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Review

Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects

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ABSTRACT

Background: Acetylcholinesterase (AChE), a serine hydrolase, is primarily responsible for the termination of signal transmission in the cholinergic system, owing to its outstanding hydrolyzing potential. Its substrate acetylcholine (ACh), is a neurotransmitter of the cholinergic system, with a predominant effect on motor neurons involved in memory formation. So, by decreasing the activity of this enzyme by employment of specific inhibitors, a number of motor neuron disorders such as myasthenia gravis, glaucoma, Lewy body dementia, and Alzheimer's disease, among others, can be treated. However, the current-available AChE inhibitors have several limitations in terms of efficacy, therapeutic range, and safety.

Scope and approach: Primarily due to the non-compliance of current therapies, new, effective and safe inhibitors are being searched for, especially those which act through multiple receptor sites, but do not elicit undesirable effects. In this regard, the evaluation of phytochemicals such as flavonoids, can be a rational approach. The therapeutic potential of flavonoids has already been recognized agaisnt several ailments. This review deals with various plant-derived flavonoids, their preclinical potential as AChE inhibitors, in established assays, possible mechanisms of action, and structural activity relationship (SAR).

Results and conclusions: Subsequently, a number of plant-derived flavonoids with outstanding efficacy and potency as AChE inhibitors, the mechanistic, their safety profiles, and pharmacokinetic attributes have been discussed. Through derivatization of these reported flavonoids, some limitation in efficacy or pharmacokinetic parameters can be addressed. The selected flavonoids ought to be tested in clinical studies to discover new neuro-therapeutic candidates.

1. Introduction

Acetylcholinesterase (E.C.3.1.17) is an acetylcholine hydrolase enzyme with esterase activity. It plays key role in neural functioning *via* the cholinergic pathways. Acetylcholinesterase (AChE) was isolated from Pacific electric ray (*Torpedo californica* (*Tc*AChE)) and its 3D structure was elucidated in 1991. This enzyme is mainly localized in the synaptic gaps of the central and peripheral nervous system, and on red cell membranes. It terminates nerve impulses by catalyzing acetylcholine (ACh) hydrolysis [1,2]. ACh is a neurotransmitter of cholinergic system, which mediates an array of functions, including cognition [3]. Botox (Botulinum toxin), an exotoxin (botulinum toxin type A) from the bacterium *Clostridium botulinum* can bock the release of ACh from the cholinergic nerves, which prevents the local neural transmission and suppresses the muscles. While it can cause muscle paralysis, Botox is harnessed for cosmetic (to suppress facial lines, creases, wrinkles) and therapeutic (headache, spasmodic dysphonia (laryngeal dystonia/ voice tremor), masticatory myalgia (a type of temporomandibular joint dysfunction), cervical dystonia (spasmodic torticollis or chronic neck pain), sialorrhoea (hypersalivation or drooling or excessive salivation), trigeminal neuralgia (vocal tics), Frey's syndrome (gustatory sweating or gustatory hyperhidrosis), strabismus (misalignment of the eyes)) purposes. Inhibition of the enzyme AChE by specific inhibitors is the therapeutic target to manage disorders such as myasthenia gravis, glaucoma, Lewy body dementia and Alzheimer's disease (AD) [4,5].

However, the accumulation of ACh leads to pathologies as well.

Abbreviations: SAR, structural activity relationship; AChEI's, acetylcholinesterase inhibitors; Ach, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease

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Overstimulation of the ACh receptors (nicotinic and muscarinic receptors) in synapses leads to neural diseases like depression, dizziness, headache, nausea, difficulty breathing and sudden death [6].

