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ASCERTAINING THE ANTIOXIDANT PROPERTIES OF QUERCETIN AGAINST OXIDATIVE STRESS IN COMBATING ALZHEIMER'S DISEASE: A REVIEW

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Review Article

ABSTRACT

Quercetin is a flavonoid compound mostly found in plants including fruits, vegetables, green tea, red wine etc. Apart from having many health effects, quercetin possesses antioxidant capacities. It can scavenge hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), nitric oxide (NO) and other free radicals. The reactive oxygen species have high potential to interrupt the functions of lipids, proteins, DNA and RNA which can lead to various epigenetic changes. These components in the neurons are more vulnerable to oxidative stress. Mitochondrial dysfunctions, amyloidopathy, taopathy, metal accumulation, synaptic dysfunctions, genetic and neuro-inflammation can cause oxidative stress in a cell, which is responsible for development of Alzheimer's disease (AD). The relationship between oxidative stress and AD suggests that oxidative stress is an important contributor of the pathological process for AD and antioxidants may be useful for the treatment. It is proposed that quercetin would be the best choice to act against oxidative stress and hence to AD as it has antioxidant properties as well can inhibit the crowding of macromolecules such as Aβ, Tau protein etc. The neuroprotective effects of quercetin are regulated through nuclear factor (erythroid-derived 2)-like 2 (Nrf2), Paraoxonase-2, c-Jun- N-terminal kinase (JNK), Protein kinase C, Mitogen-activated protein kinase (MAPK) and PI3K/AKt pathways. Therefore the aim of this review is to study the antioxidant effects of quercetin against oxidative stress and hence to prevent the pathogenesis of AD.

Keywords: Oxidative stress; alzheimer's disease; mitochondrial dysfunction; aβ plaque; tau hyperphosphorylation; antioxidant; quercetin.

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1. INTRODUCTION

"Alzheimer's disease (AD) is the most common type of neurological disorder mostly observed in older people" [1]. "This disorder ranks 3rd as the main cause of death in elderly populations, after heart disease and cancer. A German physician Dr. Alois Alzheimer first described the disease in 1906. Nowadays about 60-80% of dementia cases are reported to be caused by AD" [2]. "Almost 35.6 million people worldwide are reported to be affected with AD and about 4.6 million new cases are diagnosed each year" [3]. "It is estimated that AD can grow up to fourfold to 106.8 million with one in 86 individuals by the year 2050" [1]. At the early stage of the disease, a person starts to forget the recent events or conversations which gradually lead to mild and severe cognitive impairments and at last loss of the ability to maintain the daily life schedule.

"The main causes of AD are not clearly understood but many molecular pathways lead to the pathogenesis of AD. It is reported that in most people, AD is caused by genetic factors, environmental factors, lifestyle, age, sex, etc. Less than 1% of cases are caused by genetic changes that encode for amyloid β protein precursor (AβPP) processing" [1]. Tau proteins which are responsible for microtubule stabilization play an important role in causing AD.

"Oxidative stress is an early pathological symptom of AD. Oxidative stress can be defined as the imbalance between pro-oxidant and antioxidant. It is associated with increased production of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) including superoxide radical anion ($O2^-$), H_2O_2, HO^-, NO, $ONOO^-$ etc. An alternative explanation for AD pathogenesis has emerged, that links mitochondrial dysfunction and increased Reactive Oxygen Species (ROS) production. In embryonic mice models with AD were reported to have mitochondrial dysfunctions before the development of any macromolecular crowding i.e. amyloid β plaques and hyperphosphorylated tau proteins. Mitochondrial dysfunctions detected in multiple animal models show disruption in glucose metabolism which leads to oxidative stress in AD patients" [4].

"ROS can act as an ambiguous blade within the biological system and can serve crucial functions like signalling molecules, but also can cause injury to the biological system once available in excess quantity. Not only the increase of free radicals but changes in the functions of antioxidant enzymes (SOD and catalase) in the Central Nervous system and peripheral tissues are also reported to cause AD. In the CNS they are capable of oxidizing all major biomolecules as well as nucleic acid, proteins, and lipids. The brain, especially the neurons is very susceptible to oxidative stress because of the higher consumption of energy for a higher metabolic rate" [5]. For these reasons oxidative stress is considered a key treatment target for AD.

"Various therapeutic approaches are provided for preventing oxidative stress as well as AD. Few medications such as Aduhelm have been approved by U.S.FOOD and DRUG ADMINISTRATION (USFDA) for the treatment of AD [2] but these drugs are not very effective and also have some side effects". "A wide variety of natural products from different origins have been evaluated preclinically and clinically for their neuroprotective mechanisms in preventing and attenuating the multifactorial pathologies of AD. Hence, the use of natural products is recommended nowadays" [6].

Phytochemicals such as Flavonoids are reported for having various therapeutic effects. The antioxidant and anti-inflammatory activities of flavonoids are two major properties for which they are being studied extensively.

Quercetin (3,4,7,3',4'- pentahydroxyflavone) is one such product that is a type of polyphenolic flavonoid compound, that has robust antioxidant activity. Quercetin is mainly found in fruits, vegetables, and in many beverages. In addition to antioxidant properties, it also shows anti-carcinogenic, anti-inflammatory, anti-infective and psycho stimulant, anti-anxiety, and cognitive enhancement effects. It has high solubility and bioavailability and also can penetrate the blood-brain barrier. These features make quercetin a potent therapeutic agent for neurodegenerative diseases [7].

2. MECHANISMS THAT INDUCE OXIDATIVE STRESS LEADING TO ALZHEIMER'S DISEASE

Though oxygen consumption is required for the body to function properly, excessive consumption can also cause the production of free radicals. Especially, the brain is very vulnerable to free radicals because of its high oxygen demand. Various mechanisms can induce oxidative stress.

Dysfunctional mitochondria are not efficient producers of energy or ATP but can form ROS efficiently and can cause oxidative stress [8]. It is found that in AD all the functions related to mitochondria get impaired. Hence, proper mitochondrial function is very necessary for preventing oxidative stress as well as AD [9].

