

## VIEWS AND REVIEW

# Pharmacotherapeutic potential of soy isoflavones in neurological disorders

<sup>1</sup>Melika Mokhtari, <sup>2</sup>Fatemeh Qolipour, <sup>3</sup>Amir Hossein Yousefi, <sup>4</sup>Dorsa Alijanzadeh, <sup>4</sup>Qumars kasnavi, <sup>4</sup>Tina Sadeghi, <sup>4</sup>Diba Akbarzadeh, <sup>5</sup>Mohammad Sadegh Fallahi, <sup>4</sup>Niloofar Deravi

<sup>1</sup>Student Research Committee, Dental Faculty, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran; <sup>2</sup>Student Research Committee, School of Medicine, Ardabil Branch, Islamic Azad University, Ardabil, Iran; <sup>3</sup>Student Research Committee, Babol University of Medical Sciences, Babol, Iran; <sup>4</sup>Research committee, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; <sup>5</sup>School of medicine, Tehran University of Medical Sciences, Tehran, Iran

### Abstract

In specific populations, soy products have been a part of their diet for many centuries. Soybeans are known to be beneficial mostly because of being rich sources of isoflavones. Various studies showed that Soy isoflavones such as daidzein and genistein have positive effects on gastrointestinal health, cancer prevention, and health promotion in postmenopausal women. In recent years, many studies focused on the neuroprotective effects of soy isoflavones in animal models and humans. This review includes the latest literature on the effects of soy isoflavones in various neurological disorders. In conclusion, soy isoflavones have neuroprotective, anti-inflammatory, and anti-oxidant effects and can be used to prevent stroke, improve memory and cognitive function, reduction of Parkinson's disease symptoms, and also as a therapeutic agent in multiple sclerosis.

**Keywords:** Neurological disorder, isoflavones, genistin, daidzin, complimentary medicine, natural compound

### INTRODUCTION

In recent years, soybean was introduced as a neuroprotective element in the handling of brain disorders. Soy isoflavones are detected in soybean and soybean supplement. They have been announced that improve memory adroitness. Soy isoflavones include 12 various isoforms that are distributed into four chemical forms. These forms are aglycone (glycitein, daidzein and genistein), glucoside (glycitin, daidzin and genistin), acetylglucoside (acetylglycitin, acetyl daidzin and acetylgenistin), and malonylglucoside (malonylglycitin, malonyl daidzin and malonylgenistin).<sup>1</sup> Obviously, phytoestrogens, which are obtainable doses of isoflavones, can motivate and stimulate the neuroprotective efficacy of estrogens. According to some research, eating high soy foods for a long time can reduce the risk of stroke in rats, and genistein exhibits estrogen-dependent neuroprotective effects as

well as estrogen-independant neuroprotective effects on mouse ischemia models.<sup>2</sup> Furthermore documents indicate neuroprotective activity of genistein on retention and spatial acquirement among animal models.<sup>3</sup>

Former studies have shown that soybean isoflavone has a neuroprotective effect and an anti-inflammatory and anti-oxidant function on cells.<sup>4</sup> It is affirmed that equol, an active metabolite of daidzein, exhibits antioxidant properties and possess anti-inflammatory effects on microglial cells and assists in controlling neurodegenerative disorders. In neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), oxidative stress as pathogenesis can cause neuronal cell death.<sup>5</sup> And genistein showed promising results in reversing oxidative stress.<sup>1,3</sup> Thus, this narrative review intended to digest the neuroprotective efficacy of soy isoflavones on AD and cognitive impairments, stroke, PD,

Address correspondence to: Niloofar Deravi, SBUMS, Arabi Ave, Daneshjoo Blvd, Velenjak, Tehran, 19839-63113, Iran. Tel: +989193195717, E-mail: niloofarderavi@sbm.ac.ir

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migraine, multiple sclerosis (MS), seizure, and Huntington's disease (HD).

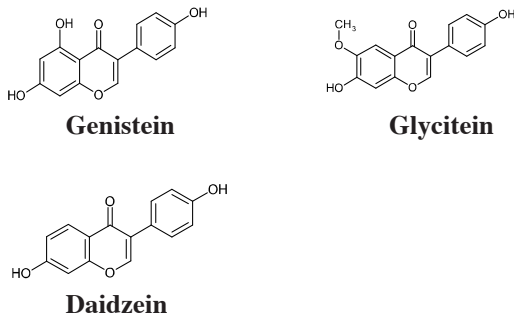


Figure 1. Chemical structures of major soy isoflavones

## METHODS

This study will review and summarize information about the Neuroprotective effects of soy isoflavones on AD and cognitive impairments, stroke, PD, migraine, multiple sclerosis, seizure, and Huntington's disease. In this study, the keywords searched included soy isoflavones, Alzheimer's disease, cognitive impairments, stroke, Parkinson's disease, migraine, multiple sclerosis, seizure, and Huntington's disease. We searched English published and reported articles in journals up to 2020 using various databases, including ISI Web of science, SID, Google Scholar, PubMed, Scopus, and Science Direct. Related articles were reviewed.

## RESULTS

### Protective mechanisms

#### *In vitro studies*

A study performed by Varinska *et al.* suggests that genistein has pleiotropic molecular mechanisms such as inhibition of tyrosine kinases,  $5\alpha$ -reductase, DNA topoisomerase II, G2/M arrest induced by galectin, cyclin-dependent kinases, protein histidine kinase and regulation of several signaling pathways like NF- $\kappa$ B, Akt and MAPK that are connected with the growth of cancerous cells.<sup>6</sup>

In a study by Ma *et al.*, primary neurons of newborn rats were exposed to oxygen-glucose deprivation (OGD) as a hypoxic-ischemia model. OGD exposure resulted in reduced cell viability, increased apoptosis, an increase in  $K_v$  currents, and a decrease in  $Na_v$  current. Also, OGD exposure decreased GluR2 expression and increased NR2 expression, resulting in changes in the glutamate signal pathway. The study indicated that genistein

reversed the mentioned effects of OGD to some extent.<sup>7</sup>

Results of a study by Wu *et al.* show that genistein has neuroprotective effects in SH-SY5Y cells intoxicated with rotenone. Rotenone treatment resulted in oxidative stress, increased MDA levels, and decreased HMOX1 activity in SH-SY5Y cells overexpressing A53T mutant  $\alpha$ -synuclein. HMOX1 acts as a neuroprotective agent against oxidative stress and is controlled by the NFE2L2 transcription factor. Genistein showed neuroprotective activity by reversing the oxidative stress and apoptosis caused by rotenone, increasing BCL-2 and Beclin 1 levels, and increasing NFE2L2 and HMOX1 expression in SH-SY5Y cells.<sup>8</sup>

#### *Animal studies*

Soy isoflavones induce anti-inflammatory, anti-apoptotic, neuro-trophic and anti-oxidant effects.<sup>9</sup> According to a study in the Institute of Cancer investigating the neuroprotective effects of soy isoflavone on memory impairments caused by scopolamine in mice, Cholinergic system function was enhanced and levels of oxidative stress in the hippocampus were suppressed by SI. Moreover, extracellular signal-regulated kinase phosphorylation, the expression of brain-derived neurotrophic factor and the protein of cAMP response element-binding phosphorylation levels of extracellular signal-regulated kinase (ERK), cAMP response element-binding protein (BDNF) and brain-derived neurotrophic factor (BDNF) expression levels in the hippocampus were upregulated by SI treatment. As a result, soy isoflavones exerted remarkable neuroprotective effect.<sup>10</sup> Zhao *et al.* discusses that pro-inflammatory cytokines production was suppressed and gliosis was alleviated in the spinal cord of mice by genistein.<sup>11</sup> Another study exploring the SI underlying mechanisms effecting chronic cognitive deficit induced by ethanol in mice, shows that SI regulated the activity of acetylcholinesterase (AChE) and the level of acetylcholine (Ach), elevated the expressions of synaptic plasticity-related protein inhibited the expressions of neuron apoptosis-related protein in hippocampus and the cortex of mice.<sup>9</sup>

In rats, genistein and daidzein have an estradiol-like mechanism that encompassed anti-inflammatory efficacy against LPS-activated microglial cell line.<sup>12</sup> Anti-inflammatory effects were interceded by inhibition of iNOS interpretation through inhibition of IRF-1,

phosphorylation of STAT1, and a decline of MCP-1 and IL-6 presentation.<sup>13</sup> Some studies presented the mechanism and potency of estrogen to reduce brain harm and recover neuronal retention in experiential models of cerebral ischemia, MS, PD, and AD.<sup>13</sup> Estrogen's ability to prevent the generation of pro-inflammatory molecules and decrease microglial activation can cause neuroprotective effects.<sup>13</sup> Although estrogen administration may have a side effect, it is necessary to find safe ways; to this intention, a low dose of genistein and daidzein can decrease complexity, retaining the neuroprotective attributes.<sup>13</sup> The isoflavones are described as phytoestrogens because they have a similar structure to estrogens, including the B ring bonded to position 3 of the C ring.<sup>14,15</sup>

