



Review article

Molecular signaling pathway targeted therapeutic potential of thymoquinone in Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease with rapid progression. Black cumin (*Nigella sativa*) is a nutraceutical that has been investigated as a prophylactic and therapeutic agent for this disease due to its ability to prevent or retard the progression of neurodegeneration. Thymoquinone (TQ) is the main bioactive compound isolated from the seeds of black cumin. Several reports have shown that it has promising potential in the prevention and treatment of AD due to its significant antioxidative, anti-inflammatory, and antiapoptotic properties along with several other mechanisms that target the altered signaling pathways due to the disease pathogenesis. In addition, it shows anticholinesterase activity and prevents α -synuclein induced synaptic damage. The aim of this review is to summarize the potential aspects and mechanisms by which TQ imparts its action in AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and is considered to be the most common form of dementia which accounts for two-third of all dementia cases around the world [1]. It progresses by a complex multifactorial type of neurodegenerative damage which is characterized by massive neuronal and synaptic loss with gradual death of neurons in the neocortex, entorhinal cortex and hippocampal regions of the brain. Its severity is manifested with the presentation of memory loss and cognitive decline along with the formation of senile plaques and neurofibrillary tangles which are the dominant features of AD [2, 3, 4, 5].

Dementia, particularly AD is prevalent in populations all around the world but with different frequencies in the developed and least developed countries in the world [6]. It is rising with advancing age at a rate of 10–30% that accounts for approximately 10 million new cases annually with a percentage of minimum 5–8% in the general mass aged above 60 [7]. A recent census study predicted that, by the year of 2050, globally 13.8 million of the population will be diagnosed with AD amongst which over half will be from the United States [8]. Apart from over 65 age criterion, about 200,000 people under 65 years of age develop early onset AD in the US with the newer population predicted to add about 10 million in the upcoming decades [9]. However, another study showed that 58% of the individuals with AD are from the low and middle-income countries of the world, which is predicted to rise to about 131.5 million

by the time it reaches 2050 [10,11]. The rise of AD cases for middle- and low-income countries possess a great challenge in respect to its treatment expenditure and the development of cost effective novel therapeutic strategies [12].

Despite the vast knowledge that exists regarding AD, the correct treatment still remains a challenge. Currently available FDA approved treatments are cholinesterase inhibitors (ChEIs) namely rivastigmine, donepezil and galantamine that prevent acetylcholine (Ach) degradation by increasing their concentration at the synapses and partial N-methyl-D-aspartate (NMDA) receptor antagonist memantine which functions by blocking elevated glutamate levels which is postulated to lead to neuronal dysfunction. Till date, the established forms of treatment are only symptomatic in nature and are mainly focused on counterbalancing the neurotransmitter disturbance features of the disease [13]. Over several years, a large number of diseases have been treated by alternative systems of medicine including herbal preparations. Due to the rising of treatment complications including adverse effects, continuing research prevails to develop alternative systems of medicine to complement or replace existing conventional treatment.

Nigella Sativa (NS) plant, an annual herbaceous plant, belonging to the family Ranunculaceae has been utilized as a medicinal plant due to its strong traditional background in Southwest Asia, Middle East and Northern Africa by being used as a spice, food preservative as well as a protective and curative remedy for multiple disorders [14].

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Thymoquinone (TQ), 2-isopropyl-A 5-methylbenzo-1, 4-quinone, an aromatic hydrocarbon, is a potential chemical constituent of NS plant, has been proven to have a wide range of pharmacological interventions, including antidiabetic, anticancer, cardioprotective, retinoprotective, renoprotective, neuroprotective, hepatoprotective and antihypertensive effects [15]. It has been suggested to be a potential anticancer agent due to its potential ability to inhibit tumor growth by stimulating apoptosis as well as by suppression of the P13K/Akt pathways, cell cycle arrest and by inhibition of angiogenesis [16].

The aim of this review article is to explore the potential ability of thymoquinone in Alzheimer's disease by targeting the signaling pathways underlying the pathogenesis of the disease and its validity as a better treatment option.

2. Pathogenesis of Alzheimer's disease

The neuro-pathology of AD can be described by the accumulation of hyper-phosphorylated tau proteins containing neurofibrillary tangles (NFTs) and amyloid beta ($A\beta$) containing plaques with degenerating and frequently swollen axons, neuritis and glial cells [17]. The pathophysiology of AD is complex involving disturbances and imbalances occurring in a variety of mechanisms including amyloid beta plaque formation, hyper-phosphorylated tau protein aggregation, disturbance in the homeostasis of calcium and other metal ions, lowered expression of BDNF, insulin resistance, low choline uptake [5, 18] (Figure 1). Apart from this, there are some other pathways in which AD mediates its severity by neuronal loss and memory decline [19, 20].