Cleaving of Amyloid β precursor protein (AβPP) by β-secretase and γ-secretase can form Aβ monomers and oligomers that lead to Aβ plaque formation. Amyloid–β plaque formation is one of the major sources of oxidative imbalance in AD. It is reported that various AD transgenic mice models that carry mutant amyloid precursor protein (APP) and presellien-1(PS-1) exhibit increased amounts of H_2O_2 and NO production as well as oxidative modification of lipids and proteins [10]. "Studies in knock-in mice reveal that apoE isoforms can modulate oxidation of Aβ 1-42 and can induce oxidative damage in mice expressing human apoE4 followed by apoE2 did not exhibit any significant change in ROS generation, lipid peroxidation, nonprotein oxidation compared to wild type mice. Hence, the apoE-associated risk of AD may be related to the relationship between the apoE isoform and Aβ" [5]. "The link between Aβ plaque formation and induction of cellular ROS generation is not restricted to CSF-residing neurons only, but this observation extends to endothelial tissue and swish muscle cells in cerebral blood vessels" [3].

Tau protein is a microtubule associated protein which can stabilize the neuronal mitochondria under the normal conditions. But its function can be injurious to neuronal cells when it gets hyperphosphorylated. Hyperphosphorylation of tau protein can form neurofibrilary tangles which are correlated with neurodegeneration and cognitive decline. Although maximum studies state that tau hyperphosphorylation and NFT formation may play a significant role in destroying neurons from oxidative insult, some studies deny those results [10]. In P301S mice, coenzyme Q10 significantly increases the activity of complex I and reduces lipid peroxidation which consequently improves the survival and behavioural deficits. A deficiency in mitochondrial SOD 2 or reduction of cytoplasmic SOD1 induces tau hyperphosphorylation in Tg2576 AD transgenic mice. Although hyperphosphorylation of tau makes it susceptible to conformational changes by the production of paired helical structures and subsequent NFTs, further studies are needed to fully clarify the role of oxidative stress in tau pathology [11].

The cell with senile plaques in AD also shows iron accumulation that increases oxidative stress. Similar to Cu – Aβ interaction, iron-binding with Aβ results in a reduction of $Fe3^+$ to $Fe2^+$ and the generation of H_2O_2 [12]. Aluminium is also associated with AD neurodegeneration by oxidative stress and inflammation. Zinc is also considered to be related to oxidative stress in AD as it is a key component in amyloid plaques and cerebrovascular amyloidosis [13].

Synaptic dysfunctions can cause caspase-mediated degradation of synaptic proteins that can cause neuronal apoptosis. Increased caspase-3 levels are reported in the postsynaptic density fraction of the brain. It can effect temporally with memory impairment, decreased spine density and size, altered stimulatory transmission, and enhanced long term disability (LTD). Thus caspase-3 activation can contribute to neuronal apoptosis and synaptic dysfunction and hence can progress AD [14].

Clusterin (CLU) is a multifunctional glycoprotein expressed from apo-lipoprotein genes, which is a genetic determinant in AD. Genome-wide studies reported that CLU can affect the clinical progression of AD as well as longitudinal brain atrophy in MCI. [15]. In neuroblastoma N2a and SH-SY5Y cells knockdown CLU by shRNA interference was found to cause a decrease in antioxidant capacity [16]. CLU can increase the formation of SDS-resistant Aβ assemblies that can again induce oxidative imbalance in PC12 cells. From the above statements it can be concluded that CLU can cause oxidative stress in AD and vice-versa i.e., oxidative stress can also indirectly induces the expression of CLU mRNA and proteins. Klotho protein which is encoded by the KL gene is responsible for the aging process as well as in increasing oxidative stress in AD patients [10].

Inflammation also participates in the production of ROS and can cause AD. It is reported that AD patients have an abundant number of microglia cells that cannot phagocytize amyloid β and contribute to Aβ plaque formation. Both microglia and astrocytes release proinflammatory mediators such as cytokines, chemokines, ROS and complement proteins that can induce inflammation [17].

3. EFFECT OF OXIDATIVE STRESS AT CELLULAR AND MOLEULAR LEVEL IN AD

Oxidative stress is an early characteristic of AD and also plays a significant role in the pathogenesis and progression of the disease. An imbalance of ROS levels can affect both cellular and molecular levels.

Oxidation of lipids is a chain reaction where free radicals generally steal electrons from lipids and cause oxidative damage to the lipids. Peroxidation of lipids leads to apoptosis, autophagy, or ferroptosis of a cell. The increased amount of free radicals decreases the level of PUFAs (docohexaenoic acid and arachidonic acid) in the brain which causes improper functioning of the brain. The most famous lipid peroxidation products, which are studied in AD are reactive aldehydes (4-hydroxynonal), malondialdehyde (MDA), 2–propenal (acrolein), etc. High levels of 4- hydroxynonal are reported in the

hippocampus, entorhi cortex, temporal cortex, amygdale, parahippocampal gyrus, ventricular fluid and plasma in AD patients. Some studies showed no increase of malondialdehyde in the basal level however showed significantly increased levels in the hippocampus, pyriform cortex, temporal cortex and also other parts of the AD brain regions. Peroxidation of arachidonic acid and docosahexaenoic acid produce F2- isoprostanes and F4- neuroprostanes respectively in the frontal or temporal lobes, body fluid, urine, and plasma of AD patients [1].

Oxidation of proteins in AD can take place through various mechanisms such as ROS attachment to the proteins or attachment with the end products of glycation, glycoxidation, and lipid peroxidation reactions. An increased level of protein carbonyl, for example, Creatin enzyme, amino alkanoic acid synthase, ubiquitin carboxy-terminal hydroxylase L-1 was reported in AD brain regions together with the hippocampus, parietal lobe, and superior middle temporal gyrus. The end product of the interaction between peroxynitrite and tyrosin is 3- nitrotyrosine, which is a major oxidative modification generally found in the brain region and cerebrospinal fluid of AD patients [12].

Oxidative damage of DNA can cause double-stranded breaks, DNA/DNA, or DNA/protein cross-linking and base modification. High levels of DNA breaks were found in each hippocampus and cerebral cortex of AD patients. The commonly used DNA oxidative markers in AD are 8- hydroxydeoxyguanosine (8-OHdG) and 8- hydroxyguanosine (8-OHG). The increased level of DNA double-strand breaks and free carbonyl levels are first thought to be a result of apoptosis, but it is now proven to be caused by oxidative stress [18].

The reaction of sugar with long-lived protein without any enzyme can produce Advanced Glycation End Products (AGEs). Maillard in 1912 demonstrated that spontaneous condensation of ketone or aldehyde groups of sugars with a free amino acid group can lead to the glycation of proteins. In vitro study suggests that tau protein can be glycated by preventing its attachment to the microtubules. Free radicals can interact with these glycation products and can promote the pathogenesis of AD [18].

