Phytosterols and its neuroprotective effect – an updated review

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DOI:10.48047/ecb/2023.12.si4.701

Abstract

In this article the beneficial effect of phytosterols in the management of neurodegenerative disorders have been discussed with respect to anti-inflammatory effect and antioxidant effect of phytosterols. Regarding the anti-inflammatory effect of phytosterols it is found that they regulate Apolipoprotein-E which activates microglia and regulate PPAR which in turn is necessary for synthesis of interleukins. Phytosterolsexhibit antioxidant effect by eliminating reactive oxygen species (ROS) and multiplying enzymatic antioxidants. A special mention about how phytosterols are useful in Alzeimer's disease has been discussed in this article. Phytosterols inhibit

the formation of amyloid-beta as it arrests the cleavage of amyloid precursor protein (APP) by β-Secretase and prevent the formation of toxic oligomers**.** Thus this review is a consolidated collection of information regarding the therapeuticpotencies of phytosterols in the area of neurodegenerative disorders.

Key words – Apolipoprotein-E, Peroxisome Proliferator- Activated Receptor (PPAR), Amyloid Precursor Protein (APP), Alzeimer's disease

Introduction:

There are billions of neurons in our brain and infinite number of involuntary electrical activity is happening in a nonterminating fashion within our brain. There exists a wide range of chemical messengers between the synaptic junctions of the nerves showcasing their inherent potency. Function of the brain is the algorithm of the functional units – the neurons. Like any other cell in our body neurons are also subjected to wear and tear process due to various reasons. The main etiological factor is neuroinflammation which can precipitate in neurodegeneration.

Some of the neurodegenerative disorders include Alzeimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic Lateral Sclerosis.Genetic inheritance, Environmental factors or Interaction of both may be the etiology for neurodegenerative diseases.Globally prevalence may reach 2 % in persons aged 65 years & older [1]. There is an increasing trend seen recently with preponement of age. In India, 30 million people suffer from neurological disorders with an average of 2394 per 1 lakh population [2].There is a wide range of allopathic medicines to treat symptomatically the signs of neurodegenerative disorders but not without side effects. Nowadays extensive research has been conducted in the field of phytochemistry to discover newer compounds from nature which can be useful in the field of management of these diseases. Here in this review article we like to discuss the medicinal value of phytosterols emphasising the neuroprotective effects of these compounds.

PS are existingubiquitiously in many plant foods. Phytosterols are bountifully present in unrefined plant oils, including vegetable, nuts and olive oils, sesame, safflower, soybeans, peas, and almonds. They are also present in nuts, seeds, whole grains, and legumes [3]. Nature expresses 250 kinds of phytosterols in plants. Phytosterols exists in saturated and unsaturated forms which are known as stanols and sterols respectively (Piironen et al., 2000). Commonly existing plant–derived sterols in human diet include β-sitosterol, campesterol, and stigmasterol. The core skeleton of the phytosterols consists of cholesterol and variation in the side chain decides the molecule. β-sitosterol and stigmasterol have an ethyl group at C-24, whereas campesterol is equipped with a C24-methyl group. C-22 desaturases is required to derive stigmasterol from sitosterol. Brassicasterol, and D-7-avenasterol are fractional constituents. Only 10% of total dietary phytosterol (PS) is constituted by stanols. (Jones and AbuMweis, 2009).

Recently, quite reasonable neuroprotective effects of Phytosterols derived from marine origin were brought to the limelight in a review article by Schepers et al. (2020). The authors candidly indicated the ability of fucosterol, 24(S)-saringosterol, sitosterol, and stigmasterol to inhibit Aβ plaque formation; the ability of fucosterol and 24(S)-saringosterol to rectify memory deficits; the ability of fucosterol to raise the acetylcholine levels in the brain; and the ability of 24(S)-saringosterol to favor Aβ clearance.[4]

Biosynthesis of phytosterols

The biosynthesis of phytosterols in plants consists of three major steps. As highlighted in Zhang et al.

Step one involves the mevalonate or isoprenoid pathway: In the biosynthesis of isoprenoids an intermediate called mevalonate is formed from acetyl -CoA.

