

# Role of Formononetin (Isoflavone) in Parkinson's Disease

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**Abstract:** Neurodegenerative diseases are the most complex diseases, which include Parkinson's, Alzheimer's, and other various diseases. In Parkinson's disease, the level of dopamine is decreased, and ACH is increased in the brain. Dopamine is a neurotransmitter that is mainly involved in a human's memory, motivation, and movement. In European countries, the prevalence of Parkinson's has increased by about 10% in the last 25 years. Parkinson's disease generally develops between the ages of 55 and 65, of which about 1%–2% of people are above 60 years old. Generally, when motor symptoms become evident, there are about 30–70% of cells lost in the substantia nigra parts. In our body, the level of ROS is increased, which increases all the pro-inflammatory cytokine levels, which leads to Parkinson's. Levodopa is the most common medication used for first-line therapy, but it is not a permanent treatment. So, researchers have to increase the variety of drugs that are used for the treatment of Parkinson's. Formononetin is an isoflavone that reduces ROS through various mechanisms and pathways that help in neuroprotection and anti-Parkinson activity. This paper describes how formononetin will protect neurons or cells from the increased production of ROS.

**Keywords:** introduction; neurodegenerative disease; formononetin; chemistry; mechanism of action.

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## 1. Introduction

Neurodegenerative disorders are characterized by progressive loss of selectively vulnerable neuron populations, and they were classified broadly by their clinical presentation with extrapyramidal movement disorder and cognitive or behavioral disorder. Out of that, these are the most common effects that occur in neurodegenerative disease [1]. The progressive deterioration of the structure and function of the central or peripheral nerve systems is a characteristic of a diverse set of disorders known as neurodegenerative diseases. Here are some examples of neurodegenerative disorders, including Alzheimer's and Parkinson's disease. The neurodegenerative disease represents a major threat to human health. It has increased in recent years because the elderly population has increased [2]. The collapse of the structural and functional loss of neurons results in the breakdown of core communicative circuitry, cognition, behavior, sensory, or motor functions [3]. Neurons the brain function properly since they play a critical role in communication. Neurons in childhood have a higher number of neurons as compared to adulthood. Synapse dysfunction, neural network dysfunction, and protein deposition in the brain are all linked to neurodegeneration. Those diseases that involve the degeneration of neurons are termed neurodegenerative diseases. Huntington's disease, Parkinson's disease, motor neuron disease, Alzheimer's disease, spinal muscular atrophy, and spinocerebellar ataxia are among the most prevalent neurodegenerative illnesses [4].

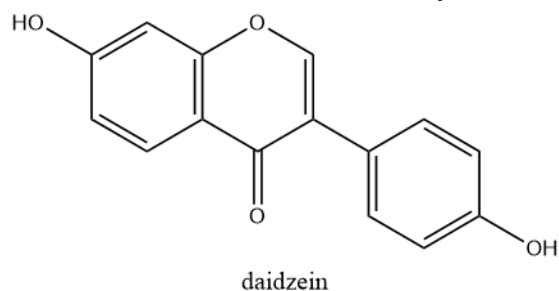
Phytoestrogens are naturally occurring nonsteroidal and phenolic plants, which are further divided into two main groups, which are named flavonoids and non-flavonoids [5].

Further flavonoids are classified, which include isoflavones, coumestans, prenylflavonoids, and non-flavonoids, which also include lignans. In the first classification, which is named isoflavones, they are classified into various molecules or compounds like Daidzein, Genistein, Formononetin, Glycitein, and Biochanin A. Isoflavones are made up of two benzene rings that are linked with each other by a heterocyclic pyran at the 3<sup>rd</sup> position, which helps to distinguish them from flavones [6, 7]. All the family of isoflavones consists of their glucoside forms, and these glucoside forms are heat-sensitive and are also present in our soya. These are also known as bioactive compounds [8].