2.1. Amyloid β pathway of protein aggregation

AD is characterized by malfunction of amyloid precursor protein (APP) processing pathway which leads to the formation of amyloid proteins generating plaques. The proteolytic processing for amyloid peptides occurs in a chronological manner through two pathways-the α pathway and the β pathway [21]. The α pathway is conducted by cleavage with alpha secretase and produces sAPP, soluble fragment which functions in regulation of enhancing synaptic plasticity, neuronal excitations, protection of neurons from oxidative and metabolic stresses and improvement of learning and memory [22]. This is the non-amyloidogenic pathway as it occurs under normal physiological state. On the contrary, in the β pathway in the neuropathological state, an additional 99-amino acid fragment is generated which is further cleaved by gamma secretase to form soluble $A\beta(1-40)$ or insoluble $A\beta(1-42)$ peptides leading to senile plaque production [22]. Comparative study

between AD patients and normal individuals reported higher levels of $A\beta(1-40)$ (>60–70%) than $A\beta(1-42)$ (~15%) in normal individual's brain [17]. This insoluble $A\beta(1-42)$ peptides exert an opposite effect to that of the sAPP causing synaptic degradation, reduced neuronal plasticity, alternation in energy metabolism pathway and oxidative stress with mitochondrial dysfunctions [23, 24]. This $A\beta$ overproduction is also accompanied with insulin degrading enzyme down-regulation (IDE), in the common non-autosomal dominant forms of AD, which has the function of degrading $A\beta$ [25].

2.2. Phosphorylation of the tau proteins and destabilization of microtubules

Along with APPs, tau proteins play a role in AD pathogenesis by bringing alternations of neurofibrillary degeneration [26]. These tau proteins are robustly expressed disorganized proteins encoded by microtubule associated protein tau (MAPT) gene on the 17q21 human chromosome [27] and are engaged in the activity of facilitating intracellular transport, preservation of the integrity of cellular structures as well as neurite outgrowth and their formation processes [28, 29]. Insoluble forms of fibrillary tau may exert its neurotoxic effect as a result of hyperphosphorylation which makes it resistant to calcium activated proteases, calpains and the ubiquitin-proteasome pathway [30]. After hyperphosphorylation of these proteins, they segregate from the microtubules and keep burgeoning up in the somatodendritic neuronal regions and eventually forms NFTs [31, 32]. These NFTs remain organized in paired helical filaments showing their emergence in the later stages of AD [33]. Moreover, studies demonstrated that hyperphosphorylated tau are capable of disrupting neuronal and synaptic functions as well as suppressing pre-synaptic protein expressions even before their deposition in the form of NFTs [34]. Besides these, a number of studies depicted the phosphorylated forms of these proteins to be responsible for amyloid induced neurotoxicity and cognitive deterioration [35].

2.3. Depletion of acetylcholine storage

The most significant neurotransmitter system that is involved in AD pathogenesis is the cholinergic group of neurons which is involved in cortical activity, blood circulations in the cerebral area, memory and learning-oriented functions, modulation of cognition [36]. This neuro-cognitive decline is characterized by selective loss of choline acetyl transferase enzyme, ACh neurotransmitter as well as nicotinic and muscarinic receptors in brain of patients with AD [37].

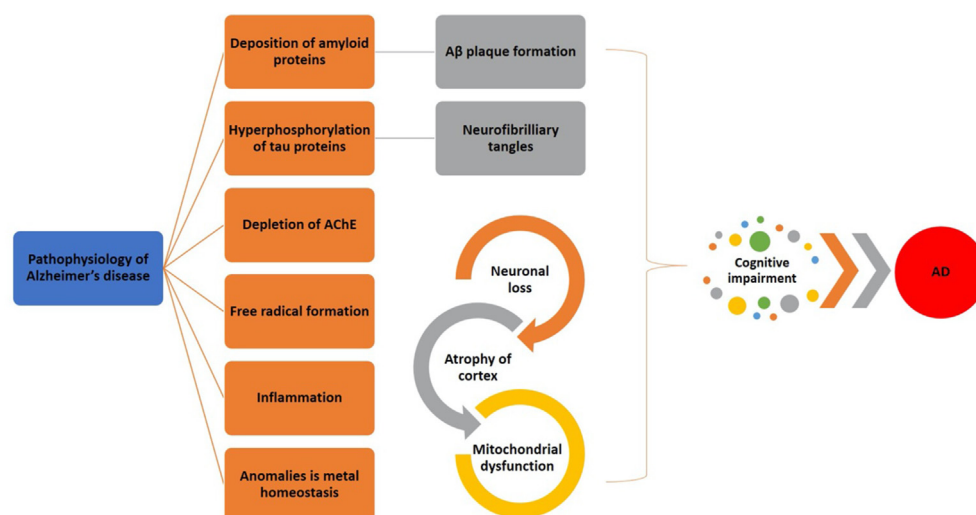


Figure 1. Pathophysiology of Alzheimer's disease.

The cholinergic hypothesis for AD pathogenesis is supported by the fact that cholinergic disruptions in AD causes changes in the glutamatergic systems as these two systems significantly interacts with each other [38]. Glutamate induced neuronal excitability for neuro-protection is mediated by ACh and its receptor ($\alpha 7$) which in AD have an interrupted neurotransmission via defects in the entorhinal cortex, followed by defects in hippocampus amygdala, frontal and parietal cortex [39, 40]. Recent studies demonstrated that A β peptides may impact cholinergic function by combining with the cholinergic receptors [41]. The cholinergic neuronal atrophy in the forebrain of AD patients causes decline in the ACh levels, leading to cognitive decline. Another component brain derived neurotrophic factor (BDNF), which is a growth factor promoting neuronal survival, synaptic plasticity and cellular differentiation, augments the differentiation and survival of cholinergic neurons in the region [42]. Hence, BDNF synthesis ensures ACh amount within marginal levels and prevents dysfunction in AD [43]. In a similar study of transient hippocampal elevation of BDNF, mRNA levels for passive avoidance and Morris water maze (MWM) tests showed that anti BDNF antibodies, which can be generated in AD, caused weakened memory in mice [44]. Besides, diminished expression of BDNF mRNA in the nucleus basalis of Meynert, the neocortex and the hippocampus has been obtained in the postmortem brain in patients with AD [45, 46].