4. A SHORT OVERVIEW OF QUERCETIN

Plant and plant-derived products are generally used for the treatment of various types of diseases since time immemorial. Increased use of herbal products for natural therapy is regarded as the best over the last decade. This trend of herbal remedies is due to their easy availability, lower cost and also no / limited side effects. They also have anti-inflammatory,

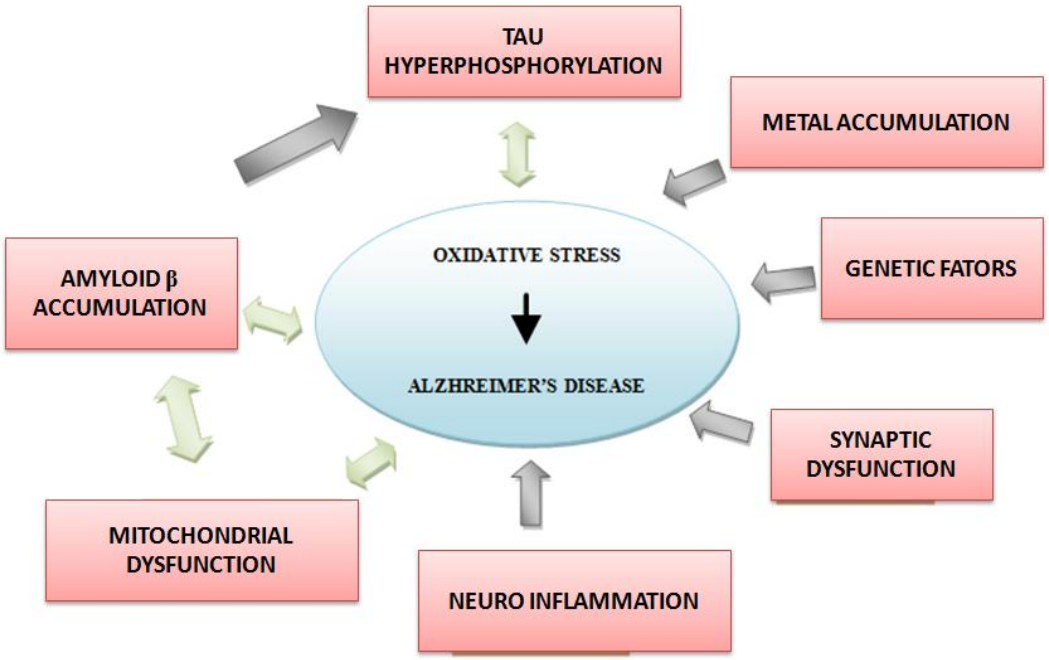

Fig. 1. Mechanisms inducing oxidative stress in AD (Chen *et al.*, 2014)

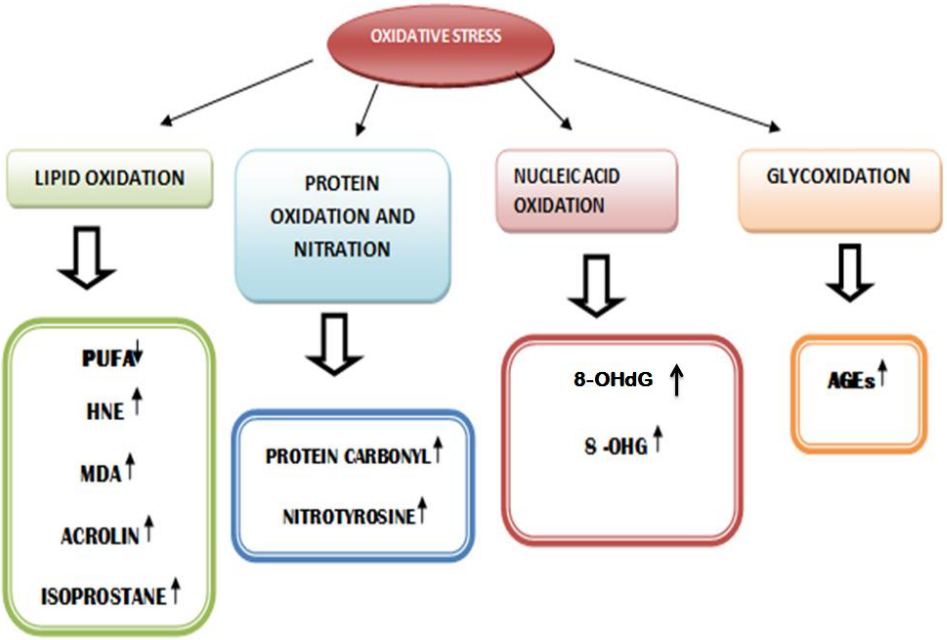

Fig. 2. Effect of oxidative stress on cellular and molecular level (Gella and Durney, 2009)

antioxidant, anti-viral, antiseptic, and anti-diabetic immuno-stimulant activities for which their demand is increasing day by day in the field of medicine/drugs [19]. Phytochemicals like flavonoids are widely used for their therapeutic potential to reduce various types of chronic diseases such as cardiovascular diseases, hypertension, diabetes and cancer [20]. They have both antioxidant and anti-inflammatory properties which are used widely in preventing neurodegenerative diseases. Quercetin is a flavonol, belonging to the subcategories of flavonoid compounds having many pharmacological properties. Quercetin is abundantly available in vegetables, and fruits like berries, apples, dill, capers, coriander, onions, etc. Because of having antioxidant and anti-inflammatory properties, quercetin can be used in preventing AD. Apart from these it also possesses anticancer, antiviral, antiseptic, and anti-allergic properties. Quercetin can prevent cardiovascular diseases, eye diseases, arthritis, platelet aggregation, and lipid peroxidation as well as can enhance mitochondrial biogenesis.

5. CHEMICAL PROPERTIES

Quercetin is yellow in color, soluble in lipids and alcohol but insoluble in cold water, whereas sparingly soluble in hot water. The chemical formula of quercetin is C15H10O7, containing OH group at positions 3, 5, 7, 3', 4' but lacks an attached sugar molecule (aglycone) [20]. The chemical stability of quercetin is maintained through oxygen concentration, pH value, temperature, the concentration of antioxidants, and concentration of metals [21].