### 1.1. Chemistry of isoflavones.

#### 1.1.1. Daidzein.

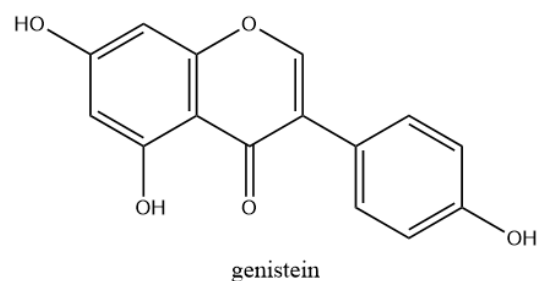
Daidzein [7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one], which is represented in Figure 1 is a naturally occurring phytoestrogen with various pharmacological activities like neuroprotection, antioxidant, and antihaemolytic activities [9].



**Figure 1.** Structure of Daidzein.

#### 1.1.2. Genistein.

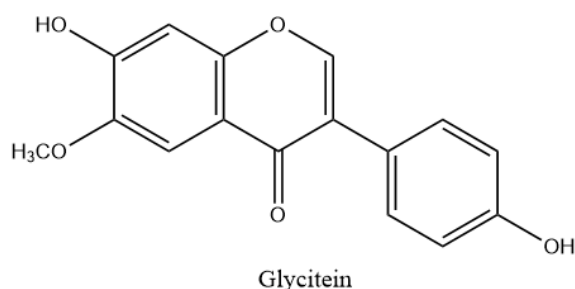
Genistein is a 7-hydroxy isoflavone at position 5, and additional hydroxy groups are present, as shown in Fig. no 2. It is a phytoestrogen isoflavone with antioxidant properties. Since Amyloid protein decreases acetylcholine production in cell lines of basal forebrain cholinergic neurons, it has been demonstrated that Genistein can alleviate cognitive dysfunction in diabetic mice [10].



**Figure 2.** Structure of Genistein.

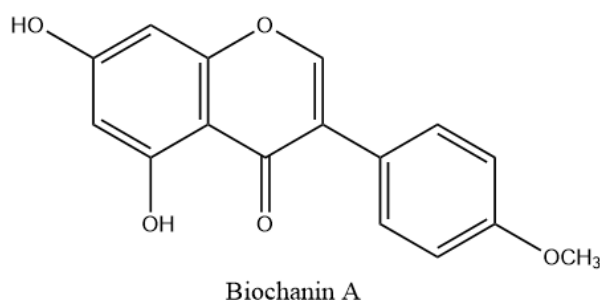
#### 1.1.3. Glycitein.

It is a 4',7-dihydroxy-6-methoxyisoflavone with anti-neoplastic, anti-neurodegeneration disease, and the structure is represented in fig. no 3.



**Figure 3.** Structure of Glycitein.

1.1.4. Biochanin A is a member of 7- hydroxyisoflavone with anti-neoplastic and anti-neurodegeneration activity. The structure of Biochanin A is represented in Fig. no 4.



**Figure 4.** Structure of Biochanin A.

#### 1.1.5. Formononetin.

It is a naturally occurring phytoestrogen and a member of the flavonoid family. It is the main and bioactive component of the legume red clover and also the active component of mainly used Chinese herbal medicines, which are named *Angelica sinensis* and *Astragalus membranaceus*. The chemical formula of formononetin is C<sub>16</sub>H<sub>12</sub>O<sub>4</sub>. The structure of formononetin is shown in Figure 5 [2]. Among the list of flavonoids, formononetin attracted more attention than any other isoflavone because it shows anti-tumor and neuroprotective activity. It has a common legume component, especially in red cloves (*Trifolium pratense* L.) and Astragalus. Formononetin is a compound of leguminous plants, and it is also an important ingredient in food. It belongs to the isoflavonoids group of phytoestrogens. Many studies show that it has an anti-tumor effect. Recently, it was also discovered that it is rapidly absorbed in the intestine and is then distributed to tissue by plasma [11]. Today's formononetin can be synthesized from resorcinol and aldol condensation [12]. It has also been reported previously that overproduction of free radicals and inflammation cause neurological disorders. Reactive oxygen species further cause oxidative stress. In much research, it has been reported that formononetin, which is a phytoestrogen, can suppress oxidative stress by various methods, like reactive oxygen species. It also fights inflammation by the NF-κB signaling pathway by inhibiting the apoptosis of neurons by Bax/Bcl-2 downregulation and upregulation of the PI3K/Akt pathway, promoting AB transport, APP denaturation, and lysis, improving BBB permeability, and activating the Nrf2 pathway [13].