Enzyme inhibitors are of great importance in diverse areas of disease management [7,8]. Clinically, acetylcholinesterase inhibitors (AChEIs) are used for the treatment of these disorders, which increase cholinergic functions, by elevating ACh quantity in cholinergic synapses [9]. In 1932, AChEIs were used for the first time in myasthenia gravis, a neuromuscular disease, that causes weakness in the skeletal muscles. These inhibitors were officially approved in 1938, for the treatment of myasthenia gravis patients [10]. Later in 1960, antibodies were produced against ACh receptors [11,12]. In glaucoma, a disease that damages optic nerves, AChEIs were used for the structural protection of retinal ganglion cells from the ocular hypertension. These drugs lowered the intraocular pressure, protected the vessels and increased ocular blood flow [13,14]. AChEIs are also used for the symptomatic treatment of Alzheimer's disease (AD), one of the most common forms of dementia, characterized by the deposition of amyloid plaques [15], resulting due to the loss of ACh functions in the brain, mostly in the geriatric populace [16]. Even though there are no available treatments that can stop or reverse AD progression, several compounds have been designed and approved in order to inhibit AChE breakdown in the brain that can increase the activity of ACh, and moderate AD symptoms [14,17].

Galantamine, a powerful AChEI, was the first compound isolated from a plant source [18,19]. Nowadays, the most commonly-used synthetic AChEIs are physostigmine, tacrine and donepezil [20–23]. However, these inhibitors are associated with a number of adverse effects such as hepatotoxicity and gastrointestinal complaints [24]. Like almost all components of the body, AChE is a double-edged sword [25,26]. It is the root cause of a gamut of pathologies, but it is vital for the functionality of neural system. So, developing compounds that can completely inhibit AChE, that will not hamper other metabolic pathways is almost infeasible [27]. For this purpose, a number of plant extracts have been examined for their potential to counteract metabolites mediating cognitive disorders and neurodegenerative diseases. Piperine, an alkaloid in *Piper nigrum* is protective of the rat hippocampus, by lowering the AChE level [28]. Several of the eburnane-type alkaloids have exerted AChEI activity, apart from cytotoxicity, anticancer, antiplasmodial, and antibacterial effects. The glycosides, E-harpagoside and 8-O-E-p-methoxycinnamoylharpagide, from the plant *Scrophularia buergeriana*, exert anti-AChE effect, which improved cognition [29]. The essential oil components such as eugenyl angelate, 2-methylbutanoate and 3-methylbutanoate, showed AChEI activity against brine shrimp. *Achillea falcata* L. essential oil components such as trans-sabinol and its esters also exerted AChEI effect in shrimp model [30,31].

AChEI are critical for managing excess AChE, but they are perilous as well [32]. Chemical warfare nerve agents are AChEI, and so are the organophosphorus pesticides [33] Figs. 1 and 2(A and B) show the mechanism of AChE function and inhibitions.

The purpose of this review is to identify new safe, effective and economical AChE inhibitors, among non-alkaloid compounds, out of which flavonoids are emerging as promising candidates. Here, we have discussed the relevance of plant-derived flavonoids with preclinical activity, mechanisms of action, and structural activity relationship (SAR), that might to lead to novel effective AChE inhibitors.

2. Flavonoids- a significant natural remedy

Flavonoids are a heterogeneous group of polyphenolics, that occur in all parts of the plants, and are quintessential components of most of plant-based foods [34]. Flavonoids occur in subclasses such as flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, and isoflavones [35]. Well-characterized flavonoids include coumestrol, kaempferol, caffeic acid, chlorogenic acid, cinnamic acids, coumarin, catechin, quercetin, hesperidin, naringin, apigenin, luteolin, rutin, casticin, myricetin, luteolin, procyanidins, epigallocatechin gallate, and anthocyanidin *etc.* Flavonoids have also been considered as health-promoting agents with proven *in vitro* and *in vivo* biological effects [36], which include nephroprotective [37], anti-arthritic [38], antidepressant [39] antibacterial, antioxidant [40,41], anticancer and antitumor [42–45], anti-ischemic [46], analgesic and anti-inflammatory [47,48]. The therapeutic effects emerge from their influence on hormones *via* enzymes such as aromatase (a cytochromes P450 enzyme), mitogen-activated protein kinase (MAPK) such as p38, JNKs (Jun n-terminal kinases) and ERK1/2 (extracellular signal-regulated kinases 1/2), among other enzymes.

