[12], antithrombotic [13], anti-diabetic [14], anti-tumor [15], neurotrophic [16] and anti-depressive effects [17]. See Fig. 1 for an overview. Until recently, geniposide was considered the main active ingredient, but more detailed studies show that it is most likely only a precursor of genipin. Therefore, genipin has been in the focus of research as a versatile therapeutic agent for multiple diseases, especially central neurodegenerative diseases. In the following paragraphs, we will review pharmacological actions of genipin for treatment of central neurodegenerative diseases, and potential underlying biochemical mechanisms of action will also be highlighted.

2. Anti-inflammatory action of genipin

A key element of disease progression in neurodegenerative diseases is the development of a chronic neuroinflammation response in the brain [18-20]. Elevated concentrations of proinflammatory cytokines such as interleukin (IL)-1ß, IL-6, and tumor necrosis factor (TNF) have been found in the brain, cerebral spinal fluid, and blood of AD and PD patients [21]. The role of the acute immune or inflammatory response is to remove damaged tissue or to inactivate potentially damaging agents or invaders. The chronic inflammation response becomes neurotoxic due to the production of free radicals and pro-inflammatory cytokines. Abnormal production of pro-inflammatory cytokines by activated microglia and astrocyte can lead to synapse dysfunction and ultimately synapse loss [22]. Moreover, the chronic neuroinflammation response is considered to play a role in promoting the formation of pathological protein plaques including amyloid plaque [23] and Lewy bodies [24]. Therefore, anti-inflammatory medication could be an effective therapeutic or preventive strategy for neurodegenerative diseases [25, 26].

The Gardenia fruit has been used for the treatment of inflammation in folk medicine for centuries in Asian countries, but the underlying mechanism of its activity needs to be investigated further. In recent studies, topical and systematic anti-inflammatory activities of

genipin have been confirmed in a variety of animal and cell studies, and some of the key pharmacological mechanisms of genipin have been uncovered.

In a variety of topical inflammatory animal models, genipin has relieved acute inflammatory responses. One study showed that genipin inhibited the acute inflammatory response in the carrageenan-induced rat paw edema, carrageenan-induced rat air pouch edema and croton oil-induced mouse ear edema models [11]. Moreover, genipin inhibited the changes in mouse vascular permeability induced by acetic acid [11], and concentration-dependently reduced lipid peroxidation induced by Fe^{2+}/ascorbate in rat brain homogenate [27]. Hence, the authors believe that genipin, rather than geniposide, is the major anti-inflammatory component of the gardenia fruit.

In several inflammatory cell models, genipin also has shown anti- inflammatory activity, and the hypothesis that nitric oxide synthase (NOS) and nuclear factor κB (NF-κB) maybe the potent targets of genipin has been proposed. In RAW 264.7, a murine macrophage cell line, stimulated by lipolysaccharide (LPS) or interferon, genipin may reduce inflammation by inhibiting the expression of inducible nitric oxide synthase (iNOS) and the production of nitric oxide (NO), as well as by inhibition of NF-κB activation [27]. Moreover, genipin has stronger anti-inflammatory activity than geniposide, and geniposide did not show any decreasing effects in the iNOS expression in RAW 264.7 magrophages [11]. In a murine microglial cell line named BV-2, genipin also inhibited LPS-induced increases in NO production and mRNA levels of inducible NOS (iNOS), COX2, IL-1β and IL-6 [8]. Another study demonstrated that genipin can reduce the inflammation response by inhibiting the inflammasome activation. This inhibition is dependent on the suppression of autophagy [28]. Furthermore, genipin also inhibited LPS-induced inflammatory responses in primary rat microglia cells and in the cerebral cortex and hippocampus in mice [29]. Since genipin can react with the amino group of amino acids to form stable blue pigments, the anti-inflammatory effect of these blue pigments also was confirmed in LPS- stimulated RAW 264.7 macrophages, and the anti-inflammatory mechanism of these pigments might be same as those of genipin [30].