Step two - cyclization of squalene and its subsequent conversion to 2,3-oxidosqualene in which various triterpenes and the phytosterols (lanosterol and cycloartenol) are its sequelae

Step three, cycloartenol is converted to 24-methylene cycloartenol by the action of C-24-sterol methyltransferase 1 (SMT1). As shown in Figure 1, the production of the 24-methylene cycloartenol would lead to the biosynthesis of the major plant sterols β-sitosterol, stigmasterol, and campesterol.[5]

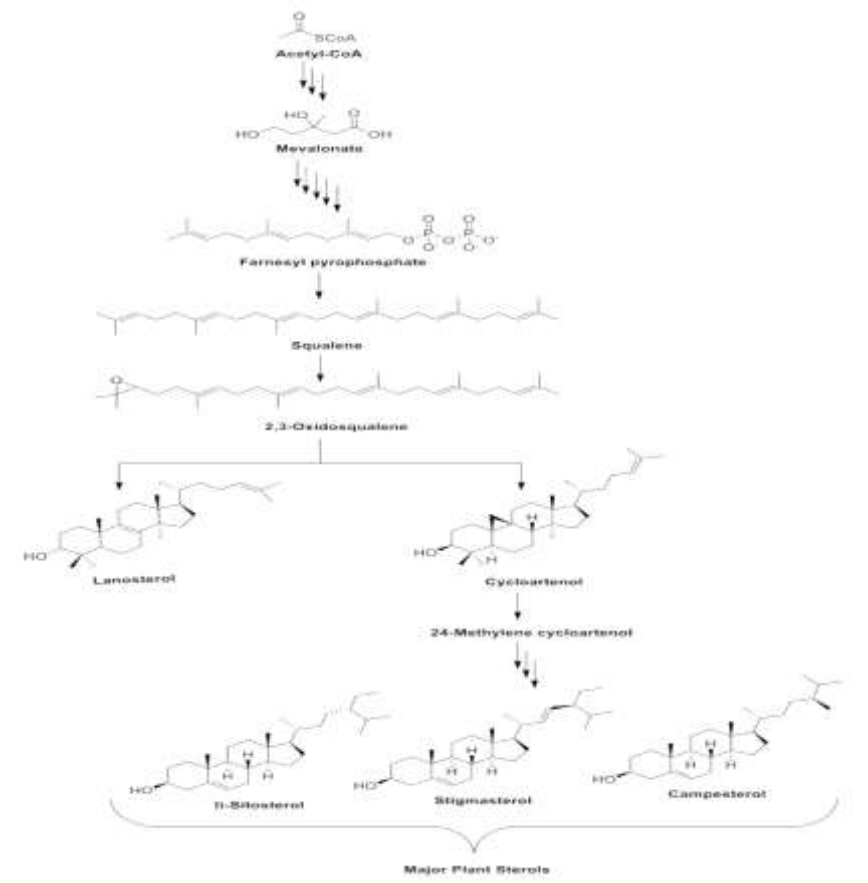


Figure 1

A simplified sketch of the biogenerative pathway of the major phytosterols in plants.[5]

Anti-inflammatory effects of phytosterols

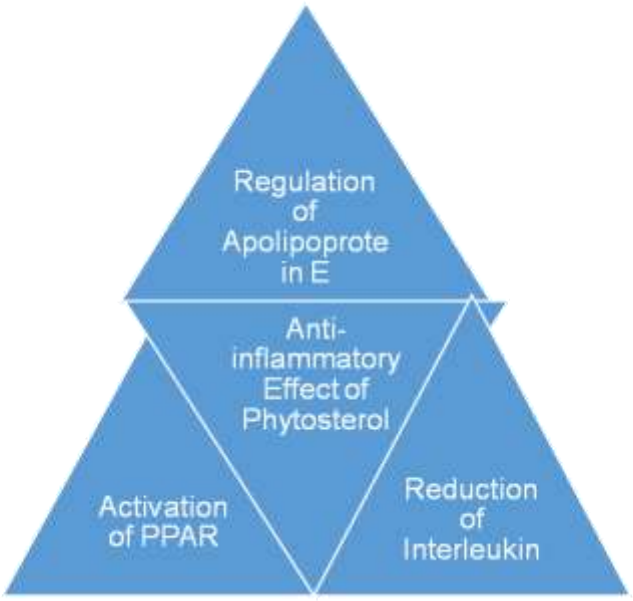
Inflammation occurs due to synchronous participation of various cytokines in a programmed pattern. The immune response to a foreign object or a parasite or body's own cells results in inflammation. Loss of function is one of the cardinal signs of inflammation along with redness, swelling, pain and heat. Neuroinflammation is an innate immune response due to various reasons like trauma, aging, neurodegenerative diseases etc. It has been deciphered that activation of microglial cells due to neuroinflammation is the primary root-cause for neurodegenerative disorders like Alzeimer's disease as the activated microglial cells lose their inherent capacity to phagocytose amyloid deposits. In the similar way neuroinflammation in the cells of substantia nigra results in dopamine deficiency precipitating Parkinson's disease. The failure of intactness of blood brain barrier due to the release of cytokines in the process of inflammation has allowed the transit of B cells and plasma cells into the brain which can destroy the myelin sheath of the nerves. Demyelination is one of the cardinal signs of multiple sclerosis. Thus, neuroinflammation is the causative factor behind neurodegenerative disorders like Alzeimer's, Parkinson's disease and Multiple sclerosis.

