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Role of Calycosin (A Derivative of Formononetin) in Parkinson's disease

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Abstract: Flavonoids are polyphenolic phytochemicals produced in fruits, nuts, and vegetables; dietary consumption of these structurally diverse compounds is associated with multiple health benefits. Literature suggests that the neuroprotective mechanisms of flavonoids in protecting the dopaminergic (DA) neurons reduce the symptoms of this movement disorder and thus act as anti-Parkinson's mediators. Flavonoids activate endogenous antioxidant enzymes, suppress lipid peroxidation, and inhibit inflammatory mediators. Calycosin, an isoflavone phytoestrogen, exhibits a protective role in preventing dopaminergic neuronal cell death and locomotor deficits by reducing oxidative stress. To date, limited research has explored the anti-inflammatory properties of calycosin in relation to neurodegenerative diseases. Moreover, calycosin showed an effect on increased oxidative stress by inhibiting SOD, CAT, GSH, and MDA levels. Various research studies revealed that calycosin also modulates MAPK and TLR/NF-κB signaling pathways. Henceforth, drawing upon existing literature elucidating the neuroprotective attributes of calycosin, the current review endeavors to provide a detailed analysis of its mechanistic underpinnings in neuroprotection.

Keywords: introduction; neurodegenerative disorder; calycosin; chemistry; mechanism of action.

1. Introduction

The neuronal loss primarily characterizes neurodegenerative disorders. They are the pivotal pathophysiological change in various brain-related disorders [1]. The progressive degradation of the structure and function of the central or peripheral nerve systems is the hallmark of a diverse set of illnesses known as neurodegenerative disorders. Here are a few instances of neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. A serious risk to human health is neurodegenerative illness. It has increased recently due to an increase in the elderly population [2]. In India, with a mean of 2394 per 100,000 persons, the prevalence rates of the spectrum of neurological illnesses from various parts of the nation ranged from 967 to 4,070, roughly estimating the number of people with neurological disorders to be over 30 million (excluding neuroinfections and traumatic injuries). Regional differences are significant when it comes to the prevalence and incidence rates of common conditions such as epilepsy, stroke, Parkinson's disease (PD), and tremors, as established by population-based surveys [3]. Non-communicable neurological illnesses accounted for 4·0% (95% UI 3·2–5·0)

of all DALYs in India in 1990; by 2019, that percentage had doubled to 8·2% (6·6–10·2), whereas neurological disorders attributable to injury accounted for 0·2% (0·2–0·3) and 0·6% (0·5–0·7) of all DALYs. On the other hand, throughout the same time period, the contribution of communicable neurological illnesses dropped from 4·1% (3·5–4·8) to 1·1% (0·9–1·5) [4]. According to research by de Lau and Breteler from 2006, PD affects an estimated 10 million people worldwide (or roughly 0.3% of the global population) and 1% of people over the age of 60. The precise incidence and prevalence of PD in India have not been well studied in population-based studies. In 2004, a door-to-door study in Bangalore district, South Karnataka, India, determined the crude and age-adjusted prevalence rates of Parkinsonism to be 33 and 76 per 100,000, respectively. The frequency was higher in the rural than in the urban populations. However, it was less than other very common neurological conditions such as mental retardation, headache, epilepsy, and stroke. According to a survey conducted in Kolkata in 2006, 45.82 out of 100,000 people have PD [5].

Astragalus membranaceus is the source of calycosin, an isoflavone phytoestrogen with a variety of medicinal applications, including anti-inflammatory, anti-cancer, and antioxidant properties. [6]. Often extracted from the dry root extract of Radix astragali, the hydroxy isoflavone compound calycosin, also known as 7-hydroxy-3-(3-hydroxy-4-methoxyphenyl) chrome-4-one, as shown in Figure 1 [7]. Furthermore, calycosin has been shown to reduce oxidative stress by inhibiting the activity of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels [8]. Moreover, calycosin suppressed the loss of dopaminergic neurons and apoptosis [9]. Various research also revealed that calycosin also modulates MAPK and TLR/NF-κB signaling pathways [10]. Therefore, by supporting published reports, the neuroprotective capabilities of calycosin in this review aim to give a mechanistic neurological potential as shown in the Table. 1.