Apart from inhibiting NOS, NO and NF-κB expression, other possible mechanisms may be involved in the anti-inflammatory properties of genipin. A study demonstrated that

inhibition of exocytosis is a novel anti-inflammatory mechanism of genipin [31]. Additionally, genipin enhanced the anti-inflammatory response via upregulation of heme oxygenase-1(HO-1) in macrophages [32]. Moreover, genipin suppressed the LPS-induced inflammation response via newly identified mechanisms, including downregulation of chemokines, chemokine receptors, and IFN-induced protein expression [33]. Further systemic inflammation studies also have indicated that genipin attenuates mortality and organ injuries during sepsis through interference with TLR signaling which is crucial for induction of hyperinflammatory responses and tissue injury during sepsis [34]. Genipin also reduced the lethality induced by D-galactosamine/LPS-induced fulminant hepatic failure through prevention of oxidative stress, apoptosis and NF-κB nuclear translocation [35]. A study also showed that genipin exerted its anti-inflammatory effects via activation of the PI3K/Akt signaling pathway [36].

In conclusion, these findings suggested that genipin might be useful as a potential therapeutic agent for the treatment of topical and systematic inflammatory diseases. Genipin would be an ideal starting point for the development of a new non-steroidal anti-inflammatory drug (NSAID) with fewer side effects. In contrast, geniposide did not show any effects in anti-inflammatory activity. Hence, genipin may be a better anti-inflammatory strategy for neurodegenerative diseases.

3. Neurotrophic and neuritogenic action of genipin

Neuronal loss in specific brain regions and synaptic failure are the main causes which result in most of the typical symptoms of neurodegenerative diseases. In AD, the basal forebrain, the hippocampus and its neighboring cortical structures within the temporal lobe lost a large number of neurons, and a reduced acetyl choline (ACH) activation, which is one of the reasons for the decline in cognitive ability of AD patients [37]. In PD, the substantia nigra loses a large number of dopaminergic neurons, and causes reduced dopamine transmission, which is a key element in the decline of motor activity of PD patients [38]. Therefore, the therapeutics that can promote neurotrophic processes and neurogenesis to remedy neuronal loss will be a promising therapeutic strategy. It is known that the physiological roles of neurotrophins on the nervous system span from neuronal development,

to growth, repair, blocking of apoptotic pathways and cell survival. Reduced levels of endogenous neurotrophic factors, such as GDNF, NGF and BDNF has been observed in several neurodegenerative diseases [39]. Therefore, any drug that has neurotrophic activity may play a therapeutic effect in neurodegenerative diseases [40].

Recently, both neuroprotective and neuritogenic (neurite outgrowth) action of genipin have been demonstrated in a series of studies in which the molecular mechanisms of the neuritogenic effect of genipin has been defined further [16]. The studies found that genipin does not enhance the expression of any endogenous neurotrophic compounds such as NGF or other neurotrophins at effective concentrations and that genipin extends neurites without activation of any neurotrophin receptors, including the Tropomyosin receptor kinase A (TrkA). Indeed, genipin induces neurite outgrowth by activating neuronal NO synthase (nNOS), cyclic GMP-dependent protein kinase, and mitogen-activated protein kinase (MAPK) in PC12h cells [41] and Neuro2a cells [42]. This suggests that nNOS plays a crucial role in the observed neurotrophic activities of genipin, as it has also been reported that genipin has structural and electron transferring properties as an activator of nNOS. Genipin also protects neuronal cells against cytotoxicity induced by various agents including amyloid-β (Aβ), 6-hydroxydopamine (6-OHDA), hydrogen peroxide, and endoplasmic reticulum stress inducers *in vitro* [42, 43]. In contrast, the authors found that the precursor geniposide did not show any neuroprotective or neuritogenic action. In addition, other studies showed that genipin can protect cells against damage from ROS and Reactive Nitrogen Species (RNS) production in organotypic hippocampal slice cultures, demonstrating its potential as a free radical scavenger [44]. Genipin also significantly reduced cell death due to rotenone exposure, providing evidence for genipin's ability to distribute within cells to prevent the widespread damage following the internal production of ROS and RNS. These findings suggested that genipin may be a novel potential treatment for a range of neurodegenerative diseases.