## 2. Mechanism of Action of Formononetin

### 2.1. Inhibition of oxidative stress by formononetin.

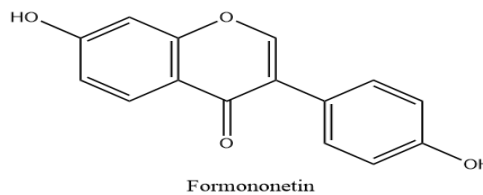
Formononetin obviously has inhibitory effects on reactive oxygen species and free radicals [13]. It has been studied that inducible nitric oxide synthase (iNOS) and <https://nanobioletters.com/>

cyclooxygenase-2 (COX-2) can cause ROS, which further damage the cellular proteins and also the nucleic acid [14]. SOD (superoxide dismutase) can reduce ROS production through the GSH-Px pathway. Formononetin can increase the activity of SOD, which can further reduce ROS and reduce oxidative stress in our nervous system [15].

Malondialdehyde (MDA), if the indexes were decreased, it is suggested that formononetin can also inhibit, which will cause a reduction in ROS, which shows the antioxidant effects. Li *et al.* studied the MDA (malondialdehyde) level in a group of rats that were treated with formononetin. This result showed that formononetin provides antioxidant effects and reduces oxidative stress [16,17]. Formononetin treatment protects the neuronal cells by downregulating COX-2 mRNA levels, which is able to increase further the activity of GSH-Px and SOD, which decrease the MDA content in the brain [18]. If these activities are reduced or inhibited by formononetin, it will also help treat Parkinson's disease.

### 2.2. Formononetin acts as a COX-2 inhibitor and causes inhibition of the NF- $\kappa$ B signaling pathway, leading to a neuroprotection effect.

When any neurological disease exists in our body, it will directly affect the tumor necrosis factor (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-6, prostaglandin E2 (PGE2), nitric oxide free radicals, and various other inflammatory factors. It was discovered by Chen *et al.* that formononetin, which is an isoflavonoid, can inhibit these inflammatory factors, which are discussed above as follows: it can also protect the dopaminergic neurons in an LPS that induces injury to our nervous system [19-21].



**Figure 5.** Structure of formononetin.

### 2.3. Possible activities of formononetin.

#### 2.3.1. Formononetin enhanced the survival of H9c2 cells.

The author demonstrated that after 8 hours of OGD and 16 hours of reoxygenation, cell viability was reduced. Moreover, FRT treatment at a 25 mg/kg dose significantly increased the viability of OGD-induced injuries. The author also demonstrated that H9c2 cells in rats were measured by a standard colorimetric MTT assay [22].

#### 2.3.2. Protein signaling.

The author documented that formononetin at a dose of reverted the decreased level of protein expression of p-Akt. However, formononetin had little effect on p-ERK1/2 protein expression [23]. The author documented that formononetin at a dose reverted the decreased level of IL10 in the serum of TBI cortical neurons and cytokines. The author demonstrated that the brain IL10 mRNA level in TBI rats was notably reduced. In comparison with the TBI control, FN-treated cortical neurons showed increased expression of IL-10 mRNA. Furthermore, the author demonstrated that formononetin at a dose reverted the reduced levels of IL-10 shown by immunoblotting data. The author documented that formononetin at a dose

of reverted the lowered expression of Nrf 2 expression in TBI-induced rats [24]. The author demonstrated that formononetin at doses of 5 and 10um reverted the decreased ER $\beta$  [25].