#### *In vitro studies*

Based on a study done by Chacko and colleagues, genistein is involved in monocyte rolling inhibition. Also, genistein has an antiadhesive effect on cytokine-activated endothelial cells that is determined by flow and mediated by activation of PPAR- $\gamma$ .<sup>16</sup> An article studying three kinds of isoflavones and their metabolites that are metamorphosed by the intestinal microflora in humans, demonstrates that the release of NO induced by LPS, (TNF)- $\alpha$  in primal cultured microglia and also BV2 microglial cell lines were suppressed.<sup>17</sup> Another study shows that isoflavones are involved in inhibition of LPS-induced inflammation, decrease in quantity of leukocytes and reduction in production of IL-1 $\beta$ , PGE2, IL-6 and NO in peritoneal exudate fluid and cell supernatant in mice.<sup>18</sup> A study performed by Yu *et al.* provides evidence that isoflavones exert anti-inflammatory effects in animals and human as a result of increasing antioxidative activities, regulating NF- $\kappa$ B, reducing cytokine levels and pro-inflammatory enzymes activities.<sup>19</sup> An article conducted by Mirahmadi *et al.* suggests that genistein reduces cognitive dysfunctions induced by LPS and decreases neuroinflammation caused by oxidative stress and alleviates AChE activity. In addition, the modulation of NF- $\kappa$ B, TNF $\alpha$ , GFAP, IL-6, Inos, Nrf2, TLR4, and COX2 is reduced by genistein.<sup>20</sup>

### **Therapeutic effects on human neurological disease**

#### **Alzheimer's disease and dementia**

AD is a neurodegenerative condition distinguished

by amyloid- $\beta$  (A $\beta$ ) peptide extracellular deposition and Tau protein hyperphosphorylation, which eventually contributes to the development of intracellular neurofibrillary tangles and cell death. Growing evidence suggests that there are neuroprotective effects of genistein, a soy isoflavone, against A $\beta$ -induced toxicity.<sup>21</sup> The main isoflavones found in soybeans are genistein, daidzein, and glycitin.<sup>22</sup> At concentrations above 1  $\mu$ M, genistein stimulates the peroxisome proliferator-activated gamma receptor (PPAR $\gamma$ ), and PPARs are described as neuroprotective targets for neurodegenerative diseases. Genistein eliminates A $\beta$ -induced inflammation in cultivated astrocytes.<sup>23-27</sup> Genistein stops the accumulation of A $\beta$  and behavioral deficits through interactions with PPAR $\gamma$  in a mouse with AD.<sup>28</sup> Several cognitive features (recognition memory, hippocampal learning, odor discrimination, and implicit memory), as well as a decrease in the number and region of A $\beta$  plaques, have been enhanced by genistein therapy.<sup>29</sup>

#### *In vitro studies*

In 2018 Youn *et al.* studied on b-site amyloid precursor protein cleaving enzyme 1 (BACE1), which causes amyloid b (Ab) generation, inhibition by genistein. Genistein interacted with major amino acid residues in BACE1 through hydrogen bonding. This study suggested that genistein may help inhibit and/or treat AD.<sup>30</sup>

Valles *et al.* reported in pre-treated with genistein, A $\beta$  produces pro-inflammatory chemicals, which estradiol or genistein protects astrocytes against, and In astrocytes treated with A $\beta$ , increased PPAR and iNOS expression was observed.<sup>26</sup>

Genistein can impressively castrate the detriment of nucleic acids by activating estrogen receptor (ER), an estrogen hormone (17 $\beta$ -estradiol) reactionary membrane-bound protein, which are often G-protein coupled receptors.<sup>31-33</sup> Furthermore, genistein can arrange the pro-survival pathways by actuating the Nrf-2 and HO-1.<sup>31</sup> At physiological concentration, genistein can prevent the hyperphosphorylation of tau protein via compilation of the calcium invasion.<sup>34</sup>

Genistein is a significant phytoestrogen in soy. It has been broadly administrated as an anti-inflammation and cerebrovascular drug because of its anti-oxidation and anti-acetylcholinesterase effects.<sup>35</sup> Herein, Ren *et al.* assay the suppressive efficacy of genistein on the association of A $\beta$

(aggregated with AD) and hIAPP (aggregated with type II diabetes) and A $\beta$ - and hIAPP-induced neurotoxicity using a composition of experiential and calculative approaches.<sup>35</sup> According to communal experimental from ThT, AFM, and CD, genistein indicates strong prevention ability to inhibit the conformational transition of both A $\beta$  and hIAPP monomers to  $\beta$ -sheet structures decreasing final amyloid fibrillization from A $\beta$  and hIAPP monomer association by 40-63%.<sup>35</sup>

Hong *et al.* studied on anti-Alzheimer effects of genistein derivatives. These derivatives could inhibit acetylcholinesterase (AChE). The compound 5-hydroxy-7-(2-(pyrrolidine-1-yl) ethoxy)-3-(4-(2-(pyrrolidine-1-yl)ethoxy) phenyl)-4H-chromen-4-one (5a) show strongest AChE inhibiting. 5a targeted the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. The compounds did not change the cell's viability at the 100  $\mu$ M concentration, so these can use as a treatment of AD.<sup>36</sup>

In 2019 Morelli *et al.* implemented an advanced engineered system, like membrane bioreactor, to better understand neurodegeneration pathways. This neuronal membrane bioreactor used to test neuroprotective effects of isoflavone. Glycitein protected neurons against the activation of apoptotic markers, production of ROS, and other incidents caused by  $\beta$ -amyloid aggregation.<sup>37</sup>

In 2019, Petry *et al.* induced a neural damage model treated with various concentrations of amyloid- $\beta$  to assess the genistein against A $\beta$ -induced cell death and also assess the probable participation of protein kinase B (PKB, also termed Akt), glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ), and Tau in this neuroprotective mechanism. Genistein partially prevents A $\beta$  induced cell death through the prevention of A $\beta$ -induced PKB inactivation and Tau hyperphosphorylation.<sup>21</sup>

#### Animal studies

Soy isoflavones have an estrogen-like mechanism, so soy products prevent cognitive deficiency and disorders.<sup>12</sup> In a perusal with cortical neurons, isolated from the cerebral cortex region of the 15-day old murine fetuses, 0.5  $\mu$ M genistein showed suppressive action against A $\beta$  induced cytotoxicity through the modulation of the p38 MAPK pathway.<sup>38</sup> Furthermore, various pre-clinical discussions on genistein show its undertaking therapeutic activity against AD's pathogenesis.<sup>39</sup> A study has defined that in A $\beta$  intoxicated rats, genistein at 10 mg/kg dosage can barricade the formation of A $\beta$  plaques in the brain and amend

the acquirement and memory deficits via the compilation of the estrogenic signaling cascade.<sup>39</sup>

#### Human studies

In general, cognitive function is classified into four classes: learning and memory, reception, expression, and thinking. While all these functions are essential and interconnected in cognitive processes, the role of learning and memory is crucial, and many studies have therefore concentrated on learning and memory.<sup>40</sup> Isoflavones are diphenolic compounds that are structurally similar to estrogens. Since biological effects in the brain and other tissues may be estrogenic or antiestrogenic, they are often categorized as selective estrogen receptor modulators.<sup>41</sup> Soy isoflavones in postmenopausal women tend to enhance cognitive function.<sup>40</sup> Isoflavone can imitate the estrogen's efficacy in postmenopausal women, whose strogen stages are diminishing, more than the coeval men in whom estrogen is generated from androgens.<sup>42</sup> A case-control study suggested that in men, supplementation with soy isoflavone improved cognitive function.<sup>43</sup> According to former reports, there are several estrogen receptors in the central nervous system. These receptors have a significant role in cognition and memory operation.<sup>44</sup> Significant enhancement in mental flexibility and short-term and long-term memory were observed in young healthy adults who received high soy dosages. These results were observed in both males and females. While there are few clinical data to investigate soy isoflavones' effects on cognitive function, clinical trials suggest that soy isoflavones might enhance cognitive function in young adult and postmenopausal women and young adult men.<sup>40</sup>