1.1. Chemistry of calycosin.

1.1.1. Structure of calycosin.

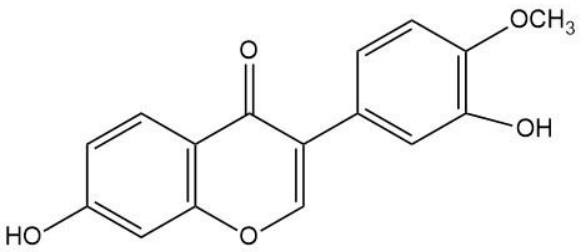

Figure 1. Structure of calycosin.

1.1.2. Synthesis of calycosin.

Generally, liquirtigenin is treated with IF/HID to form diadzein. Furthermore, in the presence of IOHT, it will be converted into formononetin. Calycosin can be synthesized from formononetin with the addition of 13’ H. Based on this synthesis, calycosin is known as a derivative of formononetin, as shown in Figure 2 [11, 12].

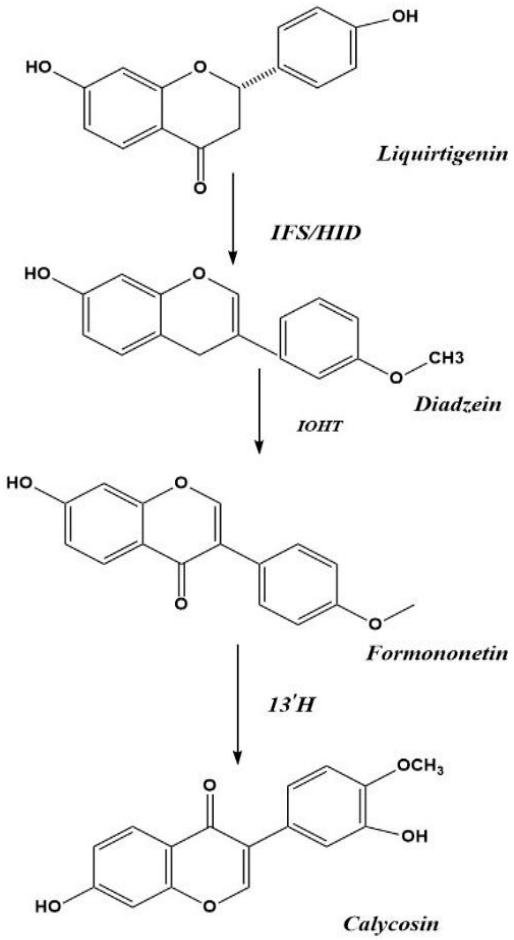

Figure 2. Synthesis of calycosin.

Table 1. Various In-vivo and In-vitro studies of calycosin confer neuroprotection and antiparkinson activity.

Sr. No	Model	Molecule	Dose	Effect	Ref.
1.	Paraquat –induced neurodegeneration drosophila model	Calycosin	0-100 µM	Reduced oxidative stress Increased SOD and Catalase Reduced MDA level. Reduced JNK and Caspase-3 Increased mitochondrial complex I and complex III activity	[8]
2.	α-synnuclein amyloid fibrils induced oxidative stress neural-like cell model	Calycosin	1 -20 µM	Increased the SOD, GSH level Reduced neurotoxicity. Downregulate caspase-3 activity	[9]
3.	MPTP-induced Parkinson's disease mice model	Calycosin	15 and 30 mg/kg	Reduced the TNF- α, IL-1β, IL-6 expression. Reduced the phosphorylation of p38, JNK, and ERK. Reduced the increased TLR2, TLR4. Suppress the activity of MAPK.	[10]

2. Mechanism of Action

2.1. Inhibition of oxidative stress by calycosin.

Pieces of evidence suggest that increased oxidative stress plays a crucial role in PD [13, 14]. The literature review suggests that decreased level of SOD and CAT leads to increased oxidative stress [15, 16]. Several studies showed the effect of calycosin on increased oxidative stress [8, 9]. By using the paraquat-induced neurodegeneration drosophila model, Chaouhan et al. documented that calycosin (0-100 µM) significantly reduced the increased oxidative stress

and DHR's fluorescence intensity in flies. In addition, it also significantly increased the reduced SOD and CAT levels and reduced the MDA level in the brain of flies [8]. By using α-synuclein amyloid fibrils-induced oxidative stress neural-like cell model, Pan et al. documented that calycosin (1, 10, and 20 μM) increased the reduced SOD and glutathione (GSH) levels [9].

