

Neuroprotective Potential of Silymarin against CNS Disorders: Insight into the Pathways and Molecular Mechanisms of Action

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SUMMARY

Silymarin, a C₂₅ containing flavonoid from the plant *Silybum marianum*, has been the gold standard drug to treat liver disorders associated with alcohol consumption, acute and chronic viral hepatitis, and toxin-induced hepatic failures since its discovery in 1960. Apart from the hepatoprotective nature, which is mainly due to its antioxidant and tissue regenerative properties, Silymarin has recently been reported to be a putative neuroprotective agent against many neurologic diseases including Alzheimer's and Parkinson's diseases, and cerebral ischemia. Although the underlying neuroprotective mechanism of Silymarin is believed to be due to its capacity to inhibit oxidative stress in the brain, it also confers additional advantages by influencing pathways such as β -amyloid aggregation, inflammatory mechanisms, cellular apoptotic machinery, and estrogenic receptor mediation. In this review, we have elucidated the possible neuroprotective effects of Silymarin and the underlying molecular events, and suggested future courses of action for its acceptance as a CNS drug for the treatment of neurodegenerative diseases.

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Introduction

Silymarin, a plant-derived flavonoid from the plant *Silybum marianum* [1,2], is considered the most potential drug to treat almost all kind of liver diseases [3–6], particularly alcoholic liver disease [7,8], acute and chronic viral hepatitis [9–11], and toxins-mediated liver dysfunctions [12,13]. Silymarin is basically a mixture of lignan-derived flavonols, containing mainly silybin followed by silydianin, silychristin, and isosilybin [14–18]. It was first isolated as a mixture from the seed extract of *Silybum marianum* in 1968 [19] and all the constituents were purified [20], and their structures elucidated using techniques like X-ray crystallography and NMR [21]. Since then myriads of research were undertaken to understand the mechanisms of action of different constituents or its mixtures in cellular and animal models, as well as in human subjects. Several studies have reported that oral absorption of Silymarin is about 23–47%, and the peak plasma concentration is achieved in 4–6 h [6,22] while its serum half-life is approximately 6 h [23,24]. However, the bioavailability of

Silymarin in brain is not known yet in spite of the fact that it is shown to be protective against several CNS disorders.

Silymarin is reported to have a good safety profile with no adverse side effects in either humans or animals in high doses [23]. The potential benefit of Silymarin in the treatment of liver disease is associated with its antioxidant property [25] and its ability to block hepatotoxicant binding sites, along with tissue regenerative capabilities [26]. Besides hepatoprotection, Silymarin has recently been reported to be a putative neuroprotective agent against several neurodegenerative diseases including Alzheimer's disease (AD) [27], Parkinson's disease (PD) [28], and cerebral ischemia (CI) [29]. Although, the underlying neuroprotective mechanism of Silymarin is mainly due to its capacity to inhibit oxidative stress in brain [30], it also confers additional neuroprotection by influencing other pathways such as inflammatory pathways [31,32], inhibition of β -amyloid (A β) aggregation [33], apoptotic mechanisms of cell death [29], and estrogenic receptor-mediated pathways of neuronal death [34]. Additionally, Silymarin also exhibits the potential to recover psychomotor and

cognitive abnormalities [35] in animal models. In this review, we have explained the possible pathways of neuroprotective effect of Silymarin and the underlying cellular and molecular events.

Silymarin in Neurodegenerative Disorders

Neuroprotective evidences in support of Silymarin have been documented not only in animal models of neurodegenerative diseases [28,34,36,37], but also in neuronal and non-neuronal cellular models [33,38] of AD, CL, and PD. The *in vitro* and *in vivo* doses, and the routes of application, the preparations used, and time window of treatment of Silymarin, which are very important for its potential clinical purpose are mentioned in Table 1. Surprisingly, reports on the effect of Silymarin on other central nervous system disorders where oxidative stress plays a pivotal role, such as Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis [39,40] are lacking.