2.4. Induction of oxidative stress

Presence of extensive oxidative stress (OS) is one of the identical features of AD brains where the accretion of damage is induced by free radicals and variability in the expression and functions of the antioxidant enzymes, catalase and super-oxide dismutase [47, 48]. Among the unpaired electron containing mediators, $\cdot\text{OH}$ is considered as the most harmful mediator of oxidative injury to neural and non-neural cells [49]. Besides, other reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by neurons, microglia and astrocytes which lead to neurodegeneration and cognitive decline [49]. When these ROS levels exceeds the physiologic capacity, the vitamin E and glutathione levels are significantly compromised which is reported in the brain and CSF samples from AD patients [50]. A study demonstrated that these ROS can be generated by A β plaque aggregation by NMDA receptor activation and OS could also mediate A β and phosphorylated tau generation and their polymerization leading to a vicious cycle of AD progression [51]. In addition, mitochondrial dysfunctions of energy generation, loss of antioxidant enzyme activity and membrane potential, caspase stimulation etc. are caused by elevated ROS levels taking turn to neuronal death [52, 53, 54].

2.5. Mediation via the inflammatory pathway

Neuroinflammation is the one of the most significant factors in the cycle of AD pathogenesis characterized by extensive activation of the glial cells and cytokine release at the damage site [55]. Cytokines are a group of proteins generated in the inflammatory response conditions whereas chemokines are the pro-inflammatory cytokine proteins which take part in inflammatory cell recruitment [56]. Both of these are elevated in AD as a part of the inflammatory pathway [57]. Microglial cells express a variety of receptors such as TREM 2, Fc γ Rs, MHC-II, CD200R, RAGE, CX3CR1 (fractalkaline), toll-like receptors 2 and 4, CD47, integrins, galectins 1 and 3 by which these cells mediate both pro-inflammatory and anti-inflammatory response depending on certain environmental influence [58, 59]. The secreted inflammatory molecules also recruit monocytes and lymphocytes to promote neuroinflammation by crossing the blood brain barrier (BBB) in the CNS in AD patients [60]. Lipopolysaccharides (LPS) serves as an activator to microglia causing a two-fold increase in APP expression levels, 18 fold increase in β -cleaved carboxy-terminal fragment of APP (β CTF) and up to three-fold increase in A β -40 and A β -42 amounts [61, 62].

Furthermore, the risk factors that aggravate AD, overexpression of NF- κ B occurs which is an important regulator in the transcription pathway conducting communication between immune cells and inflammatory responses and contributing to TNF- α secretion. This generates a new homeostatic alternation and thereby brings epigenetic changes in the neuroendocrine genes with the production of pro-inflammatory cytokines. On the other hand, NF- κ B induced iNOS, COX-2 and inflammatory cytokine production that paves the way to the anti-inflammatory activity [49, 63, 64]. Studies show that the concentration of cytokines such as IL-1 α , IL-1 β , IL-6 and type B receptor IL-8 (IL-8RB) is elevated close to the sites where amyloid plaques are located. Another similar study reports that, IL-6 remains more commonly in the diffuse plaques and IL-1 β and IL-10 impacts elevated basal neuroinflammation [65]. Overexpression of IL-1 protein occurs in AD brain causing elevated neuronal acetylcholinesterase (AChE) activity along with further microglia and astrocyte activation [66, 67]. The cholinergic anti-inflammatory pathway depicts that electrical stimulation of the vagus nerve suppresses TNF production with subsequent reduction of the inflammatory response. This regulatory mechanism exists for AD-induced neuroinflammation for the neuronal type acetylcholine receptors (AChRs) [68, 69].

2.6. Dysregulation of calcium homeostasis

The maintenance of intracellular calcium homeostasis is significantly important for regulating concentration gradients for cellular pathways, physiological activities of attaching to various proteins, receptors and ion channels [70, 71]. Calcium also efficiently contributes to ACh synthesis, alpha-secretase pathway of APP degradation and in hippocampal neurogenesis [72]. In AD, variations of calcium buffering capacities, deregulation of calcium channel activities, deviations of normal mitochondrial activities occur which can be attributed to the inability of the neurons for subcellular compartmentalization of calcium as well as their influx and efflux mechanisms [73, 74]. Age-related OS, metabolic impairments, A β aggregations and presenilin gene mutations are thought to be the reasons for such aberrations in calcium homeostasis [75]. Accumulation of these A β oligomers may promote pore generation and OS on neuronal membranes to cause calcium overload [73, 76].

2.7. Insulin resistance

Insulin and insulin-like growth factor (IGF) levels are important in memory-related activities by maintaining neuronal and glial functions of growth, metabolism, gene expression, protein synthesis, cytoskeletal assembly, synapse generation, neurotransmitter function and plasticity [77, 78, 79]. Several studies reported that AD patients exhibit a neuro-metabolic disorder characterized by the decline of the insulin capacity to stimulate glucose use owing to lack of insulin or under secretion/utilization [77, 79, 80].