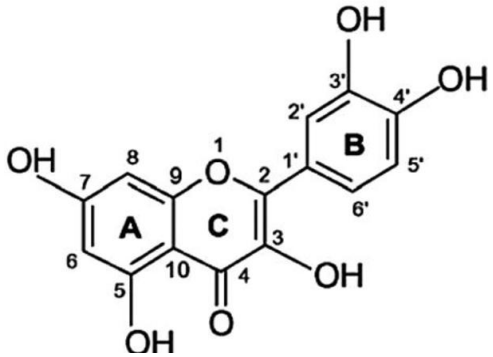

Fig. 3. Chemical structure of quercetin (source: google images)

6. OXIDATION – DEGRADATION OF QUERCETIN

Quercetin can be oxidized into various oxidation products, such as quercetin-quinones, which contain one ortho-quinone and three quinon methides. Cleavage of quercetin can give rise to protocatechuic acid. In low GSH levels, quercetin can interact with protein sulfhydryl to form unstable glutathionyl quercetin. But at higher concentrations quercetin can directly form glutathionyl-quercetin [21].

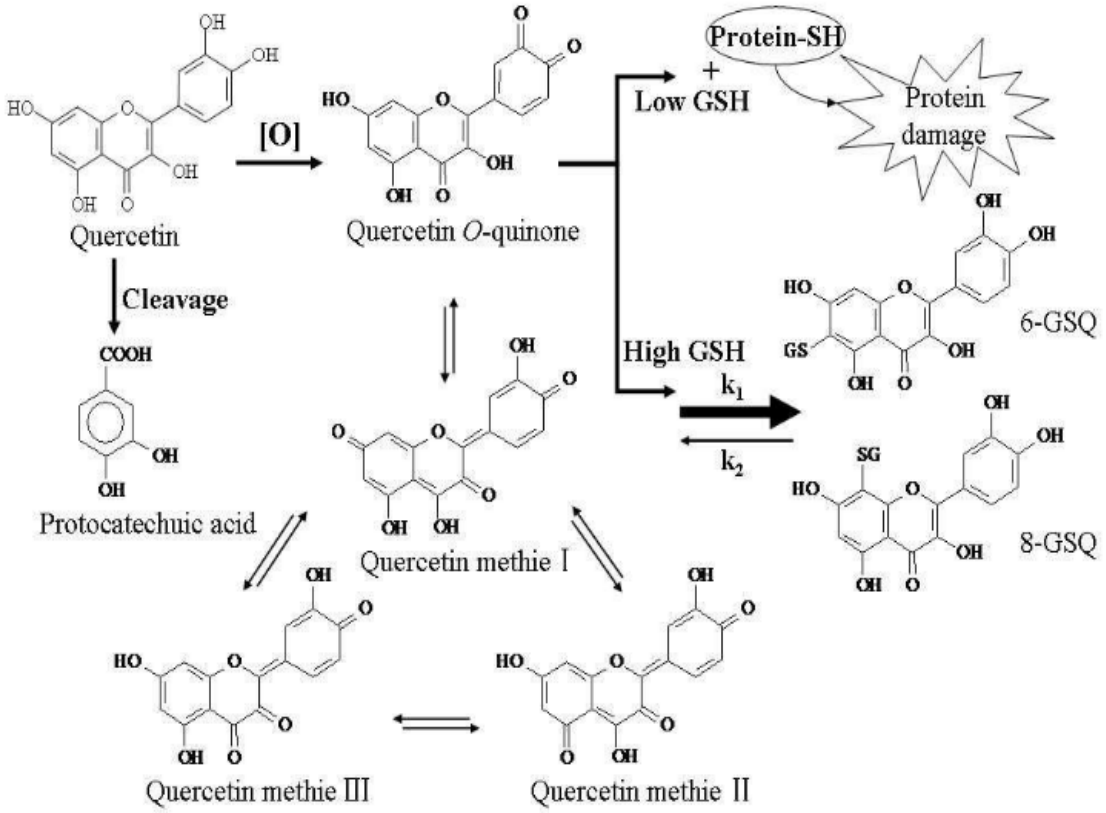

Fig. 4. Oxidation and degradation pathways of quercetin (source: Boots *et al.*, 2003)

7. ABSORPTION AND EXCRETION OF QUERCETIN

Though only limited studies have been done on quercetin, these studies reported that quercetin and its metabolites tend to accumulate within the organs that are related to its metabolism and excretion [22]. *In vitro* studies with blood-brain barrier models show that quercetin can enter the brain tissues whereas *in vivo* studies on rats and pigs suggest that low levels of quercetin are found in the brain [23]. Mitochondria might be an area of quercetin concentration inside the cells. The average terminal half-life of quercetin is 3.5 hours. In male rats though 93% of quercetin was metabolized in the intestine, liver can also metabolize 3.1% of quercetin [24]. The report also revealed that about 59.1% of total quercetin including free and conjugated quercetin as well as its metabolites was adsorbed after an oral administration of a single dose of 10 mg quercetin/kg body weight in rats. A long-term treatment (11 weeks) of rats with quercetin fed in diet (500 mg/ kg BW rat) demonstrated that quercetin and its metabolites were distributed in several organs (e.g., lung, kidney, heart and liver), with the highest level of quercetin in the lung and the lowest level in the brain and spleen. It implies that the intake of quercetin from daily diet can lead to the accumulation of quercetin throughout the body [25]. It was also reported that iso-quercetin i.e. the glycosylated quercetin is more absorbed than quercetin in the aglycone form [22].

In the mouth quercetin first, react with salivary proteins and form soluble quercetin–protein complex. In the stomach, quercetin interacts with the highly acidic condition and can be degraded to phenolic acids. These acids are absorbed by the stomach or can be excreted through exhalation. In the small intestine, quercetin can be glucuronidated by the action of uridine di-phosphate, methylated by the action of catechol-o-methyltranferase, and can be delycosylated to quercetin by the action of microbiota-derived β-glucosidase. These derivatives are transported to the liver for further metabolisms like methylation, glucuronidation, and sulfation through the hepatic portal vein. The resulting quercetin can be released into blood circulation via the portal vein of the liver. The absorption of quercetin and its derivatives takes place in the large intestine due to the presence of microorganisms that can break down quercetin and form absorbable metabolites. The absorbed quercetin and its derivatives were excreted through bile, urine, or through feces [22].