PSs tune up the inflammatory process; they possess therapeutic benefits which include antioxidant, antiulcer, immunomodulatory, antibacterial, and antifungal effects; and also facilitate wound healing and platelet aggregation inhibition [4]. The macrophage- and neutrophil-mediated inflammatory responses are decelerated by phytosterols. *In vivo* studies in mice have indicated that PSs can induce a cytokine mediated immune response- Th1 response. Oedema and proinflammatory cytokine concentrations are reduced (Nashed et al., 2005; Brüll and Mensink, 2009). Production of proinflammatory cytokine, namely, IL-6 and tumor necrosis factor (TNF)-α, found a low titer and an increase in indicator Th1/Th2 ratio [6].

The anti-inflammatory action of various phytosterols are substantiated and are known to terminate synthesis and stimulation of chemical messengers nitric oxide (NO), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and phosphorylated extracellular signal-regulated protein kinase (p-ERK) [7]. Hence, phytosterols may possess therapeutic implications in neurodegenerative diseases.

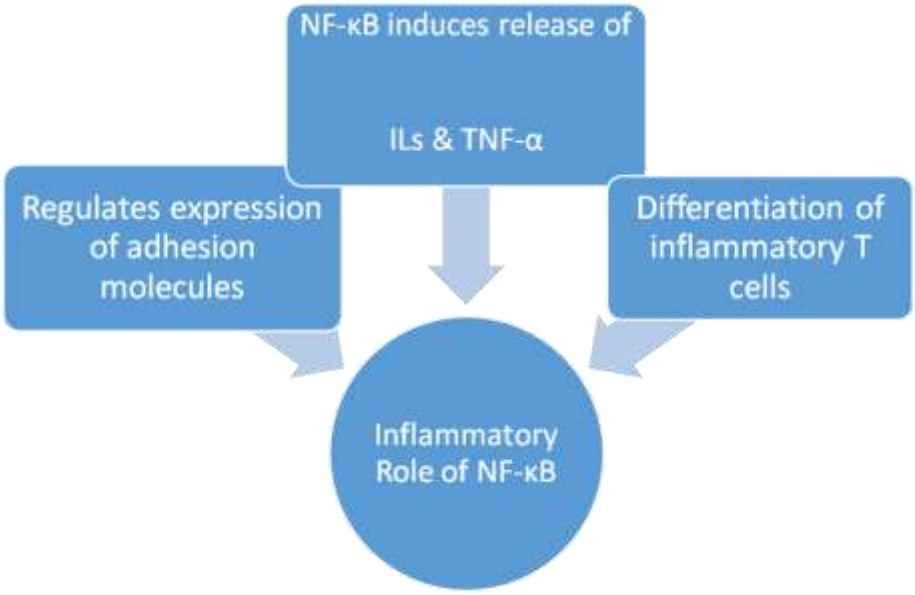
The structural integrity of the phytosterols makes it accumulate within the brain and gets it incorporated within the neuronal cell membrane and ameliorates the inflammatory process within the brain [8]. The regulation of apolipoprotein E and activation of peroxisome proliferated activated receptor (PPAR) by phytosterols along with reduction in interleukins like IL-18 seems to be responsible for its anti-inflammatory effects [9]. PPAR regulates inflammation by monitoring the expression of genes involved in inflammation [10]. Phytosterol activates PPAR and thus regulates inflammation. Apolipoprotein E seems to possess anti-inflammatory role in CNS through its ability to modulate beta amyloid induced glial activation [11]. There is a

direct link between ILs like IL-18 and NFκB which is involved in the expression of adhesion molecules and acts as mediator for the process of chemotaxis involved in inflammation [12]. Reduction of interleukins by phytosterols thus produces anti-inflammatory effects.