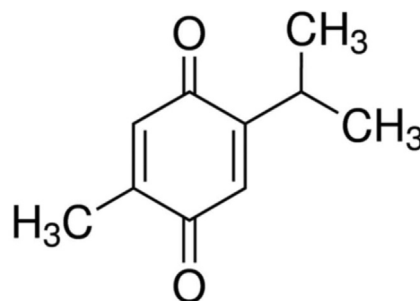


Figure 2. Chemical structure of thymoquinone.

3. Chemistry of thymoquinone

TQ (IUPAC name of 2-isopropyl-1,4-dimethyl-5-quinone) (Figure 2) is a plant-derived, potential bioactive monomer compound (about 30–48%) in the volatile oil of NS seeds with the molecular formula $C_{10}H_{12}O_2$ and molecular mass of $164.20 \text{ g mol}^{-1}$ [81]. Chemically, NS contains 30% fixed oils (mainly fatty acids), 0.40–0.45% volatile oils, vitamins, amino acids, proteins, carbohydrates, alkaloids, saponins, crude fiber, and minerals. The oil part is enriched in polyunsaturated fatty acids (PUFA), phytosterols, TQ, carvacrol, t-anethole, sesquiterpene longifolene and terpinen-4-ol [82]. The concentration of TQ in the oil has been reported to be 18–25 $\mu\text{g/mL}$ [83].

Due to the presence of a basic quinone ring moiety, TQ exhibits keto-enol tautomerism [84] in which the keto-version is the major form showing pharmacological capabilities such as antitussive, gastro-protective, anti-inflammatory, antinociceptive, antihistaminic, antibacterial, anthelmintic, antioxidant, immunomodulatory, anticancer, hepatoprotective, cardioprotective, antidiabetic, ototoxicity protective, and nephroprotective effects [81]. It also provides neuroprotective effects by reversing memory impairments and enhance cognitive functioning as well as attenuate drug tolerance and dependence. It exhibits low stability in aqueous solutions particularly in alkaline pH and possess a solubility range of 549–669 $\mu\text{g/mL}$ in all aqueous solutions [85].

4. Effects of thymoquinone in Alzheimer's disease

4.1. Anti-inflammatory effect

The anti-inflammatory activity of TQ is mediated through the Toll-like receptors (TLRs) which are transmembrane proteins possessing an extracellular leucine-rich domain followed by an intracellular toll-interleukin-1-receptor (TLR) domain [86]. The activation of these receptors trigger two separate downstream signaling pathways one of which includes the Myeloid differential factor 88 (MyD88), where TLRs dimerization stimulates NF- κ B in order to generate pro-inflammatory cytokines such as TNF- α and IL-1,6,7 [87]. NF- κ B is primarily associated with the CNS microglia and its activation causes nuclear translocation of p65 subunit followed by its phosphorylation, acetylation and

methylation, as well as DNA binding and gene transcription. Afterwards, the activated NF- κ B binds to particular DNA sequences of these target genes for neuro inflammatory cytokine production [88, 89, 90]. TQ inhibits nuclear translocation of NF- κ B which subsequently blocks the production of NF- κ B mediated neuroinflammatory cytokines [90].

In the other pathway, there occurs the activation of interferon-regulatory factor-3 (IRF-3) which causes multiple Type-1 interferon inducible genes formation by utilizing the TIR-domain-containing adapter-inducing interferon- β (TRIF) pathway independent of MyD88 [91]. Exhibiting the anti-inflammatory effect, TQ administration at different doses (10, 20, 40 mg/kg) significantly down-regulated the mRNA expression of TLR-2, TLR-4, MyD88, TRIF and their downstream effectors Interferon regulatory factor 3 (IRF-3) and NF- κ B which consequently caused a decline in protein levels of the pro-inflammatory cytokines such as IL-1B and TNF- α [19]. Similarly, TQ also inhibits LPS induced pro-inflammatory cytokine release like IL-1B, IL-6 and IL-12 p40/70 via its interaction with NF- κ B [92].

Nuclear erythroid-2 related factor/antioxidant response element (Nrf 2/ARE) being an upstream signaling pathway of NF- κ B signaling pathway, its activation by TQ has been linked to the inhibition of p65 translocation to the nucleus followed by consequent anti-inflammatory action [93]. On the other hand, the downstream signals of NF- κ B was reported to be attenuated by TQ through targeting the interleukin receptor associated kinase-1 (IRAK-1) enzyme activity as IRAK-1 possesses NF- κ B activating function. The validation of such findings was confirmed in a NF- κ B driven luciferase assay where TQ lowered such type of stimulation by 80% in a dose-dependent manner. Moreover, TQ reduced the IRAK-1 mediated p65 and c-Jun phosphorylation, ubiquitinated IRAK-1 generation as well as promoted its complex generation with TNF receptor associated factor-6 (TRAF-6) [94]. It also attenuates NF- κ B dependent neuroinflammation by obstructing phosphoinositide 3-kinase (PI3K)/Protein kinase B (PKB) or Akt/NF- κ B signaling pathway for stalling of cytokine release including the messenger RNA (mRNA) of IL-1 β , IL-6, and TNF- α for the BV-2 microglial cells [55, 93, 95].