Based on these findings, a series of genipin derivatives have been designed and the neurotrophic activity of these derivatives has been confirmed to be superior to genipin. Gardenamide A (GA) is a stable genipin derivative that was shown to have greater neuroprotective effects than genipin in PC12 cells when exposed to serum deprivation and 6-OHDA exposure. GA attenuated the accumulation of intracellular ROS and the loss of

mitochondrial membrane potential. The mechanism was mediated by both the PI3K/Akt and ERK1/2 signaling pathways [45]. Some authors claimed that the direct activation on endothelial NOS may be the reason for the stronger activity of GA when compared to that of genipin [46]. Recently, Koriyama et al. also design a novel long-acting genipin derivative, (1R)-isoropyloxygenipin (IPRG001), which showed significant neuroprotective activity in RGC-5 cells, a retinal precursor cell line, against oxidative stress, such as hydrogen peroxide [47]. Furthermore, IPRG001 promoted staurosporine-induced neurite outgrowth from RGC-5 cells in a dose-dependent manner [48]. Indeed, both the neuroprotective and neuritogenic effects of IPRG001 in RGC-5 cells were all nNOS/NO-dependent. They found that IPRG001 significantly induced RARb expression in adult rat RGCs through S-nitrosylation of HDAC2 processing mechanisms. Concomitant with RARb expression, adult rat RGCs displayed a regenerative capacity for optic axons in vivo after IPRG001 treatment [49]. Therefore, some authors postulated that genipin and its derivative most likely act as neurotrophic factor-like compounds with both neuritogenic and neuroprotective effects.

4. Antidepressant effect of genipin

Depression is a state of low mood and motivation levels that can affect a person's thoughts, behavior, feelings and sense of well-being. Depression is common in the neurodegenerative diseases [50]. It occurs in approximately 45% of all patients with PD [51] and 20-25% of AD patients [52], and is associated with greater impairment of the quality of life and an increased caregiver burden. It is hypothesized that depression is a consequence of the disease process itself, sometimes developing prior to the onset of motor symptoms or cognitive symptoms. Recent studies have suggested that some of the currently available antidepressant medications may be effective and well tolerated in PD population [53], and clinical trial data support that antidepressants have the potential to treat AD. Antidepressants are reported to regulate stem cell fate to regenerate neurons in the adult hippocampus and are effective in reducing toxic amyloid peptides and are known to increase neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) [54].

Our understanding of the pathophysiology of PD and AD associated depression remains limited. So far, the pathogenesis of depression is interpreted by two popular hypotheses. One

hypothesis is the deficiency of monoamines neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) which is a key topic in the field of biological psychiatry and which lead to the development of tricyclic antidepressants (TCAs), selective norepinephrine reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), norepinephrine reuptake dual inhibitors (NARIs) and selective norepinephrine reuptake inhibitors (SNRIs) [55, 56]. Another hypothesis is the deficiency of neurotrophins, which promotes the external application of neurotrophins, such as BDNF, for the treatment depression [57, 58]. However, current antidepressant therapies act slowly, and these drugs fail to work for 30% of patients, and can cause undesirable side effects as observed in clinical practice [59].

In TCM, herb compounds containing G. jasminoides fruits, such as Zhi-zi-chi preparations [60], the Yueju pill and others, has been widely used for the treatment of depression in East Asian countries for hundreds of years [61]. Recently, the mechanism of action of these herb medicines has been gradual uncovered by pharmacological studies. Xue and colleagues have found that acute administration of the ethanol extract of the Yueju pill rapidly attenuated depressive-like symptoms in learned helpless paradigms, and the antidepressant-like effects were sustained for at least 24 hours in tail suspension tests of ICR mice. Additionally, the Yueju pill, like ketamine, an antidepressant that blocks NMDA receptors, rapidly increased the expression of BDNF in the hippocampus of mice [62, 63]. Moreover, other authors also verified that the ethanolic or methanolic extract of G. jasminoides fruits can inhibit MAO-A/B and Dopamine β-Hydroxylase activity in in vitro assays, and the action of the extract was also observed after oral administration in rats [64]. Further research showed that iridoid compounds, geniposide and genipin, are the major bioactive ingredients for antidepressant activity in these herb compounds containing G. jasminoides fruits. The iridoid compounds could pass through the blood brain barrier (BBB) and distribute in the hippocampus, hypothalamus, premotor cortex, striatum, oblongata and cerebellum [60]. In addition, iridoid compound show significant selective MAO-B inhibition and are more potent than the other isolated compounds.