Silymarin and Alzheimer's disease

The cognitive impairment and the deposition of extracellular amyloid- β ($A\beta$) fibrils in senile plaques, which are the characteristic features of AD brain [41–43], have been reported to be attenuated by administration of Silymarin [27,33,38]. In an $A\beta$ -induced animal model of AD, the cognitive abnormalities, particularly memory impairment was significantly improved after Silymarin administration [27,36], which is suggested to be due to reduction in oxidative stress and inflammatory responses [27,44]. Additionally, in amyloid precursor protein (APP)-based

transgenic animal model of AD, chronic Silymarin supplementation was reported to recover the characteristic behavioral abnormalities, without causing toxicity to any organs [38]. Reports are also available on the protective effect of Silymarin on inhibition of $A\beta$ fibril formation and aggregation in animal and cellular models of AD [33,38; Figures 1 and 3], which is discussed in later section of the review.

Silymarin and Parkinson's Disease

The characteristic features of PD, particularly the loss of dopaminergic neurons in substantia nigra pars compacta and the motor behavioral abnormalities [45–51] generated by intrastriatal administration of parkinsonian neurotoxin, 6-hydroxydopamine (6-OHDA) was considerably attenuated by treatment with Silymarin [34]. In maneb- and paraquat-induced animal models of PD, Silymarin was also found to be protective against midbrain dopaminergic neuronal loss and associated behavioral impairments [28]. In different toxin-induced animal models of PD [28,35], and even in naive animals [52], Silymarin administration showed substantial increase in dopamine and serotonin levels in hippocampus and cortical regions of brain. Interestingly, Silymarin is reported to inhibit monoamine oxidase-B [53], suggesting additional neuroprotective mechanism of Silymarin to counter the loss of dopamine in PD [54,55; Figure 3]. However, reports on the effect of Silymarin against parkinsonian hallmark pathology, α -synuclein aggregation and Lewy body formation [56,57] are not available. Nevertheless, the molecular mechanism of neuroprotective potential of Silymarin in PD has been mainly attributed to amelioration of oxidative stress [28,34].

Table 1 The administration routes, doses, preparation, and time window of treatment of Silymarin

CNS disorders	Model system	Administration routes	Effective doses	Preparation	Time window of treatment	References
Alzheimer's disease	Mouse	Oral	200 mg/kg	Suspended in 0.3% carboxymethyl cellulose (CMC)	8 days	[27,36]
	Mouse	Oral	1%	Silymarin in normal diet	6 months	[38]
	PC12 cells	Media	100 μ M	In dimethyl sulfoxide (DMSO)	24/96 h	[38]
	SH-SY5Y cells	Media	50 μ M	In DMSO	3 days	[33]
Parkinson's disease	Mouse	Intraperitoneal	40 mg/kg	In DMSO	9 weeks	[28]
	Neuron-glia culture	Media	80 μ M	In DMSO	7/25/49 h	[31]
	Rat	Intraperitoneal	200 mg/kg	In propylene glycol (PEG)	2 weeks	[34]
Cerebral ischemia	Rat	Oral	200 mg/kg	Suspended in a 0.3% CMC	15 days	[29]
	Rat	Intragastric	50/100 mg/kg	Silibinin dissolved in 0.9% NaCl	24/72 h	[32]
	Rat	Intravenous	1–10 μ g/kg	Ethanol and normal saline	24 h	[37]
Ageing	Rat	Oral	200 mg/kg	In 1% (w/v) CMC	7 days	[61]
	Rat	Oral	200/400 mg/kg	Suspended in corn oil	14 days	[30]
	Mouse	Intramuscular	50 mg/kg	In PEG	6 weeks	[86]
Cognitive impairment	Mouse	Oral	100/200 mg/kg	Suspended in 0.3% CMC solution	7 days	[35]