TQ also inhibits the expression of all genes regulated by NF- κ B, i.e., COX-2, VEGF, MMP-9, c-Myc, and cyclin D1 which distinctively lowers NF- κ B activation making it a potentially effective inhibitor of inflammation, proliferation and invasion [96]. Besides, it prevents the rise of

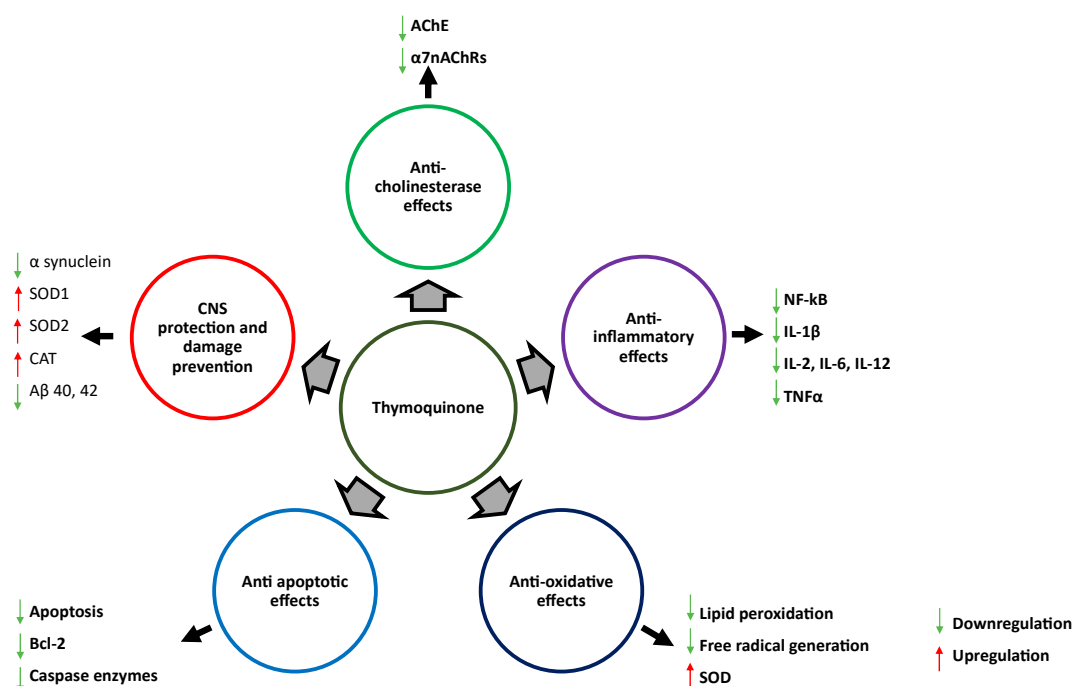


Figure 3. Therapeutic potentials of thymoquinone in Alzheimer's disease.

malondialdehyde (MDA), transforming growth factor beta (TGF- β), c-reactive protein, IL-1 β , caspase-3 and concomitantly upregulates glutathione (GSH), cytochrome c oxidase, and IL-10 levels [92]. TQ also causes reduction in the levels of NO₂ in concordance with the decline of inducible nitric oxide synthase (iNOS) protein expression as well as reduction of pro-inflammatory chemokines including IL-12p40/70, chemokine (C-C motif) ligand 12 (CCL12)/monocyte chemoattractant protein 5 (MCP5), chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemoattractant protein 1 (MCP1), and granulocyte colony-stimulating factor (G-CSF), tumor necrosis factor- α (TNF- α), IL-6, and IL-1 β expression in LPS-activated BV-2 murine microglia cells [97], indicating a strong anti-neuroinflammatory capability.

4.2. Antioxidative effect

Oxidative stress plays a prominent role in AD pathogenesis of cognitive impairments. Any means of chronic cerebral hypoperfusion generates the formation of free radicals which wanes the antioxidant defense mechanism, primarily superoxide dismutase (SOD), thereby leading to neuronal degeneration and death. In a study conducted on male Wistar rats with cerebral hypoperfusion leading to learning and memory impairments, TQ prominently mitigated hippocampal lipid peroxidation and improved SOD activity [98]. Moreover, the NS oil caused an improvement in the spatial working performance on a radial arm maze model and subsequently developed the spatial cognition activity with global cerebrovascular hypoperfusion [99].

TQ is a strong hydrogen peroxide, hydroxyl scavenger and lipid peroxidation inhibitor with a percent inhibition value of $79.5 \pm 2.12\%$, whereas it possesses a low antioxidant activity against 2,2'-diphenyl-picrylhydrazyl radical (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals which is attributed to its chemical structure providing the site for the free delocalization of electrons as well as H⁺ donation for the radical scavenging action [100, 101]. It exhibits a potential neuroprotective activity against A β 1-40 mediated neurotoxicity in rat hippocampal cells by refining the oxidative stress conditions [92]. A study by Ismail and colleagues showed that pretreatment of primary cultured cerebellar granule neurons (CGNs) with varying therapeutic levels of TQ (0.1 and 1 μ M) ensured the inhibition of free radical generation, lowering of the release of lactate dehydrogenase (LDH) along with inhibition of both extrinsic and intrinsic caspase pathways, thereby, improving conditions of oxidative damage after subsequent exposure to A β 1-40 peptide [102]. Similar phenomenon was also observed in case of differentiated pheochromocytoma (PC-12) cells of rats [103]. TQ can also act as a therapeutic agent against ethanol-induced neuronal apoptosis in the cortical neurons [104].