The antidepressant effects of genipin have been evaluated in multiple animal models of depression. Studies showed that intragastric administration of genipin for 7 days in mice significantly reduced the duration of immobility in the forced swimming test and the tail

suspension test, while it did not affect the locomotor activity in the open field test. Genipin could antagonize reserpine-induced ptosis and hypothermia and elevate the contents of norepinephrine (NE) and 5-HT in mice hippocampi significantly [17]. In addition, using ^{1}H-NMR spectroscopy in a chronic unpredictable mild stress (CUMS) rat model, they found that the levels of 5-HT and NE in the hippocampus decreased and the level of 5-hydroxyindole acetic acid (5-HIAA) increased in the CUMS-induced depressive rats. However, pre-treatments with genipin significantly increased the levels of 5-HT, NE and decreased the level of 5-HIAA in the hippocampus [65]. Hence, these results suggest that one possible mechanism of antidepressant-like effects on genipin is due to the modulation of the monoaminergic neurotransmitter system and the potential dysfunctional regulation of the post-receptor signaling pathway, which particularly affected the 5-HT1AR, 5-HT2AR and BDNF levels in the hippocampus [66].

5. Anti-diabetic effects of genipin

The incidence of central neurodegenerative diseases appears to be higher in people with T2DM, suggesting that shared mechanisms, such as insulin dysregulation or insulin resistance (IR), may underlie these conditions [67]. As a consequence, some anti-diabetic strategies, such as insulin [68], metformin [69] and incretin hormones administration [70] are being developed to inhibit pathologic hallmarks of neurodegenerative diseases. The extract of Gardenia jasminoides Ellis fruits has been used over the years in TCM to treat symptoms of T2DM. However, the explicit biological mechanism related to the anti-diabetic effect was not known until recently. New studies gave some indications that uncoupling protein 2 (UCP2) negatively regulates glucose-stimulated insulin secretion and genipin plays an anti-diabetic role by inhibiting UCP2 [14].

UCP2 is a member of the inner mitochondrial membrane anion carrier superfamily. It is known that one mechanism for sensing glucose in pancreatic endocrine ß-cells is UCP2-mediated insulin secretion. By mediating mitochondrial proton leakage and decreasing ATP production, UCP2 negatively regulates glucose-stimulated insulin secretion. It has been proposed that increased UCP2 expression in ß-cells could result in cell dysfunction and the development of T2DM. Moreover, gene knockout of UCP2 restores first phase insulin

secretion, increases serum insulin levels, and greatly decreases levels of hyperglycemia in ob/ob mice. There is also evidence that increased amounts of UCP2 expression in humans can downregulate insulin secretion and increase the risk of type 2 diabetes [71]. In conclusion, these results suggest that UCP2 negatively regulates glucose-stimulated insulin secretion.

One study [14] found that genipin could stimulate insulin secretion of pancreatic ß-cells, and this effect was dependent on the presence of UCP2. In UCP2-deficient islets, genipin did not stimulate insulin secretion. Moreover, genipin increases the mitochondrial membrane potential, ATP levels, and closes K_{ATP} channels by which genipin ultimately stimulates insulin secretion. Genipin also reverses high glucose and obesity induced ß-cell dysfunction [14]. Given that genipin is a naturally occurring cross-linking agent by reacting with the amino group of proteins, it is theoretically possible that the cross-linking activity of genipin could be required for the inhibition of UCP2. However, AG, a genipin derivative that lacks protein crosslinking activity, also inhibits UCP2-mediated proton leakage, closes K_{ATP}-channels, and stimulates insulin secretion in a UCP2-dependent fashion [14]. These results suggest that the cross-linking activity of genipin is not required for its biological activity as a UCP inhibitor.