The anti-inflammatory effect of an amalgam of sitosterol (60%), campesterol (25%), and stigmasterol (15%) has been demonstrated by its ability to form a stumbling block against demyelination of neurons in an autoimmune encephalomyelitis (EAE) model of multiple sclerosis [13]. Phytosterols seem to promote myelination by incorporation of a protein -PLP into the myelin sheaths and inhibits the activity of inflammatory cytokines.

The expression of pro-inflammatory markers, such as interleukin-6 (IL-6), inducible nitric oxide (iNOS), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2) was suppressed by β-sitosterol in BV2 cells, thus exhibiting its anti-inflammatory activity. It also has the potency to inhibit the phosphorylation and degradation of inhibitor of nuclear factor kappa B (IκB) and inhibited the phosphorylation of nuclear factor kappa B (NF-κB) which are key molecules to regulate various cytokines in inflammatory pathway [14]. NF-κB initiates the inflammatory response by inducing the synthesis and release of proinflammatory cytokines like interleukins and TNF-α. This also regulates the expression of adhesion molecules which is crucial for adhesion of WBC at the sites of inflammation. This factor is also necessary for differentiation of inflammatory T cells mediated by cytokines like interleukin -12 [15].



Stigmasterol has disturbing impact in the IL-1β-stimulated NF-κB inflammatory pathway as it arrests many pro-inflammatory cytokines in IL-1β-treated cells without influencing IL-6 levels. [16]

Antioxidant effect of Phytosterols

Oxidative processes are happening continuously involving various moieties at a definite rate. Uncontrolled oxidative processes are the causative factor for a myriad of diseases including neurodegenerative diseases**.** The reactive oxygen species generated during oxidative stress activates signaling pathways that trigger microglia and astrocytes which releasesproinflammatory cytokines. Thus oxidation and neuroinflammation are interlinked processes where one leads to the other. Neurodegenerative disorders are fruits of these processes.

Certain phytosterols exhibit free radical scavenging property and thus possess inherent antioxidant nature.

β-Sitosterol enacts the role of free radical eliminator by regulating glutathione levels [17,18]. In RAW 264.7 macrophage culture study phorbol esters influences the glutathione/oxidized glutathione ratio in reverse direction producing a defect. β-sitosterol normalizes the ratio substantiating its antioxidant effect [18]. These data can be correlated with a decent hike seen in antioxidant enzyme factors namely manganese superoxide dismutase and glutathione peroxidase activities. Antioxidant potency of β-sitosterol had also been proved by studying the estrogen/phosphatidylinositol 3-kinase pathway [17]. Furthermore, β-sitosterol being a lipophilic compound gets incorporated into the cell membranes and nullifies the oxidative injury caused by glucose oxidase and lipid peroxidation. Thus the neurodegenerative damage produced by oxidative insults in the brain and CNS can be attenuated by β-Sitosterol.

Normal physiological equilibrium seems to fluctuate when the yield of reactive oxygen species reaches beyond the endogenous antioxidant threshold levels.

In Wistar rats the mushrooming levels of liver lipid peroxides induced by 1,2-Dimethyl Hydrazine were reduced by β-sitosterol treatment. The DMH induced deprivation of enzymatic antioxidants in colonic and hepatic tissues of experimental animals was overcome by β-Sitosterol. Levels of nonenzymatic antioxidants (vitamin C, vitamin E, and glutathione) also shooted up. The compound normalized the histopathological changes caused by DMH [19].

The detrimental effect of ROS induced by glutamate was obtunded by Stigmasterol by inhibition of reactive oxygen species (ROS). An oxidative stress model was created in SH-SY5Y neuroblastoma cells by using H2O2 to investigate the possible neuroprotective mechanisms of stigmasterol. Apoptosis seems to be the causative factor to hike the levels of ROS. Oxidative processes induced cell death was declined in the pretreatment group with stigmasterol based on its ROS cleansing property. It also results a peak in levels of catalase, fork head box O (Fox O)3a, and induces the expression of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) in the neurons thus demonstrating its antioxidant potential [20].