Another study performed by Mehri et al. reported that TQ at concentrations of 5 and 10 mg/kg shows neuroprotectivity by altering the reduced levels of malondialdehyde (MDA), a sensitive biomarker for lipid peroxidation, and thereby elevated the levels of GSH [105]. Such evidence can be further solidified by studies of the brain tissues of microcystin (MC)-intoxicated mice, where there was elevated levels of MDA and NO which was significantly reversed by TQ (245.15%–62.77% and 190.04%–75.11%, respectively) and also caused to upsurge the levels of GSH, SOD, catalase (CAT) and glutathione peroxidase (GPX) (43.85%–169.90%, 51.33%–159.73%, 52.76%–157.73%, 41.97%–165.70%, respectively) in comparison to toxic controls [106]. In addition, in case of tramadol (TRM) treated rats with their low SOD and GSH values, they had enhanced H₂O₂ production along with stimulation of lipid peroxidation, NO, and protein oxidation. These TRM induced rise of OS were seen to be negated by TQ and also could contribute to the inhibition of NO generation by suppressing the expression of inducible NO synthase [107]. However, another recent study has revealed that the potential antioxidant characteristics of TQ can be altered due to the generation of semiquinone (one reduction), a potent pro-oxidant or thymohydroquinone (two reductions), a more potent antioxidant than parent TQ [108].

4.3. Anticholinesterase (AChE) activity

ACh is an essential neurotransmitter for memory and learning processing, serving as the primary media to carry nerve impulses between nerve cells where enzyme AChE acts to rapidly degrade ACh into choline and acetate [38]. Researchers demonstrated that elevated levels of AChE activity is strongly co-related with cognitive deficiencies due to the impact on the cerebral blood flow [109, 110]. Therefore, suppression of this enzyme may serve as a potential approach for the treatment of AD which elevates the synaptic concentration of ACh, allowing a higher occupancy rate and longer duration at its receptor [111, 112].

The NS oil seed has a prominent dose dependent impact on AChE inhibitory activity ($r^2 = 0.989$) compared to essential oils from other medicinal and aromatic plants and edible oils such as olive oil used in the Mediterranean diet. A study with methanolic extract of NS demonstrated almost identical levels of AChE activity compared to the standard drug donepezil [113]. TQ exhibited the highest AChE activity of 53.7 g/mL in which NS extract overall exhibited 84.7 g/mL, which suggests a significant AChE inhibition. In another study, L-Cys was administered to mimic the biochemical changes induced by ACh decline and found that administration of TQ (5 and 10 mg/kg; p. o.) lowered the L-Cys induced stimulation of AChE enzyme function at the same dose level of donepezil (10 mg/kg; p. o), the reference control for the study [114]. This donepezil effect was mimicked by improving cognitive capacities by suppressing AChE, TNF- α levels, inhibiting lipid peroxidation, and increasing glutathione levels [115]. Furthermore, TQ exerts an inhibitory action on α 7 nicotinic acetylcholine receptors (α 7 nAChRs) [116]. A potent agonist of this receptor, PNU-282987 along with its positive allosteric modulators show enhanced induction of the receptors which was similarly observed for TQ in the recent studies as well [92]. Besides, a significant co-relation between antioxidant and AChE activity has been demonstrated. After administration of A β fragment 25–35 (A β 25–35) into differentiated pheochromocytoma (PC12) cells in rat, there was a prominent rise in NO and AChE, where TQ [at 2 μ M ($p < 0.01$) and 4 μ M ($p < 0.01$)] administration provided neuroprotection by restoring glutathione and significant lowering ($p < 0.05$) of AChE in A β 25–35-treated cells as compared to control group [103].

4.4. Effect on alpha-synuclein induced synaptic damage

Besides tau and β -amyloid proteins aggregations, α -synuclein, a 140 amino acid protein also aggregates in AD. Insoluble versions of this protein comprise the Lewy bodies and neurites which are significantly located in the pigmented neurons of the substantia nigra and in other neuronal populations at the peripheral and central levels. Accumulation of α -synuclein in the synaptic junctions causes loss of synaptic function [117]. Synaptophysin is an integral glycoprotein in the neuroendocrine secretory system and is closely related to the synaptic vesicles for which it is widely accepted as a marker for synapse density quantification. In a study where rat hippocampal region as well as human induced pluripotent stem cell (hiPSC)-derived neurons have been α -synuclein induced, it was demonstrated to cause suppression of synaptic communication and impaired synaptic function as well as loss of spontaneous neuronal firing. This led to loss of capability of recycling of the synaptic vesicles as a result of dropping of synaptophysin levels from hippocampal neurons. The loss of synaptophysin levels and recycling of synaptic vesicles were effectively restored by TQ in the cultured hippocampal neurons and thereby provided neuroprotection against synuclein-induced synaptic damage and mutated β -synuclein (P123H) [118, 119]. Another study suggests that co-administration of recombinant human synaptophysin SN (1 M) and TQ (100 nM) saved the neurons from synuclein induced damage as well as replenished the synaptophysin levels by accelerating them to 98% of its control value, without causing any necrosis [119].