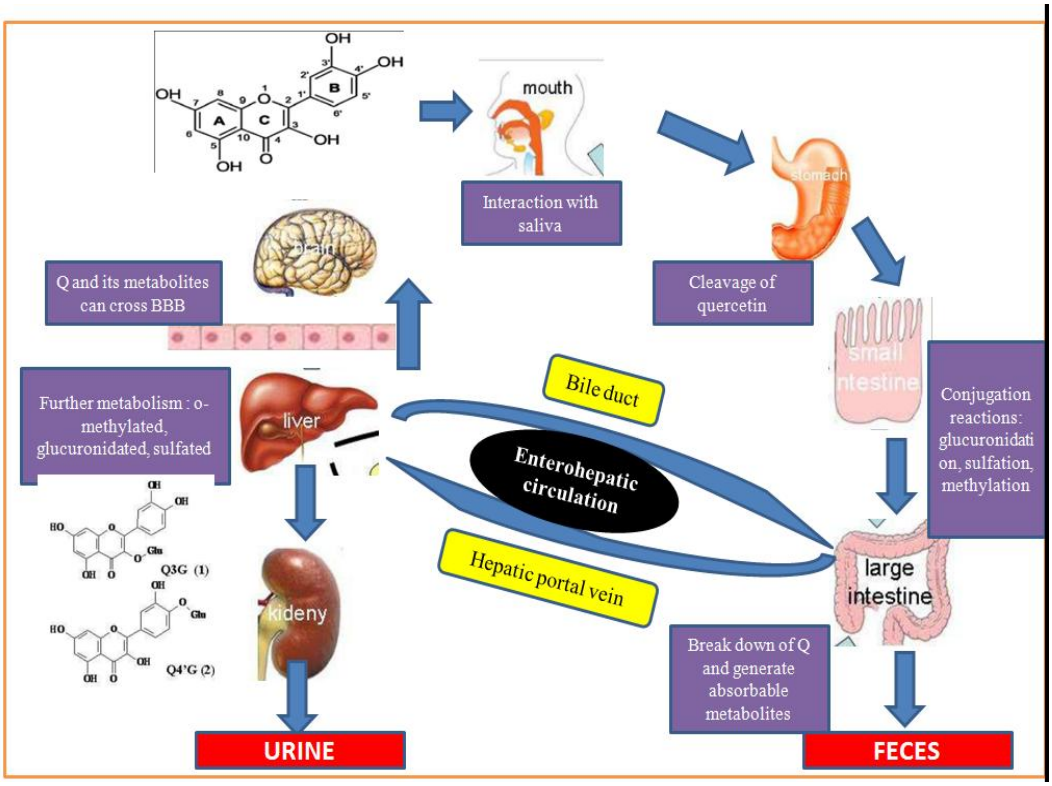

Fig. 5. Schematic representation of metabolism, absorption and excretion of quercetin (Q) (source: Wang *et al.*, 2016)

Table 1. The botanical sources of quercetin

Botanical Name	Family	Common Name	Active Parts
Punica granatum	Lythraceae	Pomegranate Fruits	Fruits
Ruta graveolens	Rutaceae	Rue	Leaves
Camellia sinensis	Theaceae	Green tea	Leaves
Allium cepa	Amaryllidaceae	Red onion	Fruits
Mangifera indica	Anacardiaceae	Mango	Fruits
Moringa oleifera	Moringaceae	Drumstick tree	Leaves
Cydonia oblonga	Rosaceae	Quince	Fruits and leaves
Solidago canadensis L.	Compositae/ Asteraceae	Goldenrod	Flowering parts
Vaccinium angustifolium and *Vaccinium corymbosum*	Ericaceae	Blueberries	Fruits
Phaleria macrocarpa	Thymelaceae	Mahkotadewa	Seeds
Lepidium latifolium	Brassicaceae	Papperweed	Roots and leaves
Achras sapota (Manilkara zapota)	Sapotaceae	Sapodilla	Fruits
Cichorium intybus	Compositae/ Asteraceae	Chicory	Leaves
Solanum lycopersicum	Solanaceae	Tomato	Fruits
Malus domestica	Rosaceae	Apple	Fruits
Vitis vinifera	Vitaceae	Grapevines	Fruits
Rhamnus alaternus	Rhamnaceae	Buckthorn	Bark
Passiflora incarnate	Passifloraceae	Passion flower	Leaves
Morus alba	Moraceae	White mulberry or Tut	Leaves
Ginkgo biloba	Ginkgoaceae	Maidenhair tree	Leaves
Hypericum perforatum	Hypericaceae	St. John's wort hypericum	Aerial parts
Achillea millefolium L.	Compositae/ Asteraceae	Yarrow	Flowering tops

(Source. Khan et al., 2019)

Table 2. Quercetin content in selected vegetables and fruits (Source. Costa *et al*., 2016)

Sources	Content (mg/100 g)
Onion	11–33
Lettuce	10–30
Pepper	10–30
Broccoli	3–5
Tomato	2–4
Asparagus	7–20
Peas	14
Apple	2-5
Cherry	1–3
Blueberry	5

8. SOURCES OF QUERCETIN

The bioavailability of quercetin is generally low in animals and its value also varies from animal to animal. In plasma, it is normally available in the nanomolar range (>100 nm) but after ingestion, it reaches the micromolar range [23]. Table 1 enlists some botanical sources of quercetin as well as Table 2 provides the quantity of quercetin available in some food sources.

9. QUERCETIN AS AN ANTIOXIDANT

Oxidative stress occurs when reactive oxygen species (ROS) build up in cells, either from high production or insufficient neutralization, causing harm to proteins, lipids and DNA. Mitochondria are a chief contributor of cellular ROS; ROS produced in the mitochondria can also target the electron transport chain (e.g., complex I), resulting in a cycle where ROS production increases, that is followed by ATP depletion and at last cell death. It is a major hallmark of Alzheimer's disease pathogenesis. On the basis of that, the detection of novel compounds which can neutralize oxidative stress as potential therapeutics is a very active area of research. Natural compounds like quercetin have received much attention in this regard due to its potent antioxidant activity. Various *in vivo* studies also suggest that quercetin prevents oxidative imbalance in a cell either through directly acting on enzymatic activity or through various signal transduction pathways.

10. AS DIRECT ANTIOXIDANT

Quercetin can directly scavenge ROS in *in vitro* condition at a concentration of 5 to 50 µM [22]. Quercetin is required in nanomolar range to be effectively used as an antioxidant. The B ring and OH group at position 3 makes quercetin an effective scavenger of ROS ($O2^{\cdot}$.) and RNS (NO, ONOO). It has been recommended that quercetin and its metabolites can act by modulating the cells own antioxidant defense mechanism, which signifies that quercetin may act as a pro-oxidant rather than an antioxidant. A mild degree of oxidative stress may certainly enhance the cells own antioxidant defenses, resulting in overall cytoprotection [26].