Table 1
Neuroprotective Effects and Antioxidant Effects of Phytosterols

S.No.	Type of study	Phytosterol	Model	Inference
1.	Invivo study	β-Sitosterol	APP/PS_1 mice	Decreased expression of amyloid β (Aβ), BACE 1. Improvement of spatial learning, Recognition of memory ability, Reverses dendritic spine loss in mice [21]
2.	Invitro study	Stigmasterol	SH-SY5Y cells	Decline in reactive oxygen species in cells, Rise in antioxidant enzymes like catalase and increase in expression of anti-apoptotic protein Bcl-2 [20].
3.	Invitro Invivo Behavioural study	β-Sitosterol	Transgenic mice model	IC_{50} value – 55 µg/ml against AChE and 50 µg/ml against BChE Free radical load in the brain tissues declined in the treated group

				Marginal and slow progress in working memory, Spontaneous alternation behaviour& motor coordination [22]
4.	Invivo study	β-Sitosterol	Wistar Albino Rats	Decrease in elevated levels of liver lipid peroxides Increase in levels of enzymatic antioxidants – catalase, superoxide dismutase and reduced glutathione [19]
5.	Invivo study	β-Sitosterol	Wistar Albino Rats	Downregulation of IKκB/NFκB and c-Jun-N-terminal kinase (JNK) signaling pathway. Decreased gene expression of IL-6, TNF-α [23]
6.	Invitro study	β-Sitosterol	HT 22 mouse hippocampal cell line	Increased availability of soluble neuroprotective soluble amyloid precursor protein – s APP α than amyloid β-Aβ [24]
7.	Invitro study	β-Sitosterol	BV2 cells	Suppression of pro-inflammatory markers and activation of NFκB[14]

Phytosterols in Alzeimer's Disease

Alzheimer's disease (AD) is the most common form of dementia, characterized by progressive memory loss, cognitive impairment, and behavioral complications. Patho-physiologically, AD is characterized by excessive amyloid-*beta* (Aβ) peptide aggregation, intracellular neurofibrillary tangles, highly phosphorylated tau protein, deficiency of essential neurotransmitters, and oxidative stress-induced neuronal damage [25].

Several studies have been conducted to analyze whether the phytoconstituents present in plants have the inherent potential to inhibit Aβ deposits or possess anticholinesterase property or demolish free radicals. Cholesterol lowering effect is seen with phytosterols which may rectify the effects of Alzeimer's disease. Blood

brain barrier is an intact barrier which allows the movement of lipophilic molecules within the brain. Phytosterols being lipophilic have the ability to navigate through the blood–brain barrier (BBB) and get accumulate in the brain [26]. They have the inherent potency to modulate the various neuromodulatory pathways operating within the brain and can attenuate the neurodegenerative processes within the brain.

There is a positive correlation between cholesterol homeostasis and amyloidogenesis. High cholesterol levels are detected by cellular cholesterol sensor APP- Amyloid Precursor Protein which has the tendency to form complex with cholesterol and initiate amyloidogenesis. Phytosterols seems to inhibit Aβ and β-secretase which plays a vital part in amyloid deposition. Inside the brain, A-β plaque clearance is escalated and neuroinflammation is decreased by the neuroprotective characteristics of phytosterol, increasing re-myelination besides reducing neuro inflammation and BACE 1 activity [27].

In HT22 mouse hippocampal cell line study substitution of cholesterol with β-sitosterol resulted in the genesis of neuroprotective soluble amyloid precursor protein – s APP α than amyloid β-Aβ suggesting the beneficial role of this compound in nullifying the effects of Alzeimer's disease [24]. Phytosterols are known to arrest disease progression and decelerate motor impairment in animal models. The incorporation proteolipid protein (PLP) into myelin membranes promotes myelination of neurons and amplifies the density of oligodendrocytes[9].

Genetic expression of apolipoprotein E in individuals seems to carry high risk for Alzeimer's disease [28]. Phytosterols having the potency to regulate apolipoprotein E are one of the potential compounds in the management of Alzeimer's disease.

Phytosterols exhibit a multitarget methodology to improve symptoms of AD.

(A) Phytosterols inhibit conglomeration of amyloid – beta as it arrests the cleavage of the amyloid precursor protein (APP) by β-secretase (BACE-I). This causes a deviation in the non-amyloidogenic pathway and declines the levels of Aβ produced.