A meta-analysis of randomized controlled trials performed by Cheng *et al.* evaluated the soy isoflavones' effects on cognition in adults. This study showed that soy isoflavones may be effective in improving cognitive function in adults. This meta-analysis of 10 placebo-controlled RCTs showed that soy isoflavone supplementation seems beneficial for enhancing postmenopausal women's visual memory and cognitive function.<sup>45</sup> Another study reviewed RCTs with nutritional intervention published in 2014-2017 systematically. The authors stated that there was convincing evidence that flavonoid supplementations had positive impact in improving some cognitive functions of healthy aged individuals.<sup>46</sup> A systematic review and meta-analysis study on four clinical trials,

which all involved healthy postmenopausal women, showed that soy isoflavone supplements enhanced memory but had no impact on overall cognitive function.<sup>41</sup> Kokubo *et al.* conducted a study to examine the effects of a commercially available soy drink on menopausal symptoms and cognitive function in post-menopausal women. This study showed that consumption of soy drink had no effect on cognitive function in post-menopausal women. However, vasomotor symptoms were considerably reduced in those with more severe symptoms at baseline after drinking 350 ml/day (35 mg IFs) for 12 weeks.<sup>47</sup> According to Nakamoto *et al.*'s studies, consumption of isoflavone and any soy product might have a desirable efficacy on women's cognitive operation.<sup>48</sup> Studies show that the soy diet and isoflavone consumption might decrease the risk of cognitive deficiency in elderly Japanese women.<sup>48</sup>

One of the main benefits in consuming genistein over the current function of applying hormone substitution therapy to battle AD in postmenopausal women is that this molecule can operate as an estrogen agonist, and it may protect neuronal cells, which does not intervene with the quick-growing pathways in the uterine endometrial cells.<sup>39</sup>

### Stroke

Genistein is one of the pleiotropic molecules, which is found in various foods, especially soy products and soybeans. It is an inhibitor of tyrosine kinase and therefore, can change several pathways.<sup>49</sup> Studies show these molecules can decrease stroke risk in Japan and China. Recent studies indicate that the isoflavone genistein as a natural combination has a potential pleiotropic factor in the prohibition and therapy of central cerebral ischemia.<sup>27</sup> Various preclinical studies of cerebral ischemia and other brain trauma patterns emphasize on advantageous impress for genistein in maintaining the brain from concussion, whether operated acutely or chronically.<sup>27</sup> Genistein is a pleiotropic molecule that operates various mechanisms to increase brain health, including immune suppression, extension of growth agent signaling, and diminution of oxidative stress.<sup>27</sup> These operations happen in neuronal, endothelial, and glial cells to prepare an effective consonant function for ischemic challenges.<sup>27</sup>

#### *In vitro studies*

The nuclear factor E2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1)

anti-oxidant protection order is a significant convergence matter for the anti-oxidant efficacy of genistein in the brain with ischemia.<sup>50,51</sup>

Various recent discussions prove that marking of the Nrf2 pathway by other meal-borne neuroprotective factors like 5-hydroxymethyl-2-furfural<sup>52</sup>, salidroside<sup>53</sup>,  $\beta$ -caryophyllene<sup>54</sup>, and phloretin<sup>55</sup>, prepares preservation against the damage of the brain. Correspondingly, genistein propels the Nrf2/Keap1 system in GCI, leading to heightened heme oxygenase 1 (HO1) and other anti-oxidant purposes.<sup>56</sup>

Genistein is a presented motivator of eNOS in vascular endothelial cells and eNOS-derived NO is neuroprotective in ischemia of the brain.<sup>57,58</sup>

In vitro study conducted by Schreihofner *et al.* on the potential ability of soy isoflavones daidzein metabolite equol, daidzein and genistein to save embryonic rat primary cortical neurons from injuries induced by an ischemic-like situation, found neuroprotective effects of soy isoflavones using ER-kinase pathways to hinder apoptotic cell death. Cells underwent excitotoxicity, hypoxia, and hypoxia, oxygen-glucose deprivation (OGD). They reported that soy isoflavones notably inhibited neural death produced by hypoxia and OGD. Soy isoflavones used in the experiment activated ER $\alpha$ -dependent transcriptions within neurons, supporting a role for ER $\alpha$ . Furthermore, the role of phosphoinositide 3-kinase (PI3K)/Akt serine-threonine kinase (AKT) was mentioned as well.<sup>57</sup>

An investigation conducted by Ma *et al.* using motor neurons of the spinal cord obtained from E14.5 rat embryos, verified that soy isoflavone daidzein could give rise to Arg1 protein expression. Furthermore, this substance was beneficial in boosting the regeneration of optic nerve axons in vivo. These promising findings indicate that using daidzein after the stroke can bring about developments in treating stroke patients.<sup>58</sup>

In vitro studies by Hurtado *et al.* reported a PPAR- $\gamma$  dependent action, which has a neuroprotective benefit by increasing synaptic performance in in vitro models. Rat cortical neurons and cerebellar granule cell cultures treated with daidzein showed PPAR- $\gamma$  accumulation in the cells' nuclei compared with the control cells suggest daidzein as a PPAR- $\gamma$  agonist. This effect, along with an increase presented in the maturation of synaptic bouton in cultured neural cells and resulting in decreased cell death, supports the idea of daidzein being a neuroprotective substance.<sup>59</sup>

Wang and colleagues connected this anti-oxidant efficacy of genistein to the operation of

endothelial nitric oxide synthase (eNOS), since the obstruction of NO generation with the nitric oxide synthase inhibitor L-NAME prohibited the protective efficacy of genistein.<sup>54</sup> At safely acquirable dosage, genistein connected and led all three familiar ERs, but with a priority for Erβ.<sup>27</sup> Genistein takes different mechanisms at premier attention, including straight action and reaction with ion channels, connecting by AhR, and dissuasion of tyrosine kinases.<sup>27</sup> These functions may be either advantageous or adverse.<sup>27</sup>

#### Animal studies

A study on rats undergoing a permanent unilateral MCAO by Stout *et al.* demonstrates a positive effect of subcutaneous daidzein administration on rats' recovery. They found a notable enhance in functional recovery on rats by using the ladder rung walking task. Rats received daidzein and vehicle showed better performance than rats which got only vehicles. Stout *et al.* also found no difference in weight between the groups and lesion volume during their study. They declared that the mechanisms of functional recovery still seem to be unclear.<sup>60</sup> A study on Sprague-Dawley rats showed that mice treated with genistein had better locomotor and cognitive function.<sup>61</sup> Another study on the Sprague-Dawley rats demonstrated that Genistein-3-sodium sulfonate protected mice brain against ischemic stroke by suppressing neuro-inflammation.<sup>62</sup>

A mechanistic study by Kim *et al.* on stroke recovery mouse model revealed that daidzein, a FDA-approved isoflavone, ameliorated the behavioral deficit following the stroke through augmenting cholesterol homeostasis without leading to adverse effects during the acute phase. For promoting functional recovery after stroke, their study suggests using chronic and early daidzein serves. Their experiments were conducted in vivo using Apoe KO (Apoe-knock-out) and C57 rats and in vitro culture system. According to their study, daidzein affects the transcription of genes engaged with cholesterol homeostasis, including downstream transporters Apoe, Abca1, and Abcg1, and Lxr, by increasing Lxr expression more strongly in neurons than the astrocytes also elevating Abca1 and Apoe but not Abcg1 mRNA. Giving treatment to the cultures with an LXR agonist, T0901317, showed increases in Lxr downstream transporter gene expression as expected. Additionally, T0901317 increased Srebp1 (sterol-regulatory element-binding proteins-1) and its gene targets

lipoprotein lipase (Lpl) and fatty acid synthase (Fas) in astrocytes. Compared with T0901317, daidzein enhanced Srebp1 transcription in the absence of changes in Fas and Lpl genes in the postischemic brain and in vitro. The association of Fas and Lpl genes with triglycerides and fatty acids synthesis results in hypertriglyceridemia and fatty liver disease (FLD). Thus findings yielded a promising advantage of chronic use of daidzein over T0901317 during stroke recovery. Findings show an Apoe mRNA increase in the contralateral hemisphere during the first three days, which can be assumed beneficial in stroke recovery. Studies on Apoe KO mice treated with daidzein after stroke did not show functional benefits, although daidzein augmented Abca1 and Lxr levels significantly. They showed a critical role for ApoE in improving daidzein-promoted functional retrieval. In vivo studies in rats yielded that daidzein does not change the infarct size but increases functional recovery at the same time. Briefly, it can be concluded that daidzein enhances genes involved in stroke-induced cholesterol homeostasis in 1 month with no neuroprotection. However, it can bring some functional benefits during recovery following stroke.<sup>63</sup>

According to Banecka-Majkutewicz *et al.*'s in vivo study, significant growth inhibition was detected in *Vibrio harveyi*, culture treated with genistein, as expected. This result was evidenced in *Bacillus subtilis* culture while homocysteine restored the growth to some extent. Demonstrating the fact that homocysteine and genistein can reduce the effects of each other. Banecka-Majkutewicz *et al.* introduced genistein as a promising agent in treating stroke patients chiefly in case of dealing with hyperhomocysteinemia.<sup>64</sup>