11. EFFECT ON ENZYMATIC ACTIVITY

During the formation of oxygen free radicals in cells various enzymes act upon them to balance the oxidative stress. When ROS is generated in the cell, the superoxide dismutase enzyme (SOD) can capture it and transform it into H_2O_2. SOD can again convert the toxic H2O2 into a nontoxic H2O form. Glutathione (GSH) is an antioxidant enzyme required as a hydrogen donor to complete this reaction. GSH accepts hydrogen and converts to GSSH i.e. oxidized glutathione disulfide. Hence, GSH is an important enzyme for controlling the level of oxidative stress in our bodies. It can control the levels of GSH and hence can increase the body's antioxidant capacity. It was reported that quercetin therapy can increase GSH levels in renal ischemia or reperfusion. Once quercetin is applied at higher doses, the equilibrium of GSH under the action of glutathione peroxidase is affected and free radicals are easily converted into nontoxic form [27].

Acetylcholinesterase (AchE) and butyrylcholinesterase (BchE) are the two key enzymes that can cause oxidative stress in cells. The hydroxyl group on the side chain of the phenyl ring of quercetin can add the necessary amino acid residues at the active site of these enzymes and can inhibit the effect of both of these enzymes [28]. Quercetin can improve the amount of manganese-induced antioxidant enzymes in rats and hence inhibiting manganese poisoning. Quercetin can significantly increase the expression level of endogenous antioxidants like Cu/Zn SOD, MnSOD, and catalase in the hippocampal CA1 pyramidal neurons of animals during ischemic injury. It can remove free radicals and upregulate the expression of heme oxygenase1 (HO-1) and superoxide dismutase1 (SOD1) which protect primary human osteoblasts exposed to cigarette smoke and hence they can promote fracture healing in smokers [29].

Lipopolysaccharides (LPS) are one of the major sources for generating oxygen free radicals. LPS can cause histopathological and biochemical damage to heart muscles in the endotoxemia model. Quercetin has been reported to prevent the LPS-induced oxidative imbalance in animals [27].

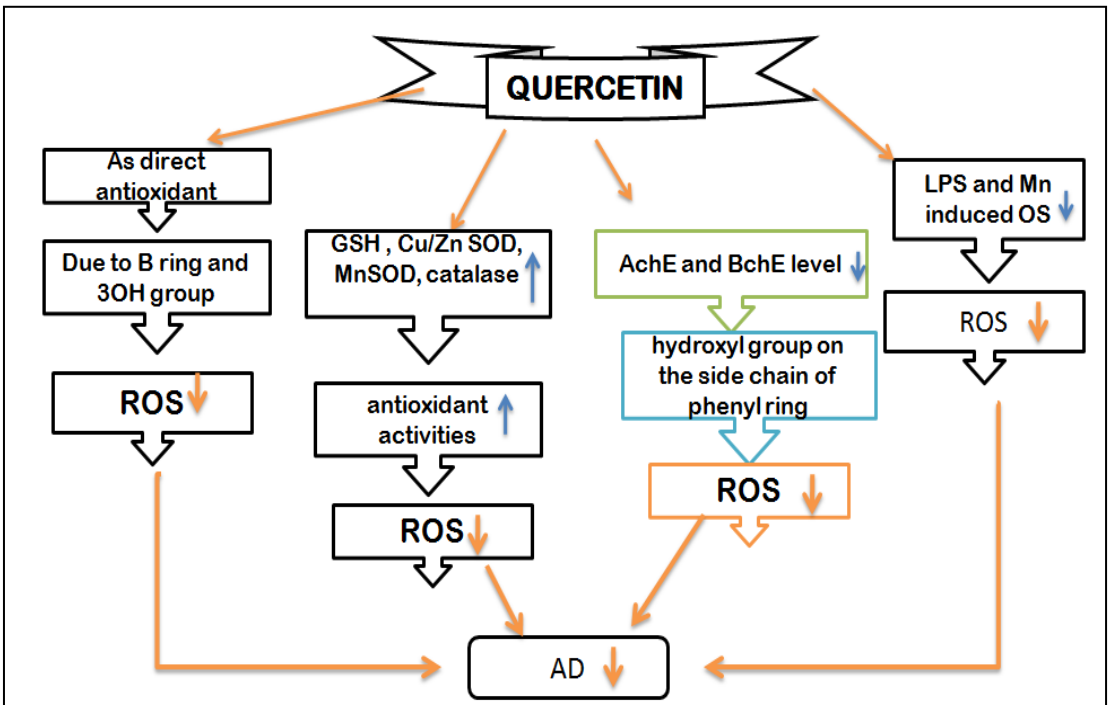

Fig. 6. Flow chart of antioxidant activities of quercetin

12. MODULATION OF ANTIOXIDANT PATHWAYS

In humans quercetin is reported in preventing or treating damage or toxicity by directly increasing antioxidant properties through signal transduction pathways.

12.1 Nrf-2 Signaling Pathway

Nrf-2 (nuclear factor erythroid 2-related factor-2) is a regulator of cellular resistance to oxidants [30]. Activation of the Nrf-2 pathway helps in providing neuroprotection against oxidative damage and cell death. Under the physiological situation, Nrf-2 is sequestered by the protein Keap1 (Kelch-like ECH-associated protein) with Cullin 3- base E3 ligase in the cytoplasm, resulting in the ubiquitination of Nrf2 protein and targeting it for proteosomal degradation. Keap1 has numerous cystein residues that make it acts as a molecular switch, responding to electrophiles and ROS with a structural change that releases Nrf2. Dissociated Nrf2 then moves into the nucleus where it binds to small Maf proteins. The created heterodimer then binds to cis-acting antioxidant response elements (ARE) and in that way activates the transcription of a broad range of phase II and antioxidant genes such as heme oxygenase1, glutamate cysteine ligase, glutathione S-transferases, glutathione peroxidase, superoxide dismutase, catalase, sulfiredoxin and thioredoxin [31].

Studies showed that quercetin could enhance the ARE binding activity and Nrf-2-mediated transcription in human HepG-2 cells. Thus, it increases the level of antioxidant enzymes by enhancing the Nrf-2 ARE pathway. Quercetin could improve mitochondrial function by promoting the translocation of Nrf-2 from the cytoplasm to the nucleus and activating the Nrf-2 ARE pathway. Lipopolysaccharide (LPS) treatment reduces the expression of protein N-Nrf-2 (nuclear) and N-Nrf2/C- Nrf2 value and it upregulates the expression of C-Nrf-2 (cytoplasmic). On the other hand, quercetin can increase the protein expression of N-Nrf2 and N-Nrf2/C-Nrf2 and can decrease the protein expression of C-Nrf-2. Hence, quercetin decreases the nuclear translocation of Nrf-2 from the cytoplasm to the nucleus. Consequently, in the mRNA and protein levels of Nrf-2 downstream genes, it was found that quercetin attenuated the levels of MNSOD2, HO-1, and NQO1, those were reduced by LPS. 200 mg/kg of quercetin could protect oxidative stress induced by LPS [32].