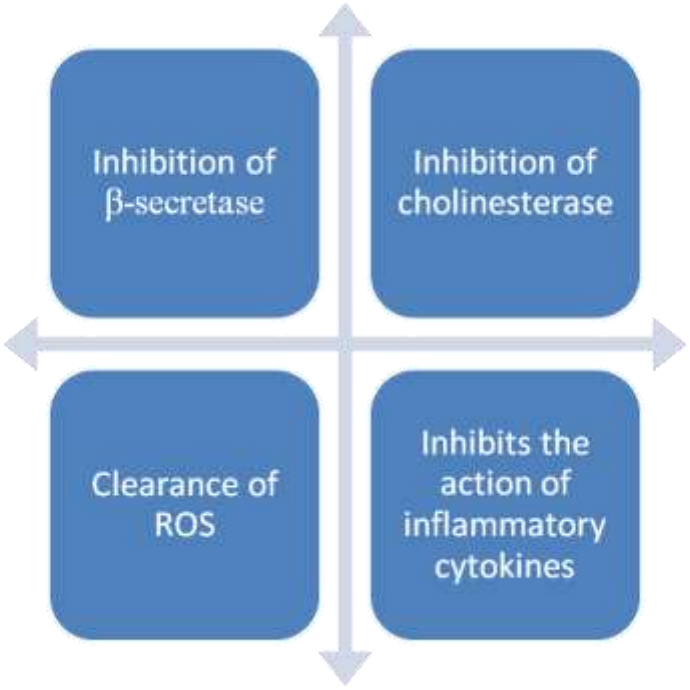
(B) Aβ can self-aggregate to form oligomers and eventually amyloid plaques. Some phytosterols are able to inhibit the formation of amyloid plaques by binding to Aβ, inhibiting aggregation, and thereby facilitating the formation of nontoxic oligomers. Microglial activation and proliferation are induced by toxic Aβ monomers and oligomers. Activated microglia induce inflammation by secreting pro-inflammatory cytokines such as IL-1β and IL-6.

(C) Lipid peroxidation is controlled to certain extent by some phytosterolsthus suppressing oxidative stress. They also increase the level of antioxidant enzymes.

(D) In Alzeimer's disease there is a trough in the concentration of autonomic neurotransmitter acetyl choline which has an influencing role in the processing of learning and memory. Declined levels of ACh can be rejuvenated by anticholinesterase activity of different phytosterols.

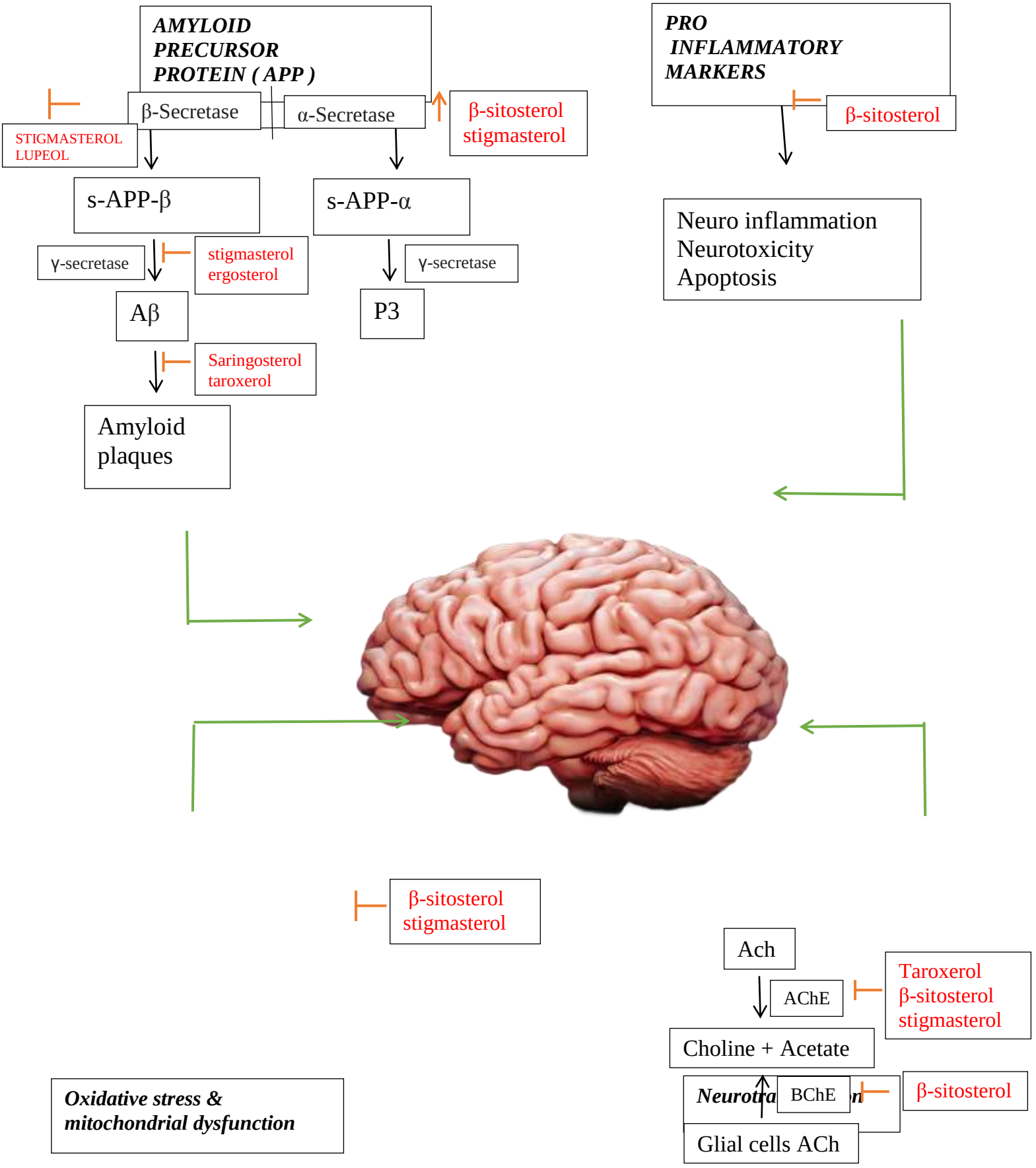
(E) Oxidation of DNA occurs irreversibly by ROS and are vital reasons for Aβ-induced neuronal cell death in the development of AD [9]