A systematic experiment by Yang *et al.* yielded a remarkable neuroprotective effect of nanoformation (OEA-SPC NPs), which is a combination of N-oleoylethanolamin (OEA) and a carrier named soybean phosphatidylcholine (SPC). According to their study, the MCAO mice treated with that substance represented satisfying results as they showed decreased neurological dysfunction. Also, researchers discovered a remarkable reduction in infarct volume and edema degree in the group treated with OEA-SPC NPs, suggesting the potential ability of this substance in treating stroke patients. The Morris water navigation task indicated that the memory of cerebral ischemia and spatial learning nearly convalesced to their normal levels. Assessment of the mice neurons condition indicated a considerable improvement in inflammation

and condition of neurons in the cortex and hippocampal CA1. The whole study exhibits a remarkable impact of OEA-SPC NPs on nervous tissue inflammation and infarct volume as well as on neurological function.<sup>65</sup>

#### *Human studies*

A study on stroke-prone spontaneously hypertensive rats (SHRSP) conducted by Pan *et al.* showed that daidzein, genistein, and glycitein inhibit DNA synthesis and multiplications of smooth muscle cells (SMC) in SHRSP. As a result, soy isoflavones possess preventive effects on atherosclerosis and subsequently reduce stroke risk. The investigators demonstrated genistein as a protein tyrosine kinase inhibitor that restrains the receptor tyrosine phosphorylation of EGF activation. The study suggests soy isoflavones as inhibitors of vascular SMC proliferation thus can prevent atherosclerosis.<sup>66</sup>

A meta-analysis performed by Lou *et al.* on both cohort and case-control studies has stated that there was limited evidence to admit that soy intake leads to a lower risk of CHD and stroke. Though, they observed that the stroke risk and soy consumption are inversely proportional. Combining cohort studies indicated no relation between soy consumption and CHD stroke risk. Furthermore, no link between soy isoflavone consumption and the risk of stroke was discovered. A meta-analysis of Cohort studies demonstrated an SRR (summary relative risks) of 0.92% (95% CI 0.70–1.10) for individuals in the highest soy intake categorization than those in the lowest categorization with low heterogeneity amongst cohort studies. A meta-analysis of case-control studies provided an SRR of 0.66 (95% CI 0.56–0.77). They did not observe heterogeneity. Lou *et al.* assumed that these inconsistent results could be due to the selection biases, which may happen in case-control studies.<sup>67</sup>

According to the Takayama Study, conducted by Nagata *et al.* that aimed the association between natto, a traditional Japanese food that contains soy, and CVD (cardiovascular disease) mortality rate, a significant relationship between the highest and the lowest quartile of natto intake was observed. This cohort study included 13,355 individuals older than 35 y. that followed-up during 16 y. with a total of 1,678 death from CVD that included 677 strokes and 308 IHD. However, there was no remarkable association between the mortality risk from total cardiovascular diseases and total soy intake. They also mentioned that

the possible benefits of soy for decreasing CVD mortality should not be narrowed down to natto, as they observed there was a significant tendency for reduced risk of ischemic heart disease. Their data suggested that natto intake may lead to a decrease in CVD mortality in men and women.<sup>68</sup>

According to the Hisayama study conducted by Ozawa *et al.* that discussed the association of dietary protein consumption with the risk of stroke in the Japanese population, the higher dietary protein consumption is related to the reduction of stroke. As the investigators mentioned, soybeans rich in isoflavones probably reduce the ischemic stroke risk. Therefore the higher intake of soybeans and their products can be a useful strategy in lowering the risk of ischemic stroke.<sup>69</sup>

#### **Parkinson's disease**

PD is an age-dependent neurodegenerative disorder. Neuroinflammation is an involved mechanism in progressing PD. Activated microglia is the prominent feature of neuroinflammation, producing significant numbers of pro-inflammatory cytokines and causing an increase in neuronal cell death. Some isoflavones like genistein and daidzein are similar to estrogens structurally and functionally. A new study shows that flavonoids might help in reducing mortality risk among patients with PD.<sup>70</sup>

#### *In vitro studies*

A multiplex related to genistein, called phenoxodiol (2H-1-benzopyran-7-0,1,3-[4-hydroxyphenyl]), demonstrates remarkable neurite-protective effects upon cisplatin at 1  $\mu$ M, a safe concentration to the PC12 cells.<sup>71</sup>

A study by Sawada *et al.* reviewed potential neuroprotective effects of estradiol in rat neuronal cells. They mentioned that Akt phosphorylation caused by estrogens results in CREB phosphorylation downstream and Bcl-2 up regulation. Also, estrogen stimulates a second-messenger mechanism like adenylate cyclase and protein kinase A (PKA). Also, PKB and PKC were stimulated, As well as a mitogen-activating protein kinase. It is considered that the mediating effects of nonnuclear estrogen receptors are causing rapid stimulation of second messengers. The expression of neurotrophins and GDNF is increased by estrogen too. ICI 182,780 was reported not to block the increase in regulation of GDNF caused by estradiol. However, inhibiting cyclic adenosine monophosphate blocked it. In conclusion, the

upregulation of GDNF is considered not to be dependent on nuclear Ers and might be relevant to nonnuclear Ers' mediatory effects on signal transduction. They also added that estrogen could suppress apoptosis caused by oxidative stress in dopaminergic neurons. They demonstrate that estrogens can have neuroprotective effects on dopaminergic neuronal degeneration by stimulating complex mechanisms such as anti-oxidant property and mediatory effects of nonnuclear Ers on protective signal transduction.<sup>72</sup>

Genistein's bioavailability is up because it can go over in the blood-brain barrier.<sup>73</sup> Accordingly, the data suggests that SI can inhibit apoptosis of dopaminergic neurons caused by ART. Occhiuto and colleagues conducted a study investigating neuroprotective effects of a natural combination of phytoestrogenic isoflavones containing daidzain, formononetin, genistain, and biochanin, a product from *Trifolium pratense* L. or Red clover on cell death caused by oxidative stress makers in HCN 1-A (a cell line of human cortical cells). HCN 1-A cell cultures exposed to hydrogen peroxide showed a concentration-related decrease in the viability of neurons. Neuronal toxicity of H<sub>2</sub>O<sub>2</sub> was observed in a concentration range between 50 and 200 200 µg/ml. An MTT test containing isoflavones extract in amounts of 0.5, 1 and 2 µg/ml as a 24 hours pretreatment resulted in increased cell survival that manifests isoflavones can protect neurons against cell death caused by exposure to H<sub>2</sub>O<sub>2</sub>. They concluded that the anti-oxidant activity of isoflavones is the reason for their neuroprotective effects. Their study indicates that despite the fact isoflavones extract causes a neuroprotective effect, it did not promote an outgrowth in the cortical neuron process.<sup>74</sup>

Moreover, genistein can decrease the activity of caspase -3 and caspase-8 in p12 cells to reduce 6-OHDA activity.<sup>75</sup> Also, its oral treatment ameliorates the mind's acting.<sup>76</sup> In 2012 Pan M *et al.* have been reported that some isoflavonoids such as daidzein may increase Brain-derived neurotrophic factor (BNDF) levels.<sup>77</sup>

The compound 6,7,4'-trihydroxyisoflavone (THIF) is a significant metabolite of an important isoflavone, daidzein. A study investigated the protective effects of 6,7,4'-THIF on neurotoxicity induced by 6-hydroxydopamine (OHDA) in the human neuroblastoma cells (SH-SY5Y). It suggests that due to the effects of 6,7,4'-THIF on the inhibition of neuronal cell death induced by 6-OHDA, the release of lactate dehydrogenase, the production of reactive oxygen species, and attenuation in the expression of Bax and Bcl-2,

6,7,4'-THIF may have therapeutic or preventive impacts on neurodegenerative diseases like PD.<sup>78</sup> In another study investigating possible neuroprotective effects of diadzein on PD, Chinta *et al.* tested the effects of diadzain on levels of NO and IL-6, two of the pro-inflammatory mediators, on BV-2 cells. MAP kinase-NFjB signaling pathway could be inhibited by diadzain leading to the prevention of synthesis and release of neurotoxic factors. Moreover, the study demonstrates that diadzain can cause neuroprotective effects by moderating microglial activation and inhibiting the release of pro-inflammatory factors. The pretreatment of LPS-activated microglia by diadzain resulted in significant inhibition of the production of pro-inflammatory factors like IL-6, NO, ROS, and TNF- $\alpha$ .<sup>79</sup>

Genistein usefully stimulates the synthesis of glial cell line-derived neurotrophic factor (GDNF), NGF, and their salvation.<sup>80</sup> Genistein is known for protecting against neurodegenerative diseases by estrogenic properties and can decrease the hurt of oxidative stress and apoptosis in SH-SY5Y cells.<sup>81</sup>

Another study performed by de Rus Jacquet *et al.* showed that neurotoxicity induced by insults associated epidemiologically or genetically to PD could be alleviated by isoflavone-rich red clover and soy extracts, as well as the individual isoflavones daidzein and equol (produced from daidzein by the intestinal microbiota). Their findings indicates that the studied extracts and isoflavones have the potential to reduce the risk of PD or make the progression of the disease slower in humans.<sup>82</sup>