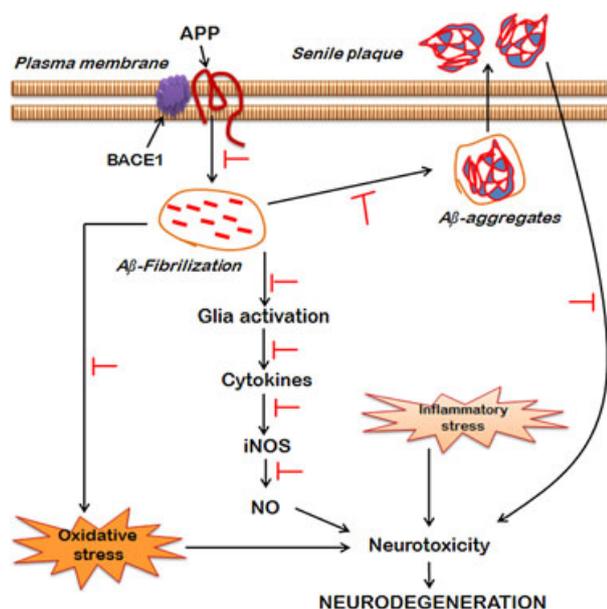


Figure 1 Neuroprotective pathways of Silymarin in Alzheimer's disease (AD). In AD patients' brains, proteolytic cleavage of amyloid precursor proteins (APP) by β -secretase (BACE1) results in the formation of toxic amyloid-beta ($A\beta$) fragments, fragments of which undergo fibrilization as well as oligomerization resulting in the deposition of intracellular $A\beta$ aggregates, and as senile plaques in the extracellular space. Both $A\beta$ fibrils as well as senile plaques mediate neurotoxicity either by enhancing oxidative stress or by exaggerating glial cell-mediated production of inflammatory markers such as cytokines. The cytokines in turn induce the production of nitric oxide (NO) by activating inducible nitric oxide synthase (iNOS). Silymarin potentially hinders the cleavage of APP and thus impede the fibrilization and oligomerization of $A\beta$, and reduces $A\beta$ aggregates and senile plaques. The $A\beta$ -induced oxidative stress and glial cell activation due to overproduction of inflammatory markers has been shown to be inhibited by treatment of Silymarin. Thus, Silymarin, by virtue of its antioxidant and anti-inflammatory properties, confers neuroprotection in AD pathology (T indicates the inhibitory effect of Silymarin).

Silymarin and Cerebral Ischemia

The neuroprotective effect of Silymarin on CI-induced neurochemical alterations including elevated levels of free radicals, nitrite content and inflammatory mediators [58–60], and behavioral abnormalities have been convincingly established in the literature [29,61]. Silymarin showed considerable reduction in cerebral infarct volume and neuronal cell loss in CI [61]. In comparison with commonly used anti-ischemic drugs such as piracetam and protocatechuic acid, Silymarin significantly improved the brain histochemical changes and psychomotor behavior in animal model of CI [61]. Additionally, Silymarin is found to be anti-apoptotic in CI by means of downregulating apoptosis inducing molecules such as p53, apoptotic protease-activating factor 1 (apaf-1), and caspase-9 in an animal model [29; Figure 3]. Silybinin, which is one of the active constituents of Silymarin, has recently been reported to activate Akt/mTOR signaling pathway, and to downregulate the inflammatory marker, NF- κ B and to upregulate the anti-apoptotic marker, Bcl-2 in CI brain [32], thereby suggesting a

novel mode of neuroprotection. The underlying molecular mechanism of Silymarin in CI-induced neurotoxicity is mainly due to downregulation of inflammatory mediators such as inducible nitric oxide synthase (iNOS), myeloperoxidase, cyclooxygenases, NF- κ B and tumor necrosis factor-beta (TNF- β) [37], and upregulation of antioxidant enzymes [29; Figure 2].