Neuroprotective action of phytosterols

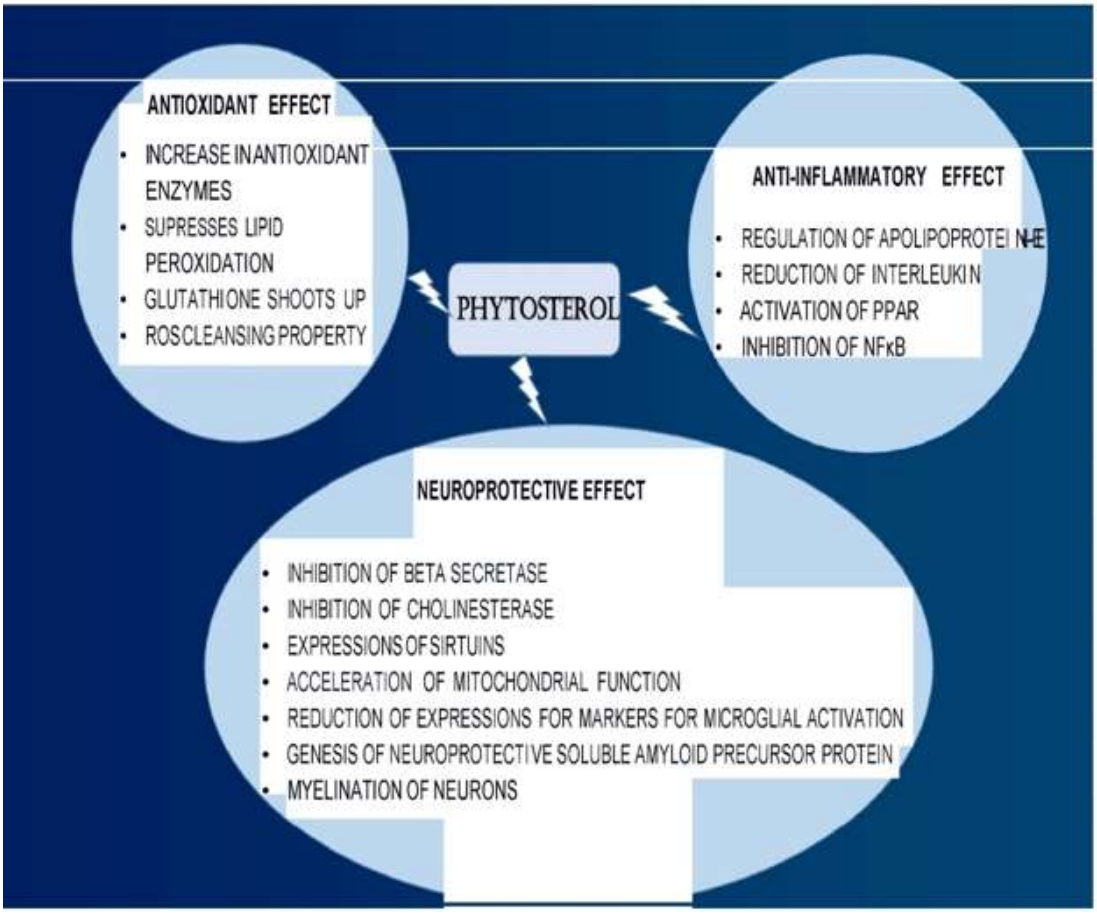


In another study it was found that there was an increase in pyramidal cells and a decrease in astrocytes reflecting neuroprotective potential of phytosterols in rat model. It was also detected that cholinergic transmission was stimulated by phytosterols as cholinesterase activity was inhibited at the cerebral cortex and cholineacetyl transferase activity was stimulated at the hippocampus and cerebral cortex by them. Thus, the cognitive deficit in Alzeimer's disease can be overcome by phytosterol esters [29].