#### Animal studies

According to an article investigating the effects of genistein on protecting dopaminergic neurons in MPTP-induced PD mice ovariectomized cells. Liu *et al.* demonstrated that phytoestrogen genistein protects dopaminergic neurons against neuronal damage induced by MPTP. Enhancing the gene expression of Bcl-2 may attribute the effects. Reduced neurotoxicity was observed after genistein treatment on the nigrostriatal system. Genistein protects against the deficits of HVA, DA, and DOPAC contents induced by MPTP in the stratum. Also, genistein reverses the reduction in TH-IR neurons induced by MPTP in the SNpc. The reverse transcription-PCR results demonstrate that genistein treatment could restore the MPTP-induced reduction of TH and mRNA expression of DAT in the midbrain.<sup>83</sup>



Moreover, a study's data suggests that genistein and quercetin at low doses or in combination have protective effects on hemoglobin and myoglobin damages caused by a radical generating system.<sup>84</sup>

Parkinsonism can cause motor and cognitive disabilities. Arbabi and colleagues experimenting with the effects of genistein on this matter in an animal model of PD, conclude that genistein improves learning and memory ability by effecting the nigrostriatal pathway and the striate nucleus. However, although genistein had neuroprotective effects, it was proven to be ineffective on motor disorders. Accordingly, the article suggests that the cognitive improvement caused by genistein may lead to positive impacts on other parts of the brain.<sup>85</sup>

Mirahmadi *et al.* compared the efficacy of dexamethasone with genistein to investigate genistein's effects on preventing cognitive dysfunction caused by lipopolysaccharide (LPS). The results show that genistein is more functional and robust in some aspects. The findings demonstrated that genistein could moderate spatial recognition and discrimination as well as memory deficits in a dose-dependent manner. Furthermore, the level of malondialdehyde (MDA) in hippocamp lowered and the activity of superoxide dismutase (SOD) increased, as well as catalase and glutathione (GSH) level after genistein treatment. They concluded that cognitive dysfunction and inflammation of neurons caused by LPS could be alleviated by genistein. Genistein attenuates oxidative stress and AchE and also modulates Nrf2/COX2/IL-6/GFAP/NF- $\kappa$ B/TLR4/TNF $\alpha$ /iNOS.<sup>20</sup>

Puerarin is a derivative of daidzin, which has a protective role on neurons and dopamine-producing cells. According to Shiyong *et al.*, 's research, function of PR have been evaluated in vitro and in vivo. In vitro, green fluorescent-differentiated cells labeled as mesenchymal stem cell secretions were administered to animal models of PD. Differentiation and proliferation of labeled cells were assessed by immunoassay as well as by fluorescence microscopy. In vitro, treatment with different concentrations of PR was performed for one week and the result was that some proteins such as TH and DAT were increased, resulting in increased mRNA expression followed by DA secretion. In vivo, such PD symptoms improved significantly 2 weeks after transplantation of DA-producing cells with PR. Subsequently, the new cells survived and migrated to more areas in the body and differentiated and distinguished into TH+ cells. To conclude, PR promulgate

survivorship, differentiation and proliferation of DA neuron.<sup>86</sup>

Siddique *et al.* exposed transgenic PD flies to genistein and observed a reduction in oxidative stress markers. Exposure to genistein not only increased GSH content but also decreased PC content, GST activity, and lipid peroxidation (LPO). Furthermore, dopamine content was increased in exposed PD flies, indicating possible genistein roles in protecting dopaminergic neurons. However, the expression of the human  $\alpha$ -synuclein protein in the neurons of PD flies was not inhibited. This study suggests that exposure to genistein results in a considerable dose-dependent increase in their survival rate and a delay in their loss of ability to climb. In conclusion, due to anti-oxidant potential of genistein, PD symptoms were reduced.<sup>87</sup>

Based on a study investigating the neuroprotective effects of soybean isoflavones (SI) on molecular mechanisms in DAergic toxicity induced by ATR in rat models of PD, we can conclude that the degeneration of DAergic neurons mediated by ATR can be prevented as a result of autophagy inducement through a signaling pathway dependent to mTOR by SI. SI attuned oxidative stress induced by ART and inflammatory damage. Also, SI pretreatment reversed the increased expression of apoptosis regulator Bax and decreased expression of tyrosine hydroxylase (a dopamine synthesis enzyme) and the antiapoptotic Bcl-2 caused by ATR in the nucleus niger and striatum.<sup>88</sup>

### Multiple sclerosis

Experimental allergic encephalomyelitis (EAE) is regarded as the multiple sclerosis murine model.<sup>89</sup> Razeghi Jahromi *et al.*, in a study on experimental allergic encephalomyelitis alleviation in C57BL/6 mice with soy daidzein, observed that daidzein could reduce the demyelination extent as well as disease severity. Chronic oral therapy using a low dose of daidzein could not prohibit experimental autoimmune encephalomyelitis. Although, high doses of daidzein prevent disease exacerbation.<sup>89</sup>

In multiple sclerosis (MS), IL-17 is generated by helper T (Th) cells stimulated by IL-6 and IL-1 $\beta$  derived from tissue cells and phagocytes such as macrophages.<sup>90</sup> Th17 and Th1 cells were promoted along with their associated cytokines IL-17, IL-1, IL-6, IFN- $\gamma$  in MS patients. Patients have substantiated dysregulation in cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expression.<sup>91</sup>

### *In vitro studies*

Miljkovic *et al.* detected that both PD98059 and A77 1726 significantly decreased IFN- $\gamma$ /LPS-induced NO synthesis in the rat astrocytoma cell line C6 cultures. The capability to suppress induction of iNOS inside astrocytes could support possible leflunomide use in the treatment of multiple sclerosis as well as other inflammatory brain disorders that are NO-dependent.<sup>92</sup>

McDowell *et al.* discovered that the exposure of motoneurons of ventral spinal cord 4.1 to microglial cytokine supernatant in vitro led to significant apoptosis and decreased mitochondrial membrane potential. A growth in calpain, reactive oxygen species, caspases, intracellular Ca<sup>2+</sup>, the bax:BCL-2 ratio, and cytochrome C were also observed. Therapy with GEN could reverse apoptotic death in addition to cellular change after cytokine exposure and also was associated with raised estrogen receptor b expression, offering that GEN could elevate neuroprotection through receptor-mediated pathways. The addition of 780, ICI 182, an estrogen receptor antagonist after therapy with GEN, could attenuate neuroprotection, offering that GEN could act primarily through estrogen receptor b to protect motoneurons of VSC4.1. They have concluded that GEN could protect ventral spinal cord 4.1 cells that were cultured from inflammatory insult and could represent a potentially useful therapy in treating neurodegenerative disorders.<sup>93</sup>

Jantaratnotai *et al.* reported that the phytoestrogens of soy, daidzein, coumestrol and genistein, reduced the generation of nitric oxide (NO) induced by lipopolysaccharide (LPS) inside the rat microglial cell line (HAPI). The rates of protein expression and inducible NO synthase (iNOS) mRNA also decreased. Transcription factors that govern the expression of iNOS involving interferon regulatory factor-1 (IRF-1) and phosphorylated STAT1 were down-regulated. These findings describe the inhibitory effect of phytoestrogens on the generation of NO. The rates of interleukin-6 mRNA and monocyte chemoattractant protein-1, pro-inflammatory cytokine and chemokine associated with diverse neurological diseases were also decreased after stimulation of LPS during the time at which HAPI cells were pretreated with phytoestrogens. Therefore, daidzein, coumestrol, and genistein may serve as anti-inflammatory factors and could have lucrative efficacies in treating neurodegenerative diseases.<sup>94</sup>

### *Animal studies*

De Paula *et al.* founded that treatment with genistein meliorated remarkably the symptoms, moderating anti- and pro-inflammatory cytokines. Furthermore, the leukocyte rolling as well as adhesion in the CNS were analyzed by doing intravital microscopy. Treatment with genistein resulted in reduced adhering and rolling of leukocytes compared to the untreated group. Their data propose that treatment with genistein could be a possible MS therapy.<sup>95</sup>

Castro *et al.* reported that 7-O-tetradecanoyl-genistein (TDG) treatment meliorates the EAE clinical signs, which correlates with a reduction of cells producing IL-17 and a growth in Foxp3+CD4+ cells in the brain. TDG can also increase IL-10 production and expression of CTLA-4 and decrease IL-6 as well as IFN- $\gamma$ . Overall, an immunomodulatory therapeutic figure for TDG in multiple sclerosis (MS) and EAE is proposed.<sup>96</sup>

IL-10 is an anti-inflammatory cytokine, and IL-12, TNF- $\alpha$ , IFN- $\gamma$  are pro-inflammatory cytokines. Jahromi *et al.* realized that administering mice with genistein, since the EAE setup, did not refract clinical signs of the disease. Although, genistein treating at the disease's early stages could alleviate the clinical signs by decreasing neuronal demyelination. Genistein could suppress the IFN- $\gamma$  production and increased the secretion of IL-10 in the brain and splenocytes. Besides, genistein decreased the secretion of TNF- $\alpha$  and IL-12 in splenocytes, reduced cell cytotoxicity, and suppressed T-cells' proliferation. Oral therapy with genistein could decrease the severity of EAE if started in the disease's early stages.<sup>97</sup>