2.2. Role of calycosin in suppressing the loss of dopaminergic neurons and apoptosis.

Some evidence suggests that the loss of dopaminergic neurons plays a crucial role in PD [17, 18]. Using the paraquat-induced neurodegeneration drosophila model, Chaouhan et al. documented that calycosin (0-100 μM) significantly increased the reduced locomotor activity in drosophila flies. Moreover, anti-tyrosine hydroxylase antibody immunostaining revealed that it increased the reduced no. of dopamine neurons in the protocerebral posterior lateral and protocerebral posterior median cluster region in flie's brain. Western blot analysis revealed that it also significantly reduced the increased levels of JNK and caspase-3 activation in the brain [8]. Apoptosis is mainly known as cell death, and it has been implicated as the main mechanism of neuronal death in PD [19]. By using α-synuclein amyloid fibrils-induced oxidative stress neural-like cell model, Pan et al. documented that calycosin (1, 10, and 20 μM) reduced the increased α-synuclein amyloid-induced neurotoxicity. Caspase activity assay revealed that it downregulated the caspase-3 activity and also apoptotic proteins [9].

2.3. Role of calycosin in signaling pathways.

By using the MPTP-induced PD mice model, Yang et al. documented that calycosin (15 and 30 mg/kg) increased the reduced motor behavior in MPTP-induced Parkinson's mice. Moreover, it inhibited the activation of microglia. Furthermore, it inhibits the loss of DA neurons in the brain of mice. Moreover, RT-qPCR revealed that it reduced the increased expression levels of TNF-α, interleukin-1 beta (IL-1β), and IL-6. In addition, western blot analysis revealed that calycosin reduced the phosphorylation levels of p38, JNK, and ERK. Western blot analysis also revealed that it reduced the increased TLR2, TLR4, and NF-κB levels in mice and reduced JNK level in the brain. In addition, calycosin suppresses the activation of the MAPK signaling pathway and also suppresses the activation of the TLR/NF-κB signaling pathway in LPS-induced BV2 cells [10]. It significantly reduced the increased level of reactive oxygen species (ROS). Additionally, JC-1 dye staining showed that calycosin significantly restored the MMP value and also recovered the mitochondrial complex I, complex III activity, and ATP levels [9]. There are various sources and methods of extraction of formononetin as shown in Table. 2.

Table 2. Sources and method of extraction of formononetin.

Sr. No	Source	Method of extraction	Part used	Ref.
1.	*Radix Astragali*	Negative pressure cavitation extraction with incubation treatment	Root	[20]
2.	*Pueraria lobatal*	Microwave-assisted extraction	Root and Leaves	[21]
3.	*Tri folium L.*	Accelerated solvent extraction and pressurized hot water extraction	Leaves	[22]
4.	*Astragalus mongholius*	Negative pressure cavitation extraction with incubation treatment	Root	[23]

2.3. Other possible activities of calycosin.

2.3.1. Role of calycosin in different signaling pathways to confer neuroprotection.

In ischemia-reperfusion rats, calycosin decreased MDA levels and ROS at a dose between 7.5 mg/kg/day and 30 mg/kg/day [24]. According to western blot studies, calycosin may also increase ERβ, miR-374, and Bcl-2 protein expression levels in middle cerebral artery occlusion rats [25]. Calycosin demonstrated possible neuroprotective effects by preventing approximately 50% of XA/XO-induced cell death, with an EC50 of 0.05 mg/L and an IC50 of around 50 mg/L. Several studies demonstrated that calycosin also involves down-regulating TNF-α expression and upregulating the expression of autophagy-related protein (p62) and neighbor of BRCA1 gene 1 (NBR1) [26]. Reduced levels of acetylcholinesterase, MDA, IL-1β, TNF-α, Tau protein, hippocampus beta-amyloid, and calycosin were seen, and the inhibitory effects were dose-dependent [27]. Results showed that the maximum blockage concentration was 40 mg/kg/day. Yang discovered that in mice with PD generated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), calycosin also reduced the symptoms of the condition. Based on the findings, Yang and colleagues verified that calycosin administration reduced behavioral abnormalities and inflammatory reactions in MPTP-induced PD animals via NF-κB/MAPK pathways [28].