Molecular Mechanisms of Neuroprotection by Silymarin

Oxidative Stress and Silymarin

Silymarin has been implicated in protecting neurons against oxidative stress [27,34,44] and nitrosative stress [36; Figure 3]. Silymarin, being a mixture of flavonoids, is reported to exert direct effect on neuronal oxidant status [1,30,44]. Silymarin offsets acetaminophen and manganese-mediated oxidative stress and neurotoxicity in animal models by elevating the activities of both enzymatic and nonenzymatic antioxidant markers [44,62]. Silymarin elicits its neuroprotective effects in manganese-induced neurotoxicity by reducing both lipid and protein oxidation, as well as by activating acetylcholinesterase activity, and inducible nitric oxide synthase gene expression [63]. In animal model of sepsis induced by cecal ligation and perforation, decreased glutathione levels and increase in malondialdehyde content, as well as myeloperoxidase activity in the brain, were reverted by administration of Silymarin [64]. In the hippocampi and the cortices of elderly rodent brain, Silymarin is reported to be neuroprotective against oxidative insults by potentially inhibiting formation of oxygen and peroxy radicals along with protein oxidation products [30]. Silymarin administration in an encephalopathy animal model produced by 4-pentenoic acid, elevated the respiratory activity in brain mitochondria and inhibited lipid peroxidation [65].

Several studies have established the involvement of oxidative stress in $A\beta$ -induced neurotoxicity [66,67; Figure 3]. Silymarin was found to alleviate the cognitive impairment induced by $A\beta$ by preventing the oxidative damage in the hippocampus in terms of lipid peroxidation and glutathione levels [27]. The level of nitrotyrosine has been used as a marker of nitrosative stress [68,69], and Silymarin significantly attenuated the elevation of nitrotyrosine induced by $A\beta$ in the hippocampus and amygdala [36].

β -Amyloid and Silymarin

The potential role of Silymarin against $A\beta$ pathology has been well reported in both *in vitro* and *in vivo* systems. In transgenic mouse model of AD, oligomerization of $A\beta$ induced by over-expression of APP was potentially inhibited by Silymarin [38]. Additionally, administration of Silymarin in animals is also reported to clear the fibrillar $A\beta$ deposits [38]. In the *in vitro* system, $A\beta$ fibrilization and aggregation were reduced significantly after incubation of $A\beta$ peptides with Silymarin [33,38; Figure 3]. It is also shown that Silymarin has the potential to revert $A\beta$ -induced oxidative stress [27,33] and cell viability [38]. The attenuation of $A\beta$ toxicity by Silymarin has been reported to be due to its antioxidative property (Figure 1), but without effecting β -secretase (BACE) [38], which is known to be involved in production of toxic $A\beta$ [70,71].