Treatment of cells with quercetin can inhibit cell growth and increases the expression of Nrf-2 at the level of both mRNA and protein. Nrf-2 knockdown mice show higher cytotoxicity due to higher apoptotic activities. Knockdown of Nrf-2 mediated enhancement of pro-apoptotic Bax and reduce the level of anti-apoptotic Bcl-2 which can increase apoptosis of the cell. In addition, Nrf-2 knockdown multiple myeloma (MM) cells are very much sensitive

toward cisplatin-induced oxidative stress [33]. Another recent study on ARPE-19 cells reported that Que-PC i.e. phospholipid- Quercetin complex has protective effects in inhibiting oxidative stress [27]. It can protect the cells from ochratoxin-induced oxidative stress and redox signaling [34].

12.2 Paraoxonase-2 Pathway

PON2 is a member of the paraoxonase family of genes that also includes PON1 and PON3 genes. PON2 is greatly found in the astrocytes of dopaminergic regions while its distribution is restricted to the mitochondria, which is a major source of generating free radicals. PON2 proteins have been detected in the mouse, rat, monkey, and human brains**.** In HeLa cells, PON2 has been shown to bind with co-enzyme Q10 that links with complex in mitochondria. A deficiency of PON2 causes dysfunctions in mitochondria and hence oxidative imbalance [31].

In mouse striatal astrocytes, neurons, and macrophages, it was reported that quercetin increases the expression of PON2. Quercetin can induce a very low level of oxidative stress, which in turn would change the JNK/AR1 pathway, causing a raise in PON2 expression. Quercetin can also induce PON2 expression through its phytoestrogen activity. The capacity of quercetin to induce PON2 may play a part in its neuroprotective actions. In striatal astrocytes of wild-type of mice, quercetin provides four-fold protection to ROS levels and cytotoxicity induced by H_2O_2 or DMNQ [32].

12.3 c- Jun- N-terminal Kinase (JNK) Mediated Pathway

JNK is a major signaling process of the MAP kinase pathway having three types of genes- JNK1, JNK2, and JNK3. Both JNK1 and JNK2 have been expressed in obesity-induced insulin resistance while JNK3 is specifically expressed in the CNS and is related to neurodegenerative diseases. In the INS-1 insulin-secreting B cells, it was reported that oxidative stress induced by H2O2 was regulated by p38 MAPK phosphorylation, which was actually positively regulated by the ERK1/2 pathway and negatively regulated by the JNK pathway. Therefore the antioxidative effects of quercetin appear to be consequent to ERK ½ hyperactivation and dependent on the balance between ERK1/2 and JNK activation. Quercetin has the property to attenuate the JNK and degrade its activator protein (AP1) transcription factor. Hence it is reported that quercetin can inhibit the generation of ROS in CaCl2- induced AAA mice models. Quercetin also attenuates H_2O_2-mediated ERK and JNK phosphorylation. Furthermore, quercetin can affect the JNK pathway through its anti-inflammatory activities [31].

12.4 Protein Kinase C (PKC) Pathway

PKC is a group of serine-threonine kinases composed of 11 iso-enzymes which are usually related to gene expression, cell proliferation, protein secretion, and inflammatory responses. ROS can affect PKCs through redox signaling and can activate them by oxidation of their cystein residues. Quercetin can regulate the expression of almost all PKCs in ascites cells of Dalton's lymphoma-bearing mice. It can thereby reduce oxidative stress and can facilitate differential localization of TNFR1 levels in ascites cells and subsequently induce apoptosis. It has been investigated that quercetin can be used as an antimanic drug in manic disorder due to its antioxidant properties [31].

12.5 Mitogen-activated Protein Kinase (MAP Kinase) Pathway

MAPK is a family of highly conserved protein kinases with serine-threonine that include a chain of proteins to amplify any signal through this pathway. MAPK-activated protein kinases can regulate various cellular processes for e.g. differentiation, movement, stress response, cell proliferation, apoptosis, etc. In HepG2 cells, quercetin can induce the phosphorylation of JNK, p35, P13kinase/Akt and can also increase the Nrf2-ARE binding activity. This can result in the expression of metallothionein (MT) proteins that are effective against toxicity induced in liver cells [35]. Quercetin can induce the activity of HO-1 in human hepatocyte cells to protect them from oxidative stress which can be done through the MAPK pathway via activating p38 and Nrf2 transcription [36].

It has been reported from the microglial cells that oxidative stress induced through LPS can be inhibited by quercetin following the MAPK pathway. This pathway can also inhibit nitric oxide (NO) production. But some studies also showed that quercetin possesses a negative impact on the MAPK pathway. A study reported that quercetin could inhibit MAPK signalling through down-regulating the expression of anti-apoptotic factor Mcl-1 of leukemia and B cells from chronic lymphocytic leukemia [31].

12.6 PI3/Akt Pathway

PI3/Akt is a family of cascades belonging to serine/ threonine kinase that can act on several target proteins upon phosphorylation. The best-known downstream

effector of Akt is mTOR i.e. the mammalian target of rapamycin. One experiment with high-fat diet-fed mice reported a heavy amount of ROS and carbonyls. These high amounts of ROS can be decreased by increasing the activity of hippocampal genes such as Akt, Nrf-2, etc [37]. Quercetin treatment can significantly induce the antioxidant and anti-apoptotic properties in the brain of ischemic rats that can be evidenced by expression of Cyt-C and GSH, GPX in the straitum and cortex of brain regions [38]. When the HT22 hippocampal neurons are treated with the same inhibitor it is reported that quercetin can down-regulate or inhibit the GSK3β activity but can activate PI3K/Akt pathway [39]. Quercetin also exerts an effect on oxidative stress-induced tau protein hyperphosphorylation. It can suppress the Akt pathway and down-regulates the phosphorylation of tau [31].

13. OTHER ROLES OF QUERCETIN IN COMBATING AD

Alzheimer's disease which is related to 60-80 percent of dementia cases can be characterized through Aβ plaque formation, NFTs formation, synaptic dysfunctions, etc. These all can cause AD directly or through inducing oxidative stress. Hence these anomalies are identified as an important drug target for AD. Various studies suggest that quercetin targets anomalies and can reduce the level of Aβ plaque formation, NFTs formation, AchE inhibition, and other attenuating oxidative stress in AD.