Degenerative processes occur in the CNS due to turbulences in the mitochondrial membrane potential. To reverse the effects of AD mitochondrial function has to be kindled by increasing the mitochondrial membrane potential ($\Delta\Psi m$) and mitochondrial adenosine triphosphate (ATP). Integration of β-sitosterol into mitochondrial membrane accelerated the mitochondrial function thus sanctioning inner mitochondrial membrane fluidity, thereby enhancing the $\Delta\Psi m$ and ATP concentrations. Hence, β-sitosterol could be a useful dietary therapy for neurodegenerative diseases such as AD [30]


AMYLOID PRECURSOR PROTEIN (APP)
β-Secretase
α-Secretase
STIGMASTEROL
LUPEOL
β-sitosterol
stigmasterol
s-APP-β
s-APP-α
γ-secretase
stigmasterol
ergosterol
γ-secretase
Aβ
P3
Saringosterol
taroxerol
Amyloid plaques
PRO INFLAMMATORY MARKERS
β-sitosterol
Neuro inflammation
Neurotoxicity
Apoptosis
β-sitosterol
stigmasterol
Ach
AChE
Taroxerol
β-sitosterol
stigmasterol
Choline + Acetate
Neurotra
BChE
β-sitosterol
Glial cells ACh
Oxidative stress &
mitochondrial dysfunction

The invitro inhibition of acetyl choline esterase by another phytosterol – Stigmasterol and its potency to reduce β-secretase activity prevented the construction of amyloid deposits [31]. It also reduced the expression of all γ-secretase components. A decrease is observed in the cholesterol and presenilin distribution in lipid rafts implicated in amyloidogenic APP cleavage. It caused a decline and the BACE1 internalization to end [9]. In another in-vitro study, Stigmasterol also increased the expression levels of sirtuin 1 (SIRT1) and decreased the levels of acetylated lysine [32]Sirtuins are highly protected NAD (+)-dependent enzymes that have therapeutic benefits in age-related neurodegenerative diseases. In vitro and in vivo studies revealed that the increased expression of SIRT1 protein ameliorated AD-like symptoms by reducing the decline in memory [33]. Similarly, APP metabolism was induced by α-secretase through activation of SIRT1 thus minimizing amyloid Aβ aggregations [34]. Moreover, manifestation of SIRT1 guarded SH-SY5Y cells from toxicity-induced cell death [35].
Transcriptomic analysis implicated the unique role of stigmasterol in upregulating genes involved in neuritogenesis (Map2,Dcx,Reln) and synaptogenesis (Arc,Egr1,Nr4a1), thus forming a scaffold for neuronal architecture in primary hippocampal neurons which processes memory and learning. Stigmasterol also caused a decline in the expression of potassium ion transport genes to sustain neuronal excitability under unfavourable conditions [36]. Thus, the wide spectrum of action has made stigmasterol a potential therapeutic agent for the prophylaxis and management of brain malfunctions, especially AD.
Saringosterol also reduced the in vivo expression of ionized calcium-binding adapter molecule 1 (Iba1), a marker for microglial activation and inflammation. It also hindered the accumulation of Aβ plaques in the hippocampus and prevented the precipitation of cognitive decline and memory loss in an in-vivo mouse model.
Taroxerol has the potential to inhibit anticholinesterase activity in an in-vivo study and it has also been found in in-silico study that it has high affinity for amyloid fibrils and Aβ plaques.
Lupeol exhibited BACE-I inhibitory activity and thus prevents accumulation of amyloid β plaques. Ergosterol inhibits β and γ secretase activity which regulates amyloid metabolism [9].



Conclusion

Phytosterols are definitely a boon to therapeutics in man. Extensive research is the need of the hour and its benefits are to be extrapolated to the entire humans so that it can improve the general health and wellbeing of the individual. Neuroprotective effects of phytosterols are being heavily investigated and its application in management of neurodegenerative disorders is to be established by multicentric trials in near future.

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