In middle cerebral artery occlusion (MCAO) rats, calycosin can exert its neuroprotective effect by increasing the expression of TRPC6 and the phosphorylation of cAMP-response element binding protein (P-CREB), as well as inhibiting calpain activation [29]. Additionally, calycosin may function as the large-conductance Ca^{2+} activated K^{+} (BKCa) channel activator in human umbilical vein endothelial cells (HUVECs), facilitating endothelium-dependent vasodilation by promoting endothelial hyperpolarisation and Nitric oxide (NO), generation [30]. By enhancing synaptic function, modulating the PI3K/Akt/GSK (glycogen synthase kinase)-3β pathway, and reducing antioxidative stress, calycosin improved learning and memory in diabetic rats induced with streptozotocin (STZ). In particular, calycosin inhibited AChE activity, increased Akt phosphorylation, decreased tau and GSK-3β phosphorylation, increased the expression of synaptic protein (SYN), post-synaptic density protein-95, or brain-derived neurotrophic factor, decreased MDA levels, and increased SOD and GSHPx activity. Moreover, by blocking the toll-like receptor (TLR)/NF-κB and MAPK pathways, calycosin reduced the signs and symptoms of PD in mice and cell lines, including behavioral abnormalities and inflammatory reactions [30].

2.3.2. Role of calycosin as an anti-inflammatory.

Isoflavones reduced NO, prostaglandin E2 (PGE2), TNF-α, IL-1β, and IL-6 releases, and possess anti-inflammatory activity via the NF-κB and MAPK signaling pathways [31]. The inflammatory cell markers CD68 and F4/80 mRNA levels may also be dose-dependently decreased by calycosin [32]. Both in vitro and in vivo, advanced glycation end products (AGE)-induced inflammation may be relieved by calycosin [33]. In both rats and HUVECs, calycosin was able to reduce the development of vasculitis by downregulating the overexpression of proinflammatory cytokines and receptors for advanced glycation end products (RAGE) brought on by AGEs, as shown in Figure 3 [34].