#### 2.3.3. Activity of formononetin at ROS level.

The author demonstrated that formononetin at doses of 10  $\mu$ M and 50  $\mu$ M reverted the determined viability level of H<sub>2</sub>O<sub>2</sub>-treated cells. Additionally, the author showed that formononetin was incapable of scavenging H<sub>2</sub>O<sub>2</sub>. These findings suggest that formononetin can shield neuronal cells from cell death caused by H<sub>2</sub>O<sub>2</sub>. [26].

#### 2.3.4. Activity of formononetin as an antioxidant.

The author documented that formononetin at doses of 10  $\mu$ M and 50  $\mu$ M increased the levels of the antioxidant proteins HO-1, NQO1, GCLM, and TXNRD1 by treatment with H<sub>2</sub>O<sub>2</sub> additionally, as compared with H<sub>2</sub>O<sub>2</sub> alone. The authors suggest that formononetin increased the antioxidant gene and protein expression in H<sub>2</sub>O<sub>2</sub>-treated neuronal cells. The author observed that the GSH-Px and SOD activities expressed in the lesioned brain zone of the TBI rat were decreased, whereas the levels of MDA, TNF- $\alpha$ , and IL-6 concentrations were elevated. The author examined that formononetin at a dose of 50mg reverted the decreased GSH-Px and SOD activities and the increased levels of MDA, TNF- $\alpha$ , and IL-6 contents, respectively [27].

#### 2.3.5. Gene level antioxidant.

The author demonstrated that formononetin at a dose of 10  $\mu$ M and 50  $\mu$ M increased the transcription level of the Nrf2 gene. According to the author, in H<sub>2</sub>O<sub>2</sub>-treated SH-SY5Y cells, formononetin increased the expression of antioxidant genes via Nrf2 and decreased intracellular ROS levels.

#### 2.3.6. Apoptotic activity of formononetin.

The author demonstrated that treatment with formononetin at doses of 10  $\mu$ M and 50  $\mu$ M reverted the elevated level of Bax/bcl-2 ratio. Additionally, the author reported that at 3 and 6 hours, the activities caspase-3/7 increased by about 4.9 and 14.8 times, respectively, when the cells were treated with H<sub>2</sub>O<sub>2</sub>. Moreover, when cells were treated with formononetin at doses of 10  $\mu$ M and 50  $\mu$ M, it reverted the enhanced level of caspase-3/7 activities by H<sub>2</sub>O<sub>2</sub>. Formononetin decreased the H<sub>2</sub>O<sub>2</sub>-mediated induction of cleaved caspase-3 and caspase-7 levels. The author also demonstrated that formononetin (12.5, 25, and 50  $\mu$ M) reversed the decreased Bcl-2 mRNA and protein level and increased the Bax protein level [28].

#### 2.3.7. Role of formononetin in the signaling pathway.

The author documented that formononetin at doses of 10  $\mu$ M and 50  $\mu$ M suppressed the phosphorylation of ERK, JNK, and p38, which was enhanced by H<sub>2</sub>O<sub>2</sub> treatment. The author suggested that formononetin inhibits the phosphorylation of MAPKs such as ERK, JNK, and p38 pathways in SH-SY5Y cells. The author also documented that formononetin inhibited the caspase-dependent apoptosis of the cell by inhibiting MAPKs in H<sub>2</sub>O<sub>2</sub>-treated SH-SY5Y cells. The author documented that phosphorylation of Akt was inhibited by treatment with formononetin. In addition, the formononetin decreased the intracellular ROS level by inhibiting the PI3K pathway. All of these findings point to the fact that formononetin activated the

PI3K/Akt-Nrf2 signaling in H<sub>2</sub>O<sub>2</sub>-treated SH-SY5Y cells, hence increasing the expression of antioxidant genes [29].