Two pathways that cause breakdown of this malformed α -synuclein protein are the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP). In AD, the proteasome inhibition causes A β

formation which can enter the cytosolic compartment and suppress the proteasome activity for neurons and subsequently causing cytosolic accumulation. These α -synuclein can also morph conformations to form oligomers (early aggregates) which can turn into well-organized fibril (late aggregates) acting as more pathogenic feature for neuronal degeneration and death than matured form of the fibrils [120]. The fibrillar protein aggregate formation known as amyloid fibrils is regarded as the prime factor for systemic amyloidogenesis [121, 122]. At variable stages of incubation, TQ suppressed fibril formation and protected SH-SY5Y cell line from pre-formed fibril induced toxicity in a dose dependent manner [123]. Furthermore, TQ dissembled preformed fibrils into their monomeric forms and halted the seeding process of monomeric synuclein by the α -synuclein preformed fibril addition [120]. In fact, the protective effect against α -synuclein induced synaptic damage for cultured hippocampal and human-induced pluripotent stem cell-derived neurons spiked by 2.6 folds and caused decrease in synaptic impairment by A β 1-42 when it had been co-administered with TQ [119, 124].

4.5. Effect on memory deficits and amyloid beta

AD is a consequence of loss of the neuronal synaptic connections in the hippocampal, cortex and subcortical regions of the brain due to the generation of amyloid plaques and NFTs between them, which results in memory deficits [125]. A study conducted by Dalli et al., with rats treated with streptozotocin (STZ) intracerebroventricularly, created conditions of memory impairments and learning deficits due to a deficit in number of neurons and neuronal connections as observed by MWM test, thereby mimicking the AD phenotype [126]. TQ was shown to reverse such condition by enhancing the fear-conditioned and spatial memory for these rats by a number of underlying mechanisms [127]. One possible way to do that is by increasing the survival rate and number of neurons by recovery of the cultured hippocampal and cortical neurons and partially reducing the numbers of degenerating neurons, rather than generation of new neurons [128]. This could possibly be accomplished by regulating microtubule associated protein DCX regulation [124, 129]. Most prominently, TQ has been found to regulate neurite maintenance for cognitive benefits by phosphorylating and thereby activating the MAPK protein, particularly the JNK proteins for embryogenesis and also lower the expression levels of pro-apoptotic Bax levels [126, 130]. TQ also reduced the aggregate forming microtubule binding protein, MAP2 in the dendrites as well is its distinct binding genetic risk factor, Apolipoprotein E (ApoE) for microtubule function and thus prevent the proteins interaction with tau hyper phosphorylation [126, 131]. There might also be a direct relation between TQ with the microRNA (miRNA), mir-124 expression levels, engaged in development and differentiation of neurons and thus reduces ERK phosphorylation and ameliorate cognitive impairments in rat hippocampus for which miRNAs may have a probable role in the early diagnosis of AD [132]. Another study conducted by the endocrine-disrupter nonylphenol (NP) demonstrated amelioration of memory decline in male Wistar rats by reducing the necrotic cells in the hippocampus by means of lowering the antioxidant parameters total antioxidant capacity (TAC), SOD and GSH. Here, TQ portrays its potential of spatial memory enhancement by reversing the conditions as observed by MWM task [133]. Experimentally induced AD rat models by aluminium chloride (AlCl₃) which caused reduction of spontaneous alternation performance (SAP%) and serum GPX levels in passive avoidance and Y-maze tests, was altered by significant memory improvement in the TQ-AD treated groups [134]. In addition, TQ attenuates D-galactose- and AlCl₃-induced cognitive impairment [135]. In a study conducted by Bargi et al., TQ ameliorated the LPS induced learning and memory impairments by lowering hippocampal IL-6, TNF- α content, reduction of hippocampal MDA levels and suppression of iNOS activity and NO generation. In fact, hippocampus serves to be the most affected area by the inflammatory mediators as it is enriched with cytokine receptors [136, 137].

A potent neurotoxic agent, glutamate, cause over activation of the NMDA receptors which ultimately leads to shifting of non-amyloidogenic pathway towards amyloidogenic processing pathway. This generates elevated levels of A β -42 peptide levels and their aggregation [51]. TQ in nanoemulsion form lowered this aggregation levels by changing the APP processing to cause reduced beta secretase and gamma secretase levels and shifting towards the non-amyloidogenic pathway back and elevated alpha-beta clearance rates This might be mediated by lowering of cytochrome c, caspase-3, LDH and A β -42 levels and ultimately restore memory and cognition caused by glutamate toxicity. TQ thus, has been shown to ameliorate the A β accumulation and maintain the ideal neuronal network connections in the cerebellar granular neurons by ensuring intact neuronal morphology and lowering synaptic impairments [138].

4.6. Prevention of neuro-degeneration and neurotoxicity

Neuroprotection can be defined as the strategic steps to protect the central nervous system (CNS) in the face of neuronal injury due to both acute (e.g., stroke or trauma) and chronic neurodegenerative disorders (e.g. Alzheimer's disease and Parkinson's disease) [139].

Studies on cultured hippocampal and cortical neurons with TQ (100 nM) efficiently suppressed A β 1-42 induced neurotoxicity by improving the cellular activity, inhibiting mitochondrial membrane depolarization and suppressing ROS [124]. In a similar experiment, Al-Majed et al showed that TQ (5 mg/kg/day, orally) has a defensive effect for these neurons against transient forebrain ischemia-mediated injury by means of diminishing the content of dead hippocampal neuronal cells [140]. TQ also provided protection against A β 1-40-induced neurotoxicity in vitro and induced cell death in neuronal cultured cells [141]. TQ has been shown to provide some neuronal and morphology-based improvements in the degenerated hippocampus of mice due to long-term toluene exposure [142]. TQ was also seen to prevent neurotoxicity by lowering the Bax, caspase -3, -8 and -9 and flourishing the B-cell lymphoma 2 (Bcl-2) levels [102]. Neuroprotective properties in nanomolecular concentrations are mediated by TQ based antioxidants which accumulates in the mitochondria as TQ inhibits A β induced hyperproduction of ROS by the mitochondria through a cascade of reactions leading to neuronal death. In fact, TQ based antioxidants can serve as the new type of neuroprotectors [103]. This can be termed as a potential neuroprotective agent by elevating antioxidant genes such as *SOD1*, *SOD2*, *CAT* expression as well as signaling related genes such as Jun N-terminal kinase (*JNK*), P38 and protein kinase B and diminishing the ROS levels [143, 144].