Spagnuolo *et al.* reviewed the inflammation role in neurodegenerative disorders, emphasizing the possible therapeutic efficacies of flavonoids as a promising approach to create a new neuroprotective strategy. Microglial activation is the principal neuroinflammation feature, increasing the release of the pro-inflammatory cytokines and causing neuronal cell death. Natural compounds, like flavonoids, have the potential to be neuroprotective that may be related to their capability to regulate the inflammatory reactions involved in neurodegenerative disorders. Pure flavonoids (e.g., genistein, hesperetin, quercetin, epigallocatechin-3-gallate) or enriched-extracts could decrease the expression of pro-inflammatory cytokines (IL-1 b, COX-2, IL-6, and TNF-a), prohibit neural damage, and down-regulate inflammatory signs. This anti-inflammatory

action is mainly related to the microglial cells' adjustment, mediated by their impacts on NF- $\kappa$ B and MAPKs signaling pathways, as indicated through *in vitro* and *in vivo* data.<sup>98</sup>

IFN- $\beta$  has a vital role in treating MS by decreasing the frequency of the relapses and the appearance of novel lesions.<sup>99</sup> Dias *et al.* have demonstrated the genistein ability to moderate the involved factors in innate immune response in the EAE preliminary phase. The therapy with genistein delayed the disease resumption, with decreased inflammatory demyelination as well as infiltration. Moreover, the TLR9, TLR3, and IFN- $\beta$  expressions were raised in the group treated with genistein, with a decline in the TH17 and Th1 cells factors. This study may show the genistein potential as a preventative strategy for preventing multiple sclerosis (MS).<sup>99</sup>

Ohgomori *et al.* figured out that GEN could act on mature oligodendrocytes in the hippocampus by increasing their myelin formation and survival and offer the phytoestrogens therapeutic possibility to treat MS patients who suffer from mental health problems.<sup>100</sup>

## Huntington's disease

### *In vitro studies*

Pierzynowska *et al.* observed that both modified huntingtin levels and the number of combinations were remarkably diminished in HD cell models treated with genistein. It caused augmented cell viability. Autophagy was up-regulated when lysosomal function inhibition via chloroquine damaged the genistein-mediated depression of the modified huntingtin aggregates. Therefore, they concluded that genistein eliminates the significant pathogenic factor of HD by stimulating autophagy. Prolonged autophagy induction was doubtful at an earlier time to be dangerous for HD patients because of supposed adverse efficacies; although, genistein has been proved lately to be suitable and safe for long-term treatments even at high doses like 150 mg/kg/day. Hence, outcomes demonstrated in their report supply a basis for applying genistein in future studies on the development of the possible HD treatment.<sup>101</sup> Pierzynowska *et al.* also represented that genistein also induced depression of mutant Htt (mHtt) in fibroblasts taken from patients suffering from HD. It was determined as a remarkable diminish in the HTT levels in HD fibroblasts measured with Western-blotting, and the evanescence of intracellular mHTT aggregates in cells observed using fluorescent microscopy. Fibroblasts

taken from control people were not influenced with treatment with genistein. These outcomes demonstrate that genistein could improve HD's phenotype in cells derived from HD patients and substantiate the requirement for future studies of this isoflavone as a possible therapeutic substance.<sup>102</sup>

### *Animal studies*

Menze *et al.* realized that early treatment with 17 $\beta$ -estradiol and genistein could attenuate locomotor hypoactivity, raised ATP levels, and enhanced retention latencies in the passive avoidance task, attenuated the augment in AChE activity, reduced the expression of COX-2 and iNOS, and meliorated the oxidative stress profile. The greater dose of genistein (20 mg/kg) was the most efficient. In summary, their findings suggest memory enhancing and neuroprotective effects for genistein in a rat model of HD. These effects could be attributed to its anti-oxidant, anti-inflammatory and cholinesterase inhibitory processes.<sup>103</sup> Menze *et al.* also designed a study to survey the possible advantageous genistein results in 3-NPA-induced HD in ovariectomized rats. Outcomes represented that 3-NPA (20 mg/kg) administration resulted in a remarkable locomotor activity of rats' disruption and prepulse inhibition. Moreover, it diminished striatal ATP rates and augmented oxidative stress, inflammatory and apoptotic signs with striatal focal hemorrhage and gliosis. Early treatment with 17 $\beta$ -estradiol (2.5 mg/kg) or genistein (20 mg/kg) caused a notable behavioral parameters improvement, diminished oxidative stress, augmented ATP production, reduced apoptosis, and inflammation. Accordingly, these findings suggest possible genistein neuroprotective efficacies in ovariectomized rats tested by 3-NPA.<sup>104</sup>

## Seizures

### *Animal studies*

Khodamoradi *et al.* did an experiment to prove the effects of genistein on seizures. According to this experiment animals who were injected by genistein after the treatment, KA throw in the left lateral ventricle to persuade seizures, after 7 days, memory and spatial learning were inquire and concluded that genistein may have tendentiously preventative results against seizure in OVX rats.<sup>105</sup> The other research done by Amiri Gheshlaghi *et al.* showed the effect of genistein on seizures in mice which ovariectomized. Their research

investigated that after 14 days of ovariectomy surgery, genistein (10mg/kg) intensified the threshold of the seizure, 30min former to infusion of seizures.<sup>106</sup> According to Elsayed *et al.* study, genistein demonstrated the powerful protective effects on seizure.<sup>107</sup> In another study, Westmar *et al.* investigated and evaluated the effect of foods containing soy, especially daidzein, on seizure disorders. By designing an experiment on mouse models the *Fmr1<sup>KO</sup>*, FRAXAD and wild type the expression level of A $\beta$ PP and A $\beta$  and its effect on the AGS phenotype and the path dependent on mGluR5/FMRP studied in *Fmr1KO* mice and found that drug interventions such as daidzein can block or reduce the mGluR5 signaling pathway and thus may be effective for the treatment of seizure disorders.<sup>118</sup>

To conclude, it is understood that there is relevancy between genistein and also daidzein and serotonergic/estrogenic systems.

## Brain tumors

### *In vitro studies*

The molecular mechanism of genistein's function in human cancer cells has been widely studied. Many studies reported multiple effects of genistein, such as triggering apoptosis, inducing cell cycle arrest, inactivating several signaling cascades and inhibiting the NF $\kappa$ B activation in cancer cells. Genistein regulates different stages of cell cycle, angiogenesis, metastasis, and apoptosis. Caspases, Bcl-2-associated X protein (Bax), B-cell lymphoma 2 (Bcl-2), inhibitor of NF- $\kappa$ B, nuclear factor- $\kappa$ B (NF- $\kappa$ B), extracellular signal-regulated kinase 1/2 (ERK 1/2), phosphoinositide 3-kinase/Akt (PI3K/Akt), Wntless and integration 1/ $\beta$ -catenin (Wnt/ $\beta$ -catenin) and, mitogen-activated protein kinase (MAPK) signaling pathway are the most important genistein's molecular targets. In addition to transcription factors, endoplasmic reticulum (ER) stress caused by genistein, induces apoptosis. Besides, peroxisome proliferator-activated receptors (PPARs) could be considered as potential therapeutic targets and genistein may induce apoptosis via aiming PPAR $\gamma$  signaling cascade. It has been shown that genistein prevents the growth of glioblastoma and medulloblastoma cells via various TP53 mutations as well as radio-responses through arresting that cells at G2/M phase. This mechanism seems to be independent of damage of DNA and this arrest is sustainable for approximately 10 days after

drug removal. Staining with Anxin V showed a reduced population of apoptotic or necrotic cells after genistein treatment. Genistein could induce inconsiderable DNA damage and this could indicate that the cell cycle arrest triggered does not cause death in cells. Genistein could induce growth arrest in association with telomerase prevention in brain tumor cells by the TR- and TERT mRNA suppression. Altogether, we can consider genistein a chemotherapeutic agent.<sup>108,109</sup>

Medulloblastoma (MB) is the most common malignancy of the CNS in children, with a maximum incidence at about five years of age.