In addition, other findings suggested that genipin has a therapeutic role for type 2 diabetes patients by improving insulin resistance and augmenting incretin hormone secretion. Some studies showed that genipin ameliorates age-related insulin resistance through inhibiting hepatic oxidative stress and mitochondrial dysfunction [72]. In another study, UCP2-deficient mice had higher plasma levels of the incretin hormone GLP-1 after administration of glucose compared with wild-type littermates, which suggested UCP2 may serve as a negative regulator of GLP-1 secretion in the gastrointestinal tract. Acute inhibition of UCP2 by genipin can improve GLP-1 secretion in ob/ob mice, suggesting that UCP2 negatively regulates GLP-1 secretion in chronic high-glucose states [73]. Moreover, a study showed that genipin is beneficial for treating complications linked to T2DM. A study showed that orally administration of genipin significantly ameliorates urinary albumin excretion, glomerular basement membrane (GBM) thickness and podocyte injury in diabetic mice. Inhibition of UCP2 expression by genipin plays an essential role in halting the progression of diabetic nephropathy [74].

However, the role of genipin on insulin signal transduction seems to differ in different types of cells. In one study, genipin was shown to cause suppression of insulin signal transduction via over-activation of c-Jun N-terminal kinase and the subsequent serine phosphorylation of the insulin receptor substrate-1 (IRS-1), thus impairing insulin-stimulated glucose uptake in 3T3-L1 adipocytes [75]. However, a study In C2C12 myotubes showed genipin can stimulate glucose uptake in a time- and dose-dependent manner. In myotubes, genipin promoted glucose transporter 4 translocation to the cell surface, and increased the phosphorylation of IRS-1, AKT, and GSK3b. Meanwhile, genipin increased ATP levels, closed K_{ATP}-channels, and increased the concentration of calcium in the cytoplasm in C2C12 myotubes. Moreover, the Genipin-stimulated glucose uptake could be blocked by both the PI3-K inhibitor wortmannin and the calcium chelator EGTA [76]. With these contradictory reports, the role of genipin in insulin resistance needs to be further explored.

Additional benefits of UCP2 activation: UCP2 is also expressed in the brain and uncouples ATP production from glucose oxidation in mitochondria to reduce oxidative stress, resulting in enhanced mitochondrial function and increased energy metabolism. UCP2 is important in the prevention of excessive generation of ROS in mitochondria, transfer of mitochondrial substrates, mitochondrial calcium uniport and in the regulation of thermogenesis [77]. Mitochondrial dysfunction is involved in the pathogenesis of neurodegenerative diseases. Increasing evidence indicates that neuronal UCP2 may well play a crucial role in neuronal survival when under stress, and numerous studies link UCP2 to the protection of neurons from mitochondrial dysfunction and oxidative damage in various mouse models of acute stress and neurodegeneration [78], including PD [79] and AD [80].

6. Conclusion and future studies

This review gives an overview of the data published on the pharmacological activity of genipin, its anti-inflammatory, neuroprotective and neuritogenic properties, antidiabetic and antidepressant effects that may be the basis of the pharmacological effects of genipin on neurodegenerative diseases. Thus, genipin shows therapeutic potential for central neurodegenerative diseases. However, compared with numerous results published from in vitro studies, genipin has not been examined in detail for its neuroprotective roles in in vivo

and in clinical studies. In addition, in regard to the inhibition of UCP2 by genipin, there are some uncertainties about the pharmacological application of genipin in neurodegenerative diseases. In conclusion, the degree of neuroprotection in vivo conferred by genipin needs further investigation by testing various animal diseases models. Furthermore, more work is required on identifying target molecules of genipin that are involved in signaling pathways that modulate neurotrophic activity.

Acknowledgement
The authors do not declare a conflict of interest.