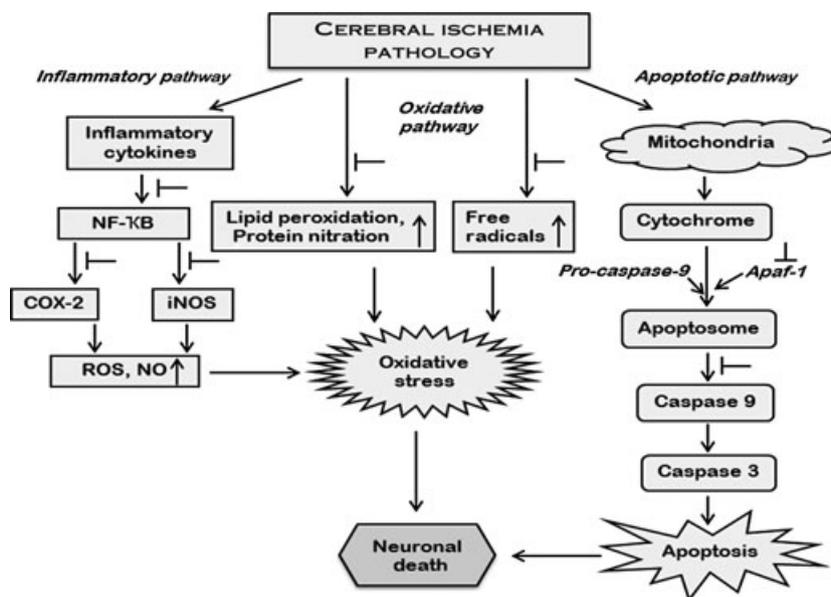


Figure 2 Neuroprotective pathways of Silymarin in cerebral ischemia (CI). The major pathological pathways that are involved in CI include inflammation, oxidative stress, and apoptosis. Silymarin elicits anti-inflammatory property in CI injury by preventing the activation of NF-κB-mediated production of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Silymarin thus by modulating NF-κB reduces both oxidative stress as well as nitrosative stress by inhibiting the generation of reactive oxygen species (ROS) and nitric oxide (NO). In addition, Silymarin also suppresses the generation of free radical-mediated lipid and protein oxidation, having the potential to change the redox state of the cell and hence ameliorate oxidative stress. The activation of intrinsic pathway of apoptosis, which is evident in CI brain, is inhibited by Silymarin, by preventing the formation of apoptosome by inhibiting apoptotic protease-activating factor 1 (apaf-1) that regulates the activation of caspases. Thus, Silymarin has the potential to prevent neuronal loss by inhibiting the oxidative stress and apoptotic mode of cell death (T indicates the inhibitory effect of Silymarin).

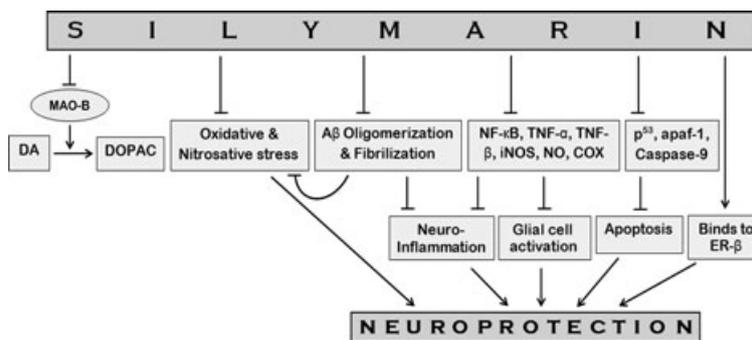


Figure 3 Schematic representation of neuroprotective pathways of Silymarin. The potent herbal antioxidant Silymarin prevents conversion of dopamine (DA) to 3,4-dihydroxyphenylacetic acid (DOPAC) by inhibiting the DA oxidizing enzyme, monoamine oxidase-B (MAO-B). The MAO-B inhibitory action of Silymarin thus leads to lesser degradation of DA and therefore would increase the extracellular concentration of this catecholamine neurotransmitter. Silymarin prevents the formation of amyloid-β (Aβ) aggregates and fibrils; as a consequence, it attenuates Aβ-induced neuroinflammation and cellular stresses. By inhibiting the production of inflammatory agents such as NF-κB, TNF-α, TNF-β, iNOS, NO, COX, Silymarin impedes neuroinflammation and glial activation resulting in increased secretions of trophic factors, leading to neuroprotection. Silymarin also showed anti-apoptotic property as revealed by inhibition of the production of apoptotic proteins, p53 and apaf-1, and reduced caspase-9 activity. Due to estrogen-like activity and binding ability to ER-β, Silymarin may provide additional neuroprotection (T indicates the inhibitory effect of Silymarin). TNF-α, tumor necrosis factors-α; TNF-β, tumor necrosis factors-β; iNOS, inducible nitric oxide synthase; NO, nitric oxide; COX, cyclooxygenase; apaf-1, apoptotic protease-activating factor 1; ER-β, estrogen receptor-β.

Aβ-induced over-expression of inflammatory mediators such as tumor necrosis factor-α (TNF-α) and iNOS mRNA in the hippocampus and amygdala of mouse brain was attenuated by administration of Silymarin [36]. Silymarin by reducing nitrotyrosine level in the hippocampus and amygdala also attenuates Aβ-induced nitrosative stress in animals [36].