**Table 1.** The method used for extraction of bioactive components in isoflavones and other phenolic compounds.

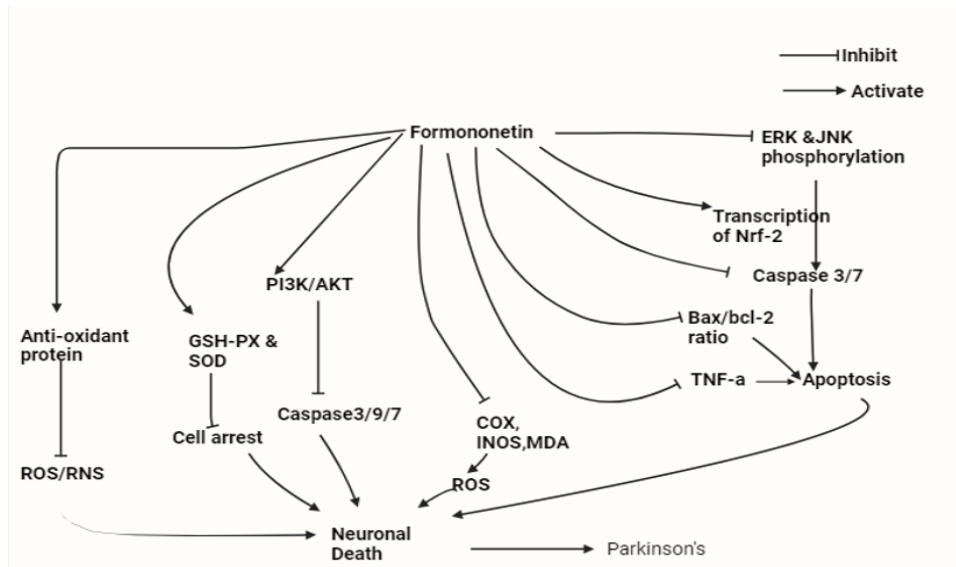
| Methods                              | Solvents                          | Description  | Advantage   | Reference |
|--------------------------------------|-----------------------------------|--|---|-----------|
| Maceration extraction                | Methanol, acetone, ethanol, water | Soybeans were treated with solvent for several days for maceration, and after that, the process of filtration cleaned off the extract from solid suspensions   | 1. Very easy<br>2. Ideal method for components that are thermally labile  | 30        |
| Microwave-assisted extraction (MAE)  | Methanol, ethanol, water          | It makes use of microwaves, which are absorbed by polar molecules like water and help the target molecule to release the solute to solvent and allow the solvent to diffuse into plant samples.                      | 1. Considerable savings in time, solvent amount, and energy consumption;<br>2. An increase in extraction efficiency | 31        |
| Soxhlet extraction                   | Ethanol, water                    | Condensed vapors are returned to the flask once the solvent is heated to the boiling point. It can be run in this manner for an unlimited number of cycles.  | 1. Easy<br>2. Cost is low   | 32        |
| Ultrasound-assisted extraction (UAE) | Ethanol, water                    | Ultrasound is utilized to develop bubbles inside the solvent. The cavitation process produces bubbles that cause wall cells to disrupt the targets, accelerating the solvent's penetration into the target material. | 1. Low power consumption.<br>2. The time of extraction is short   | 33        |
| Supercritical fluid extraction (SFE) | Carbon dioxide co-solvent         | By raising the fluid's temperature and pressure over its critical point, a supercritical condition is reached, which facilitates the extraction process.   | fast, selective, and solvent saving   | 34        |
| Accelerated solvent extraction (ASE) | Methanol, ethanol, acetone        | Solvents are used at high temperatures and pressures, but not to the point of criticality, to speed up extraction.   | better appropriate than UAE for the extraction of phenolic compounds  | 35        |