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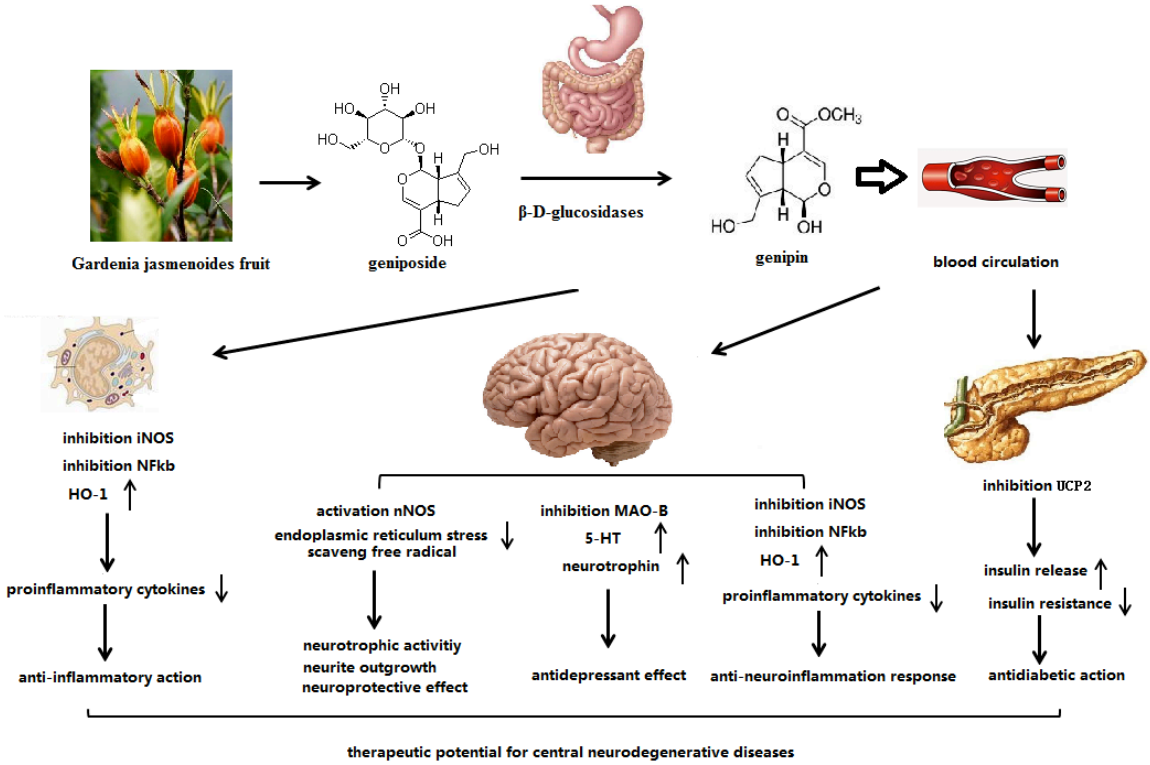

Figure 1：The pharmacological effects of genipin on neurodegenerative diseases

Geniposide is the main effective ingredient in the pharmacological preparations of Gardenia fruit. Geniposide is hydrolyzed into genipin by β-D-glucosidases in the intestine. Then, genipin is absorbed into the blood circulation to exert pharmacological effect in the body. Genipin may inhibit topical and systematic inflammation by inhibiting the expression of inducible NOS and the production of NO, inhibition of NF-κB activation, upregulation of HO-1, as well as the decrease of proinflammatory cytokines in macrophage. Genipin can stimulate insulin secretion of pancreatic ß-cells and improve insulin resistance by inhibition of UCP2, which explains genipin's antidiabetic action. Genipin can cross the blood brain barrier (BBB) and can inhibit the neuroinflammation response by inhibiting activation of glia cells and production of proinflammatory cytokines. Genipin shows antidepressant effects by promoting expression of neurotrophin and inhibiting MAO-B to increase the levels of 5-HT. Genipin also has neurotrophic and neuritogenic effects which involves activating neuronal NOS. In addition, genipin may be a free radical scavenger and can protects neuronal cells against cytotoxicity induced by various neurotoxic agents including amyloid-β (Aβ), 6-OHDA, hydrogen peroxide, and endoplasmic reticulum stress inducers. Based on these versatile pharmacological effects, genipin shows therapeutic potential for treating central neurodegenerative diseases.