4.7. Antiapoptotic activity

Improvement from apoptotic cell death is caused by the significant suppression of the apoptotic markers. AD pathogenesis involves caspase-8 as an initiator family of caspases that when stimulated initiates the downstream caspases like -3,-6 and -7, eventually breaking the primary cellular substrates causing apoptosis [145, 146]. A β contributes in this process by cross-linking with the death receptors to provoke apoptosis and simultaneous stimulation of caspase-3 and -8. Moreover, multiple studies suggest that TQ have an antiapoptotic potential as it protects the CGNs from the cytotoxic effects by inhibition of the caspase-3, -8 and -9 enzymes. However, administration of TQ at variable strengths of 0.1, 1, 10 and 100 nM in hippocampal neurons (in vitro) revealed some variable effects [138].

Over stimulation of the NMDA receptors triggers apoptotic cell death by excitotoxicity and causes the release of cytochrome c and activation of the intrinsic apoptotic pathway. Cytochrome c starts off the caspase-dependent apoptotic pathway, resulting in proteolytic processing of procaspase-9, eventually causing activation of the downstream effectors caspase-3, -6 and -7 [141,147]. A different study showed that, TQ administration to cultured cortical hippocampal neurons caused an

increase in Bcl-2 expression, an antiapoptotic protein, followed by cytochrome c suppression as well as the activity of apoptotic caspases like caspase-3 [141]. Meral et al. reported that NMDA receptor activation by pentylene-tetrazol-induced kindling could be modulated by antiapoptotic property of TQ [148]. In addition, apoptosis due to ischemic reperfusion injury and long term toluene exposure could also be mitigated by TQ through upregulated Bcl-2 expression and NF- κ B reduction [142, 149]. In summary, lowered apoptotic cell markers, maintenance of cell viability and diminished lactate dehydrogenase (LDH) levels, suppressing increased A β 1-42 by glutamate are the major implications of TQ mediated antiapoptotic activity.

5. Conclusion and future directions

Till present, there is no confirmatory pharmacotherapeutic approach for AD treatment. Currently available conventional therapeutics revolve around symptomatic relief steps to ameliorate both cognitive and behavioral symptoms. Studies report those treatments that are capable of halting or at least efficiently altering the course of AD as ‘disease-modifying’ drugs and they are still under extensive research. Ideally, in order to intervene the progression of the disease, drugs need to alter the pathogenic steps involved for clinical symptoms, including the deposition of extracellular A β , intracellular neurofibrillary tangle formation, oxidative damage, mediation of inflammatory mediators, disruption of calcium homeostasis, loss of ACh stores. These aberrant signaling pathways can be exploited as a promising therapeutic approach for the management of AD both prophylactically and from the curative point of view. Collective evidence-based studies demonstrated that the neuroprotective effects of TQ may be associated with the modulatory effects on inflammation, apoptosis and oxidative stress. Considering lesser side effects, good tolerability, ready availability and minimal expenditure, the spice component, TQ from NS exert its inhibitory effects across various mechanistic pathways specifically targeting the pathogenic process of AD development (Figure 3).

TQ mediates its curative and preventive functions in a variety of ways. The stimulation of Nrf 2/ARE signaling pathway resulting in the inhibition of NF- κ B mediated neuroinflammation [93]. Furthermore, TQ suppressed the inflammatory mediator production by blocking PI3K/Akt/NF- κ B signaling pathway as well as oxidative stress inhibition, defibrillation to disaggregate the fibril oligomers of α -synuclein, replenish ACh levels [55, 93, 95, 100, 111, 112, 123]. Experimental studies also suggest that, TQ not only reduces A β -mediated increase in the ROS, but also decreases mitochondrial membrane potential and caspase activation [102]. TQ has extended its beneficial aspects to even the prevention of newly emerging diseases including infectious ones [150]. With this in mind, Alzheimer’s pathogenic pathway can be targeted by the effects of TQ to prevent the progression of the disease. Given the low hydrophilic properties of TQ [85], lipid nanoparticle technology could be utilized for drug delivery in order for the final medication to be well-tolerated that can cross the BBB as well as diminish the risks of acute and chronic toxicities. Multiple colloidal drug delivery systems such as liposomes, self-nano emulsifying drug delivery technique, TQ loaded PLGA-Chitosan nanoparticles and nanogels have already been explored to overcome the main obstacle that comes along in case of administering of TQ [151].

In conclusion, due to fewer side effects and good tolerability, the natural remedies are the call of the day with food additives playing a vital role in curing some of the problems effectively. Hence, utilization of the spice component TQ of NS targeting AD pathogenesis, may offer a potential and assuring field to engineer as a novel scope for AD therapy.

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