Formononetin significantly alleviated the neurological deficit and the pathological state of brain tissues and reduced the volume of cerebral infarction [36-37]. Moreover, numerous *in vitro* studies have demonstrated that the safety and efficacy of FMN and its metabolites in biological systems are further confirmed *in vivo* studies [38]. Thus, SATB2 may be a viable predictive biomarker for screening OS and a therapeutic target of formononetin, whereas formononetin may dose-dependently reduce MG63 cell growth and promote apparent cell death, offering a potential treatment for OS. [39]. Moreover, we observed that FMN attenuates A $\beta$ 25-35-induced translocation of NF $\kappa$ B (p65) into the nucleus of HBMECs and found that FMN treatment induces Nrf2 expression and attenuates Nrf2-Keap1 association in a dose-dependent manner in HBMECs [40]. The beneficial effects of formononetin are achieved partially through attenuating neuroinflammation and oxidative stress *via* the related signaling pathway [41]. Molecular docking showed that formononetin is the compound of HQSJZD, which had a high affinity with AChE, and it has a good neuroprotective effect, which can improve the oxidative damage of nerve cells [42]. This study further discovered that the enhanced anti-cerebral ischemia effect resulted from natural borneol increasing the permeability of the blood-brain barrier to elevate formononetin concentration in the brain [43]. These findings suggest that formononetin's neuroprotective impact against H<sub>2</sub>O<sub>2</sub>-induced cell death arises from a reduction in ROS levels brought on by increased antioxidant gene expression via PI3K/Akt-Nrf2 signaling activation. Furthermore, formononetin inhibited apoptosis by preventing the activation of MAPKs in SH-SY5Y cells. [44]. Additionally,

formononetin promotes the expression of Nrf-2, PI3K, ApoJ, LRP1, and antioxidants. At the same time, it lowers pro-inflammatory cytokine and p65-NF- $\kappa$ B expression. Additionally, it suppresses MAO-B activity and A $\beta$  deposition. [45]. In the forced swimming test, FMN dramatically reduced the immobility period and enhanced the sucrose preference in mice receiving CORT. Additionally, FMN protected against CORT-induced neuronal impairment, increased the hippocampus's neurogenesis, decreased serum corticosterone levels, and upregulated the glucocorticoid receptor (GR) and brain-derived neurotrophic factor (BDNF) protein expression levels. [46]. Furthermore, in mice given a high-fat diet, formononetin significantly decreased the levels of the inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$ . The mechanistic investigation showed that formononetin increased anti-inflammatory Nrf-2/HO-1 signaling and inhibited pro-inflammatory NF- $\kappa$ B signaling, which may be connected to the control of PGC-1 $\alpha$  in the hippocampus of mice given a high-fat diet. [47]. Formononetin has potential use in treating oxidative stress injuries in TBI. In this study, the neuroprotective and antioxidant effects of formononetin against TBI, as well as the related probable mechanisms, were investigated [48]. According to this study, formononetin suppresses neuroinflammation by focussing on the NF- $\kappa$ B signaling pathway in BV2 microglia, potentially via ER $\beta$ -related processes. Formononetin appears to have a modest proliferative effect on MCF7 breast cancer cells by modulating ER $\beta$ . [49].