Glia and Silymarin

Few reports are also available on the inhibition and prevention of proliferation of glia by Silymarin [31,37,72]. Wang et al. [31] reported that Silymarin administration in a lipopolysaccharide-induced animal model of PD, prevented the dopaminergic neu-

rodegeneration by inhibiting activation of microglia, while other studies reported the inhibition of glial cell activation by Silymarin in cellular models possibly by inhibiting iNOS production [37,72]. Meanwhile, Silybinin has been reported to downregulate the CI-induced inflammation by activating of Akt/mTOR pathway via upregulation of anti-inflammatory markers [32]. Silymarin is also reported to protect both microglia and astroglia from oxidative insults induced by peroxide in *ex vivo* system [72]. However, Silymarin-mediated inhibition of gliosis is suggested to be due to inhibition of NF- κ B activation as well as other inflammatory mediators [31,72; Figure 3], but the exact molecular mechanism is yet unclear.

Involvement of Estrogen Receptor

Estrogen receptor- β (ER- β) is distributed predominantly in hippocampus and cortical regions of rodents brain [73,74], and is known to possess neuroprotective potential when it is activated or upregulated [34,75,76]. Apart from the involvement of ER- β in cognitive processes such as learning and memory [77–80], blockade of ER- β by antagonists has been reported to cause neurotoxicity leading to many diseases, including PD [34], epidemiologically supported by the fact that incidence of this disease in females is significantly low worldwide [81]. Estrogen-mediated neuroprotective effect has been reported to be due to its ability to bind to ER- β [82]. Silymarin administration has been reported to reduce 6-OHDA-induced rotational behavior and nigral neuronal loss in parkinsonian rodents partly by modulating ER- β [34]. One of the underlying mechanisms of Silymarin-induced neuroprotective effect may be due its estrogen-like activity [75,83], as well as its potential to bind and activate the ER- β [34,75,84,85; Figure 3].

Silymarin: Unknown Terrains

Although Silymarin has shown promising neuroprotective potential, still there are some lacunae in understanding the science of this mixture of flavonoids. The reported inhibitory potential of Silymarin on protein deposits formation in AD is not clearly understood yet. It will be exciting to know how Silymarin modulate A β fibrilization without effecting BACE that cleaves APP in AD. Similarly, it may be expected that Silymarin might have the potential to inhibit α -synuclein aggregation and resultant Lewy body forma-

tion in PD. Therefore, extensive research needs to be initiated to understand the mechanisms of macromolecular crowding in neurons and the effects of Silymarin. Another avenue that needs to be looked into is how Silymarin confer neuroprotection by interacting with ER- β receptor. Although Silymarin is a flavonoid and generally flavonoids can traverse the blood–brain barrier, yet no confirmed reports are available on the mode of transport and bioavailability of Silymarin in brain. Hence, there is a greater need to search into these avenues to understand the true picture of Silymarin-mediated neuroprotection.

Conclusions

The present article concisely reviews the antioxidant, anti-apoptotic, anti-inflammatory and enzyme inhibitory activities of Silymarin and shows how use of this molecule could provide protection of neurons against oxidative insults in the brain under distress. The neuroprotective nature of Silymarin seems to be unique as its mode of action is diverse ranging from a general antioxidant nature to specific anti-amyloidogenic, anti-inflammatory, and pro-estrogenic properties. These diverse neuroprotective actions of Silymarin on brain hold great promise to be a “wonder drug” for the treatment of neurodegenerative disorders. The nontoxic nature of this molecule warrants its urgent clinical evaluation for its potential use as an antineurodegenerative molecule in humans. However, its bioavailability in brain, including its ability to penetrate blood–brain barrier is to be established in preclinical studies, prior to any clinical trials.

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Conflict of Interest

The authors declare no conflict of interest.

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