3. Flavonoids as acetylcholinesterase inhibitors

A large number of flavonoids have been isolated from plants, of which some are tested for their possible AChE inhibition potential (Table 1). In search for potent AChEIs, a group of researchers screened a series of flavonoids, isolated from *Syzygium samarangense*. There results suggested that only the compound 2′,4′-dihydroxy-6′methoxy-3′,5′-dimethyl-dihydrochalcone show remarkable inhibitory activity of 98.5% inhibition at a concentration of 0.25 mM, while rest of compounds exert weak or negligible activity [49]. Another research group for the first time studied four flavonoids against AChE, and suggested that quercetin and tiliroside illustrated remarkable inhibitory activity with an IC_{50} 19.8 and 23.5 μM contrary to the standard dehydroevodiamine (IC_{50} 37.8 μM), while 3-methoxy quercetin and quercitrin exhibited lesser inhibitory activity with IC_{50} 37.9 and 66.9 μM, respectively [50]. Another research group screened numerous phenolic acids along with various derivatives of flavonoids such as apigenin, biochanin A, naringin, genistein, quercetin, luteolin-7-*O*-rutinoside, diosmin, kaempferol-3-*O*-galactoside and silibinin, for their inhibitory activity against AChE and butyrylcholinesterase (BChE). From the results, it was inferred that quercetin is the only compound that exhibited significant inhibitory activity of 76.2% against AChE, while genistein, luteolin-7-*O*-rutinoside and silibinin showed activity against BChE with inhibition of 65.7, 54.9 and 51.4% respectively [51]. Another research group isolated flavonoids like rutin and quercetin from the beans of *Dimorphandra gardneriana* and evaluated them for leishmanicidal and AChEI activities. The study found that rutin and quercetin exhibited potent leishmanicidal activity at amastigote stage with EC_{50} of 43.35 and 10.64 μg/ml, as compared to standard amphoteracin B (EC_{50} 19.75 μg/ml), while promising AChEI activity with a zone of inhibition 0.6 cm, as compared to the standard physostigmine (0.9 cm) [52]. The *in-silico* effect of morin along with other flavonol derivatives such as rhamnetin, isorhamnetin, rhamnazin *etc.* against AChE enzyme was studied. The results suggested that some flavonol derivatives exhibited significant binding energy than morin, which may be considered as a lead compound for the effective management of AD [53].

A series of six naturally-occurring flavonoids such as (-) pinostrobin (1), 2′,4′-dihydroxy-3′,6′-dimethoxychalcone (2), 6-8-diprenyleriodictyol (3), isobavachalcone (4), 4-hydroxylonchocarpin (5) and 6-prenylapigenin (6) isolated from *Dorstenia* and *Polygonum* species were investigated for their AChEI and anti-inflammatory activity. The results suggested that except compound 1, all the other compounds showed better AChEI activity with an IC_{50} 5.93–8.76 μg/ml [54]. The *in vitro* inhibitory activity of four flavonoids revealed that quercetin and macluraxanthone exhibited concentration-dependent inhibition of AChE. Macluraxanthone possess remarkable inhibitory activity against the enzyme with as IC_{50} 8.47 μM, as compared to quercetin [55]. A group of researchers studied AChEI activity of a number of flavonoid extracts from the whole plant of *Dryopteris erythrosora* (Autumn fern). The study showed that fern flavonoid extracts exhibited dose-dependent inhibitory activity against an AChE [56]. Similarly, a group of researchers isolated thirteen flavonoid derivatives along with two ginkgolides from the leaves of *Ginkgo biloba* [57], and screened them for possible *in vitro*