Genistein lowers the expression of the mRNA TERT and TR, resulting in a reduction in telomerase activity, as shown by the TRAP experiment; furthermore, genistein causes the cell cycle arrest by decreasing the cyclin/CDK complexes and increasing the regulation of CDK inhibitors in every type of tumor cell in the brain.<sup>108</sup>

Soybean-derived phytoestrogens such as 4',5,7-trihydroxyisoflavonoid (genistein), 4'7-dihydroxyisoflavonoid (daidzein), and the bacterial metabolite of daidzein, (s)-equol decline the activity of caspase 3 in D283 Med MB cells and protect those cells from cisplatin cytotoxic actions.<sup>110</sup>

Genistein may link specifically to DNA-PKcs and block the PKcs/Akt2/Rac1 DNA route, completely suppressing radiation-caused invasion and migration of glioblastoma multiforme which is known highly lethal type of brain cancer in vitro and in vivo.<sup>111</sup>

## Brain inflammations

In 2007 Lee *et al.* demonstrated genistein's anti-inflammatory effect on human brain microvascular endothelial damage is achieved by the inhibition of cytokine-driven upregulation of a variety of pro-inflammatory mediators such as TNF- $\alpha$  and IL-8.<sup>112</sup> Another study in this year adult Sprague-Dawley rats showed that baicalin (10-40 mg/kg) might dose-dependently protect brain tissue against brain inflammation induced by heatstroke.<sup>113</sup>

Interleukins 1 beta, 2, and 13, as well as the chemokine CXCL1, all showed significant (>2-fold) decreases that illustrates a reduction in the inflammatory response in the cerebral cortex over the intense innate during 4 and 24 hours after tMCAO, which were related to food-grade soybeans and soy isoflavones.<sup>114</sup>

## Human traumatic injury

### Animal studies

A study performed by Soltani et al. on male albino N-Mari rats using Marmarou method and a TBI induction device, indicated that soy diet might play a role in impeding intracranial pressure (ICP) during the first hours after the trauma. It is also showed that the increase of ICP on post-trauma days would be lower in soy diet rats suggesting consuming soy attenuates the ICP elevation. The assessment of vestibulomotor function by performing the Beam-walk (BW) task showed that soy diet prevented traversal time increase and it is concluded that the brain edema reduction followed by the ICP reduction might play a role.<sup>115</sup> Another study conducted by Soltani *et al.* on male Wistar rats introduced genistein as a neuroprotective agent that helped blood-brain barrier (BBB) permeability, suppressed increase of ICP and lowered the brain edema development. According to this study, brain water content increased in TBI group compared to the control group. But it was reversed by genistein administration. Also, the BBB disruption induced by TBI was reversed by administration of this agent. Furthermore, they

indicated that vestibulomotor and motor function deficits can be improved by this chemical in traumatic brain injury (TBI).<sup>116</sup>

In 2017, Hooshanginezhad and Derakhshan concluded that genistein role in TBI is due to its neuroprotective effect on cortical neurons, anti-inflammatory and anti-oxidative effects, as well as mimicking endogenous estrogen-estradiol- on brain as estrogen is proved to have beneficial effects on recovery from TBI in rat models.<sup>117</sup>

Summary of the studies are listed in Table 1.

In conclusion, the main isoflavones found in soybeans are genistein, daidzein, and glycitein. At concentrations above 1  $\mu\text{M}$ , genistein activates the peroxisome proliferator-activated gamma receptor (PPAR $\gamma$ ), and PPARs are described as neuroprotective targets for neurodegenerative diseases. Several cognitive features have been enhanced by genistein therapy. The higher intake of soybeans and their products can be a useful strategy in lowering the risk of ischemic stroke. Findings indicate that using daidzein after the stroke can bring about developments in treating stroke patients. Also, antioxidant potential of genistein may reduce PD's symptoms. There is therapeutic possibility of these phytoestrogens

**Table 1: Summary of the pharmacotherapeutic effects of soy isoflavones in neurological disorders**

Type of soy isoflavone	Disease or condition	Model	Study design	Neuropharmacological mechanisms and outcomes	Reference
Genistein	AD	A $\beta$ induced AD	In vitro: rat cortical astrocytes	$\downarrow$ COX-2 $\downarrow$ iNOS $\downarrow$ IL-1 $\beta$ $\downarrow$ TNF- $\alpha$ $\uparrow$ PPAR- $\gamma$	Valles <i>et al.</i> 2010 <sup>26</sup>
Genistein	AD	BACE1 induced AD Genistein inhibit BACE1	In vitro: enzyme kinetic predictions	BACE1 activity $\downarrow$	Youn <i>et al.</i> 2018 <sup>30</sup>
Genistein	AD	AChE induced AD genistein derivatives inhibit AChE	In vitro: IC50 value analysis	AChE activity $\downarrow$	Hong <i>et al.</i> 2019 <sup>36</sup>
Glycitein	AD	Glycitein inhibit $\beta$ -amyloid aggregation	In vitro: neurons were cultured in the extracapillary space of poly(l-lactic acid) (PLLA) microtube array (MTA) membranes	activation of apoptotic markers $\downarrow$ , production of ROS $\downarrow$	Morelli <i>et al.</i> 2019 <sup>37</sup>

Genistein	AD	A $\beta$ 25–35 induced AD	In vitro: SH-SY5Y cells	NeuN levels $\uparrow$ , A $\beta$ -induced cell death $\downarrow$ , A $\beta$ -induced decrease in pAkt and Tau hyperphosphorylation $\downarrow$	Petry <i>et al.</i> 2020 <sup>21</sup>
Genistein	AD	-	In vivo: A $\beta$ PPswe/PS1dE9 mice	Number and total region of the amyloid plaques $\downarrow$  Microglial reactivity $\downarrow$  intensity of Iba1 protein expression by microglial cells $\downarrow$  labeled microglial cell expansions $\downarrow$  brain A $\beta$ levels $\downarrow$  Plaque deposition $\downarrow$  release of ApoE $\uparrow$	Bonet-Costa <i>et al.</i> 2016 <sup>28</sup>
Genistein, curcumin, resveratrol	AD	A $\beta$ induced AD	In vivo: intoxicated rat	formation of A $\beta$ plaques in the brain $\downarrow$  learning and memory deficits $\uparrow$	Sadhukhan <i>et al.</i> 2018 <sup>39</sup>
Soy isoflavones combination	memory impairments	scopolamine (SCOP)-induced	In vivo: mice (aged 4 weeks)	$\uparrow$ Cholinergic system function/ $\downarrow$ levels of oxidative stress / phosphorylation levels of ERK, CREB, BDNF expression levels ~	Lu <i>et al.</i> 2018 <sup>10</sup>
Soy isoflavones combination	Dementia	Ethanol-induced	In vivo: male ICR mice	the AChE activity and (Ach) level ~, $\uparrow$ synaptic plasticity-related protein expressions, $\downarrow$ neuron apoptosis-related protein expressions	Lu <i>et al.</i> 2020 <sup>9</sup>
Genistein or equol	Stroke	Induced by tMCAO	In vivo: Ovariectomized Sprague-Dawley rats	Reduced inflammatory response $\downarrow$ interleukins 1 beta, 2, and 13 and the chemokine CXCL1 no effect on TNF- $\alpha$ or IFN $\gamma$	Shambayati <i>et al.</i> 2014 <sup>114</sup>
Daidzein	Stroke	Induced by transient MCAO	In vivo: C57 rat  In vitro: neurons and astrocytes  In vivo: Apoe KO rat	$\uparrow$ synaptophysin mRNA, - Psd-95*  $\uparrow$ Lxr, $\uparrow$ Abca1, $\uparrow$ Abcg, $\uparrow$ ApoE, $\uparrow$ Srebp1, -Fas and Lpl**  $\downarrow$ ApoE, $\uparrow$ Lxr, $\uparrow$ Abca1	Kim <i>et al.</i> 2015 <sup>63</sup>

Genistein	Stroke	MCAO	In vivo: BALA/C nude mice, Kunming mice and Sprague-Dawley (SD) rats.	inhibition in bacteria growth which can be restored by homocysteine	Banecka-Majkutewicz <i>et al.</i> 2017 <sup>64</sup>
Combination of isoflavones containing daidzain, formononetin, genistain and biochanin	PD	Induced by H2O2	In vitro: human cortical cell line HCN 1-A	H2O2↓ Neuronal viability↑	Occhiuto <i>et al.</i> 2009 <sup>74</sup>
Genistein	PD	Induced by 6-OHDA	In vitro: P12 cells	↓caspase-8 and caspase-3 proteins activity	Lin <i>et al.</i> 2010 <sup>75</sup>
Daidzein	PD	-	In vitro: H19-7 neural cell line	↑BDNF	Pan <i>et al.</i> 2012 <sup>77</sup>
Diadzein	PD	Induced by LPS	In vitro: murine microglial cell line (BV-2)	↓IL-6/ NO/ ROS/ TNF-a	Chinta <i>et al.</i> 2013 <sup>79</sup>
Genistein	PD	Estrogen signaling	In vitro: Primary rat astrocytes	↑BDNF, ↑NGF, ↑GDNF	Xu <i>et al.</i> 2013 <sup>80</sup>
Genistein	PD	Rotenone-induced PD	In vitro: SH-SY5Y cells	↑NFE2L2 channels activity and ↑estrogen receptors activity	Zárate <i>et al.</i> 2017 <sup>81</sup>
Daidzein	PD	CCK-8, flow cytometry, real-time PCR and ELISA induced by 6-OHDA	In vitro: MSCs In vivo: PR-treated DA-producing cells	↑TH and DAT ↑TH+ cells	Shiying <i>et al.</i> 2018 <sup>86</sup>
Diadzein	PD	Induced by 6-OHDA	In vitro: human neuroblastoma cells (SH-SY5Y),	↓lactate dehydrogenase release ↓the expression of Bax  ↑the expression of Bcl-2	Ko <i>et al.</i> 2019 <sup>78</sup>
Genistein	PD	Induced by MPTP	In vivo: mice ovariectomized cells	the expression of Bcl-2↑  reduction of DA/ DOPAC /HVA↓	Liu 2008 <sup>83</sup>
Genistein	PD	Induced by LPS	In vivo: Male albino Wistar rats	MDA↓, SOD/GSH/catalase↓  IL-6/ NF-κB p65/ TLR4/ TNFα/ COX2/ iNOS/ GFAP↓  Nrf2↑	Mirahmadi <i>et al.</i> 2018 <sup>20</sup>