#### 2.3.8. Activity of formononetin on inflammatory mediators.

The author demonstrated that formononetin (2.5, 5, and 10  $\mu$ M) significantly reduced the TNF- $\alpha$  production and IL-6 levels. Similarly, levels of IL-6 and IL-1 $\beta$  secreted into culture supernatants were significantly reduced by 5 and 10  $\mu$ M formononetin. The substance formononetin dose-dependently suppressed nitrite synthesis, according to the author's evaluation of its effect on nitrite production in LPS-stimulated microglia. Subsequent analysis using western blotting revealed that formononetin at 2.5, 5, and 10  $\mu$ M significantly reduced the levels of iNOS protein. These findings imply that formononetin inhibited the expression of the iNOS protein, which in turn reduced NO generation in LPS-activated BV2 microglia. The author also documented that LPS-stimulated BV2 cells produced detectable levels of PGE2. Formononetin (2.5, 5, and 10  $\mu$ M) significantly reduced PGE2 production concentration-dependently. The author documented that formononetin, at a dose of 10  $\mu$ M, reversed the increased production of nitrite, PGE2, and Tnf- $\alpha$  within LPS-induced. These anti-inflammatory effects were significantly ( $p < 0.01$ ) reversed in ER $\beta$  siRNA-transfected cells. The author further reported that, compared to unstimulated cells, control siRNA-transfected BV2 cells exhibited nuclear translocation of the NF- $\kappa$ B p65 subunit during LPS stimulation. When formononetin (10  $\mu$ M) was present, this activity was inhibited. On the other hand, formononetin's capacity to stop LPS-induced nuclear translocation of the NF- $\kappa$ B p65 subunit was eliminated when cells were transfected with ER $\beta$  siRNA. Western blotting demonstrated that ER $\beta$  protein was present in control siRNA-transfected cells and was downregulated in ER $\beta$  knockdown cells, indicating a high level of transfection effectiveness. The author further reported that the administration of formononetin at a concentration of 10  $\mu$ M reversed the higher levels of COX-2 mRNA in the brain in the TBI rats [50].



**Figure 6** Mechanism of Formononetin by several pathways to protect neuronal cells.

**Table 2.** Representation of phytoestrogen resources in our diet.

| Group         | Subgroup    | Dietary resource  | Reference |
|---------------|-------------|---|-----------|
| Polyphenols   |             | Red wine and grape skin   | 45        |
| Flavonoids    | Flavanones  | Citrus fruits   | 46        |
|               | Flavones    | Parsley, celery, and capsicum pepper                                    | 45        |
|               | Flavonols   | Red wine, kale, broccoli, onions, tomatoes, lettuce, apples, and grapes | 44        |
|               | Catechins   | Green tea, chocolate, beans, cherries, apricots, and berries            | 45        |
| Isoflavonoids | Isoflavones | Legumes and soybeans  | 46        |
|               | Isoflavans  | Daidzein Metabolite   | 47        |
|               | Coumestans  | Lucerne, spinach, and clover  | 47        |

Bioactive compounds are compounds, which means that these compounds are biologically active or show biological effects on any disease or disorder. Isoflavone bioactive compounds are extracted using different methods, as provided in Table 1.

### 3. Discussion

In this study, formononetin protects the neuron by activating PI3K/Akt, GSH-Px, and SOD, and transcription of Nrf-2 pathways, and further, this pathway reduced the ROS/RNS, cell arrest, and apoptosis. Formononetin also shows inhibitory action directly through inhibition of COX, INOS, MDA, TNF, Bax/Bcl-2, and ERK & JNK pathways to produce the neuroprotection effect. Various other isoflavones, like daidzein, Biochanin A, etc. were discovered and also had a neuroprotective effect but through a mechanism different from formononetin. Various studies show the action of formononetin in cells and animal models, and our study shows that Isoflavones are present in various dietary resources. This study results in the fact that formononetin has a good potential for neuroprotection. We suggest that future studies be done on its clinical uses and potential.

## 4. Conclusions

Isoflavones act as anti-neurodegenerative by various pathways like anti-inflammatory, antioxidant, and anti-apoptotic activities. However, Formononetin also has anti-neurodegenerative properties by blocking several cytokines pathways and ROS production. Here, it is concluded that Formononetin as an isoflavone possibly acts as an inhibitor of phosphorylation of ERK and JNK signaling pathways, increasing the bax/bcl ratio. All of the above-discussed activities lead to anti-neurodegeneration of neurons in the substantia part of the brain, which means that formononetin can be used as a neuroprotective agent.

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## Conflicts of Interest

The authors declare no conflict of interest.

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