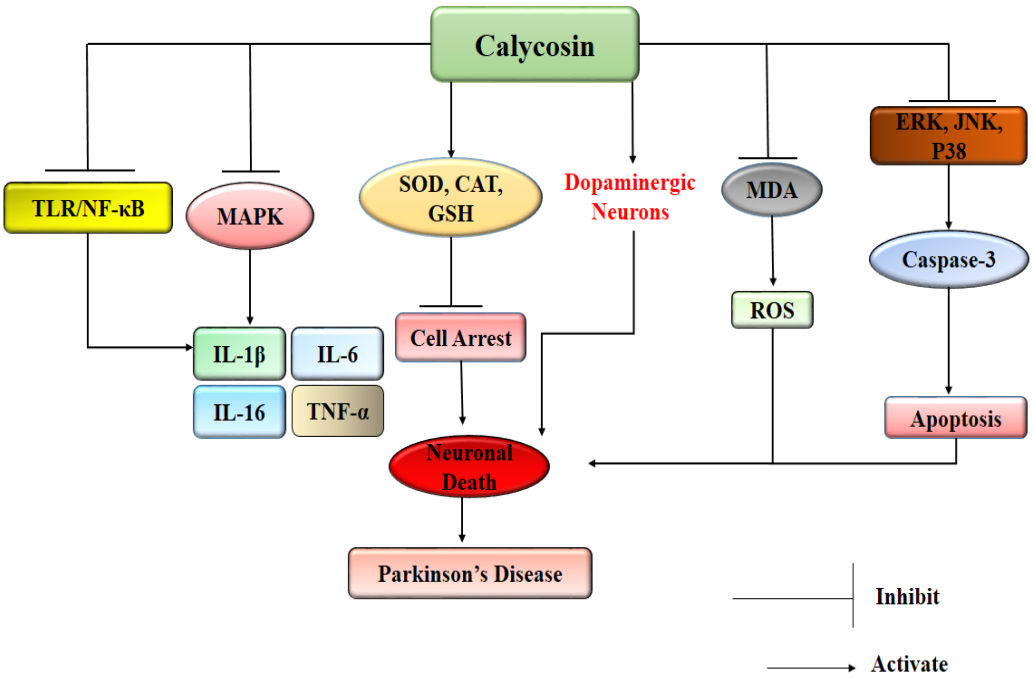

Figure 3. Mechanistic mechanism of calycosin in ameliorating Parkinson's disease.

In LPS-induced macrophage cells, calycosin suppressed the expression of proteins associated with the MAPK signaling pathway and NF-κB, which in turn reduced the production of proinflammatory cytokines, including NO, PGE2, TNF-α, IL-1β, and IL-6. Additionally, it inhibited the mRNA expression of inflammatory mediators like iNOS and COX-2 [35, 36]. By lowering IL-1β and TNF-α levels, p-IκBα expression in the cytoplasm, and blocking NF-κB/p65 expression in the nucleus, calycosin decreased inflammation in paw edema mice [37]. To have an anti-inflammatory impact, calycosin primarily controls the expression of associated inflammatory factors and the signaling pathways of NF-κB, MAPK, and p62/Nrf2.

2.3.3. Antioxidative properties of calycosin.

ROS buildup and overproduction in cells cause oxidative stress, a phenomenon that ultimately results in tissue malfunction [38, 39]. It has been demonstrated that calycosin protects cultured cardiomyocytes from doxorubicin-induced oxidative stress by boosting the activities of antioxidant enzymes such as glutathione peroxidase, catalase, and SOD, which decrease the production of ROS [40]. Moreover, calycosin may reduce the rate of H_2O_2-induced H9C2 cell death in a dose-dependent way [35]. Upon calycosin treatment, the contents of IL-33/ST2 mRNA were increased, whereas the levels of p65, TNF-α, IL-1β, and TGF-β were downregulated [41, 33].

3. Discussion

Calycosin is the most important active flavonoid substance identified predominantly within this medicinal plant. Moreover, calycosin has been reported to have anticancer, antioxidative, immune-modulatory, and estrogenic-like properties. It can be synthesized from formononetin with the addition of 13' H and possesses activity as a derivative of formononetin. Various evidence indicates that calycosin significantly reduced the increased oxidative stress and DHR's fluorescence intensity in flies. Additionally, calycosin showed an effect on

increased oxidative stress by inhibiting SOD, CAT, and MDA levels. Furthermore, calycosin also modulates MAPK and TLR/NF-κB signaling pathways. This review collected recent relevant literature on calycosin and summarized its potential neuroprotective properties in PD and the working mechanism involved, which provided a solid basis for future clinical research.

4. Conclusion

Flavonoids and isoflavonoids are versatile natural compounds and subdivisions of polyphenols that represent a large proportion of secondary metabolites produced by higher plants possessing several antioxidant, anti-inflammatory, and anti-apoptotic activities. It can be synthesized from formononetin with the addition of 13' H and possesses activity as a derivative of formononetin. Calycosin has also been reported to act against several cytokine pathways and ROS production. Furthermore, calycosin and isoflavone showed an effect on increased oxidative stress by inhibiting SOD, CAT, GSH, and MDA levels. Moreover, calycosin also modulates MAPK and TLR/NF-κB signaling pathways. All of the above-discussed activities of calycosin possess activities in the substantia nigra pars compacta part of the brain that leads to antiparkinson properties which further act as neuroprotective potential. This review compiled recent relevant literature on calycosin and summarized its potential neuroprotective properties in PD, as well as the underlying working mechanism, providing a solid basis for future clinical research.

Author Contributions

Conceptualization, A.S. and D.D.; methodology, A.S.; software, A.Y.; validation, A.S. and F.K.; data curation, A.Y. and F.K.; writing—original draft preparation, A.S.; writing—review and editing, A.S. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

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Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

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