Genistein	PD	Transgenic fly lines that expresses h- $\alpha$ S	In vivo: PD Flies	<p>↑GSH content, ↓PC content/GST/LPO</p> <p>↑Dopamine content expression of <math>\alpha</math>-synuclein &amp; formation of Lewy bodies (no change)</p>	Siddique <i>et al.</i> 2019 <sup>87</sup>
Soybean isoflavonoids	PD	Induced by ART	In vivo: Sprague-Dawley (SD) rats	pro-apoptotic Bax↓, anti-apoptotic Bcl-2↑	Li <i>et al.</i> 2020 <sup>88</sup>
Leflunomide	MS	-	In vitro: rat astrocytes and macrophages	↓IFN- $\gamma$ + LPS-induced NO synthesis in rat astrocytes	Miljkovic <i>et al.</i> 2001 <sup>92</sup>
Genistein	MS	Induced by microglial cytokine supernatant	In vitro: rat VSC4.1 motoneurons	<p>↑reactive oxygen species &amp; intracellular Ca<sup>2+</sup> &amp; calpain &amp; caspases &amp; cytochrome c &amp; the bax:bcl-2 ratio, ↑expression of estrogen receptor b</p>	McDowell <i>et al.</i> 2011 <sup>93</sup>
Genistein, daidzein, coumestrol	MS	Induced by lipopolysaccharide	In vitro: rat microglial cell line (HAPI)	<p>↓NO, ↓iNOS mRNA &amp; protein expression, ↓IRF-1 &amp; phosphorylated STAT1, ↓monocyte chemoattractant protein-1 &amp; interleukin-6 mRNA</p>	Jantaratnotai <i>et al.</i> 2013 <sup>94</sup>
Quercetin, genistein, hesperetin, epigallocatechin-3-gallate	MS	-	In vivo and in vitro data	<p>↓pro-inflammatory cytokines (IL-6, TNF-<math>\alpha</math>, IL-1<math>\beta</math> and COX-2), ↓inflammatory markers</p>	Spagnuolo <i>et al.</i> 2018 <sup>98</sup>
Genistein	MS	Induced with EAE	In vivo: C57BL/6 mice	<p>in the brain: ↓IFN-<math>\gamma</math>, ↓IL-12, ↑IL-10, ↓TNF-<math>\alpha</math></p> <p>Produced by ConA-driven splenocytes: ↓IL-10, ↓IFN-<math>\gamma</math>, ↓TNF-<math>\alpha</math></p>	De Paula <i>et al.</i> 2008 <sup>95</sup>
Genistein	MS	Induced with EAE	In vivo: C57BL/6 mice	<p>↓IL-17-producing cells, ↑Foxp3<sup>+</sup>CD4<sup>+</sup> cells in the brain, ↑IL-10, ↑CTLA-4 expression, ↓IFN-<math>\gamma</math> &amp; IL-6.</p>	Castro <i>et al.</i> 2012 <sup>96</sup>



Daidzein	MS	Induced with allergic encephalomyelitis	In vivo: C57BL/6 mice	↓interferon- $\gamma$ & interleukin-12, ↑interleukin-10, suppressed lymphocyte proliferation, ↓cytotoxicity based on lactate dehydrogenase release	Razeghi Jahromi <i>et al.</i> 2014 <sup>89</sup>
Genistein	MS	Induced with EAE	In vivo: C57BL/6 mice	↓neuronal demyelination, suppression of IFN- $\gamma$ production, ↑IL-10 secretion in splenocyte and brain, ↓IL-12 & TNF- $\alpha$ secretion in splenocytes, suppressed the T-cells proliferation ↓the cell cytotoxicity	Jahromi <i>et al.</i> 2014 <sup>97</sup>
Genistein	MS	Induced with EAE	In vivo: C57BL/6 mice	↓inflammatory infiltration and demyelination, ↑expression of TLR3 & TLR9 & IFN- $\beta$ , ↓factors of TH1 & Th17 cells	Dias <i>et al.</i> 2018 <sup>99</sup>
Genistein	MS	Cuprizone-induced demyelination	In vivo: C57BL/6J mice	↓Genes expression levels related to phagocytosis such as CD68 & lysosomal-associated membrane protein 1 in sorted microglia, ↓physical contact of microglia with myelin, the expression levels of myelin-related genes such as myelin basic protein & myelin oligodendrocyte glycoprotein in the whole hippocampal tissue recovered	Ohgomori <i>et al.</i> 2019 <sup>100</sup>
Genistein	Huntington	Transfected with a plasmid bearing mutated HTT gene	In vitro: HEK-293 cells	removes the major pathogenic factor of HD	Pierzynowska <i>et al.</i> 2018 <sup>101</sup>
Genistein	Huntington	Derived from HD patients	In vitro: fibroblasts	↓HTT, disappearance of intracellular mHTT aggregates	Pierzynowska <i>et al.</i> 2019 <sup>102</sup>

Genistein	Huntington	3-NPA-Induced Memory Impairment	In vivo: ovariectomized rats	↓locomotor hypoactivity, ↑retention latencies in the passive avoidance task, ↑ATP, ↑oxidative stress profile, ↓the increase in AChE activity, ↓expression of COX-2 & iNOS	Menze <i>et al.</i> 2015 <sup>103</sup>
Genistein	Huntington	3-NPA-induced HD	In vivo: ovariectomized rats	Improvement in behavioral parameters, ↑ATP, ↓oxidative stress, ↓inflammation & apoptosis	Menze <i>et al.</i> 2016 <sup>104</sup>
Daidzein	Seizure	Audiogenic-induced seizures (AGS)  over-express amyloid β-protein precursor (AβPP) and amyloid beta (Aβ)	In vivo: The Fmr1KO, wild type, FRAXAD mice	seizure induction was recapitulated	Westmark <i>et al.</i> 2013 <sup>118</sup>
Genistein	Brain tumors	-	In vitro: Human glioblastoma multiforme cells A172 (JCRB0228), KNS60 (IF050357), U251MG(KO) (IF050288) and medulloblastoma ONS76 (IF050285)	induces insignificant DNA damage not result in cellular death inhibit telomerase activity induces growth arrest telomerase inhibition suppression of <i>TR</i> - and <i>TERT</i> mRNA	Khaw <i>et al.</i> 2012 <sup>108</sup>
Genistein, daidzein, (s)-equol	Brain tumors	-	In vitro: D283 Med MB cells	↓the susceptibility of MB cells to cytotoxic chemotherapy  significantly protect cytotoxic effect of cisplatin  ↓caspase 3 activity	Belcher <i>et al.</i> 2017 <sup>110</sup>
Genistein	Brain tumors	-	In vitro: GBM cells	bind to DNA-PKcs block the DNA-PKcs/ Akt2/Rac1 pathway inhibit migration of GBM cells	Liu <i>et al.</i> 2021 <sup>111</sup>

Genistein	Pro-inflammatory microvascular environment	stimulated with TNF- $\alpha$	In vitro: HBMEC	↓overexpression of TNF- $\alpha$ and IL-1 $\beta$ proteins, MCP-1, IL-8, and ICAM-1, and ↓transmigration of blood leukocytes	Lee <i>et al.</i> 2008 <sup>112</sup>
Genistein	TBI	duced by Marmarou method	In vivo : Male Albino N-Mary rats	prevent the disruption of BBB, elevation of ICP↓, prevent the disturbance of vestibulomotor, inhibited the disturbance of neurobehavioral performance	Soltani <i>et al.</i> 2014 <sup>115</sup>
Genistein,	TBI	induced by Marmarou method	In vivo: Male Wistar rats	inhibited the disruption of BBB, inhibited the brain edema, increase of ICP↓, inhibited the disturbance of neurobehavioral performance	Soltani <i>et al.</i> 2015 <sup>116</sup>

for treating MS patients who suffer from mental health problems. In addition, memory enhancing and neuroprotective effects for genistein was observed in a rat model of HD. These effects might be attributed to its anti-inflammatory, antioxidant and cholinesterase inhibitory processes.

## DISCLOSURE

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