13.1 Inhibition of Aβ Aggregation

As it is known that cleavage of APP by β and ϒ-secretase results in the formation of insoluble and neurotoxic plaque of Aβ peptide, which is a major hallmark for AD pathogenesis. It is associated with causing an oxidative imbalance in our bodies. Various in vitro studies reported that quercetin could prevent the formation of Aβ plaque by forming hydrophobic interactions and H – bonds with β- sheet structure of Aβ. The hydroxyl group of the B ring in quercetin has the property to inhibit the aggregation of Aβ [26]. Quercetin can competitively bind with the metal binding site of Aβ and hence their ketoenolate group contributes to the reduction of oxidative stress induced by amyloid β metal ion complex [31].

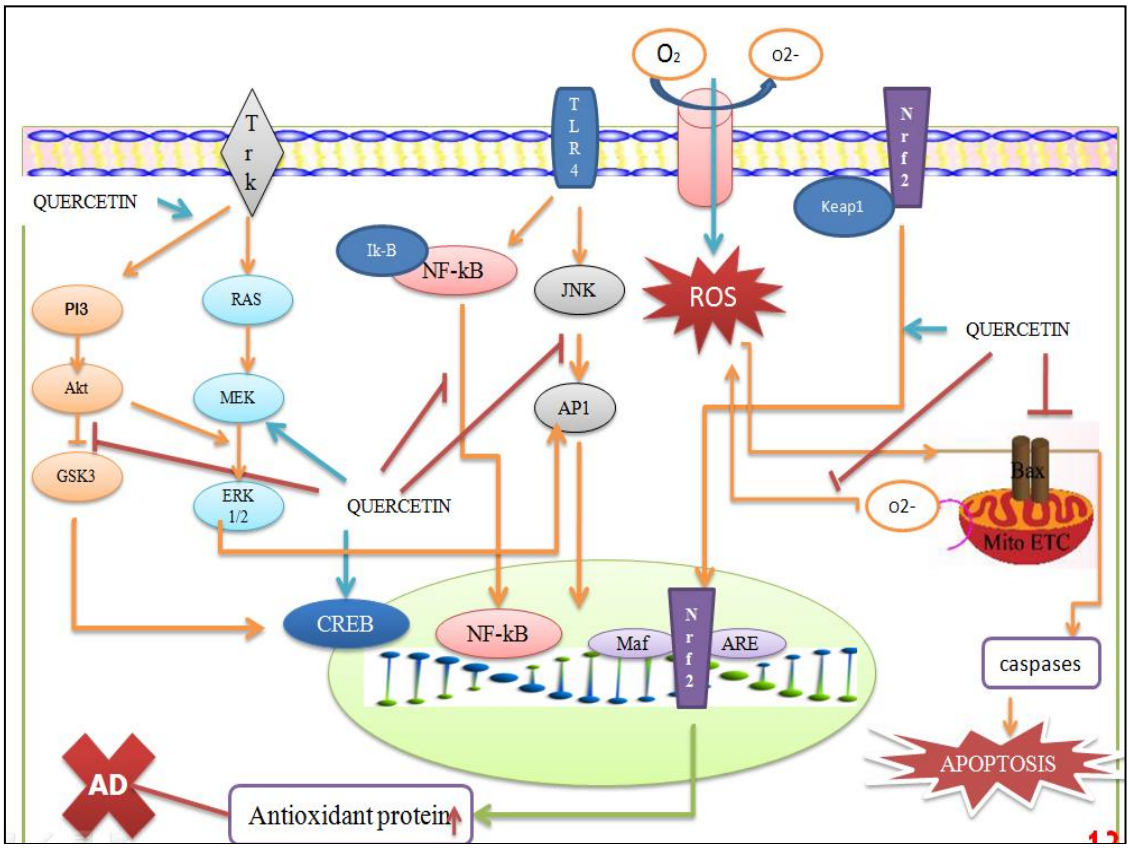

Fig. 7. Schematic representation of modulation of antioxidant signaling pathways through quercetin (Zaplatic *et al.*, 2019 Xu *et al.*, 2016 Islam *et al.*, 2013)

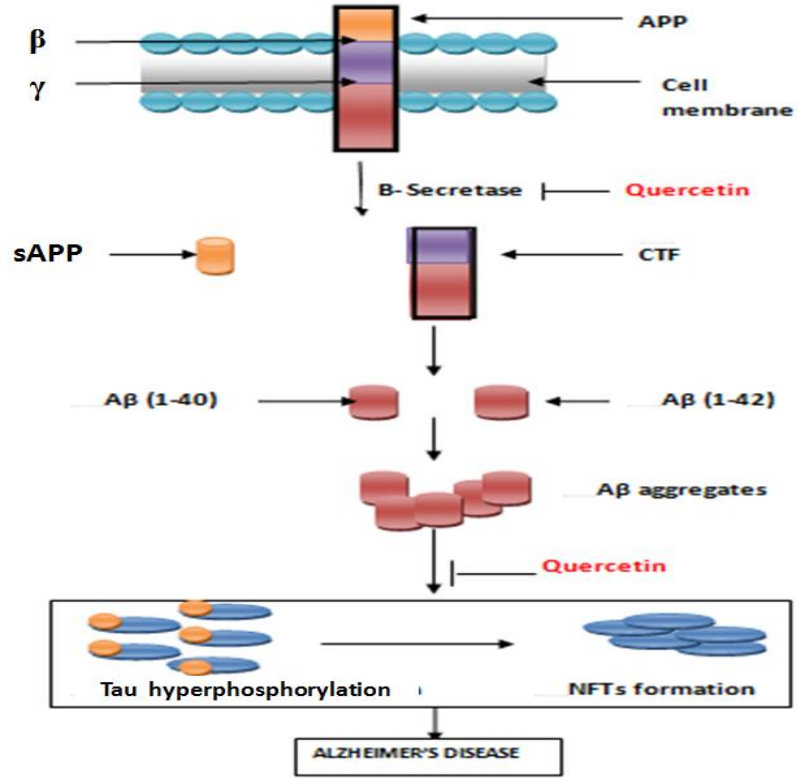

Fig. 8. Role of quercetin in inhibiting AB plaques and Tau hyperphosphorylation (Zaplatic *et al*., 2019)

The β- site of APP cleaving enzyme 1 (BACE1) is a vital therapeutic target for controlling Aβ plaque formation in AD. Studies revealed that flavonol compounds such as quercetin, rutin, kaempferol, morin, etc have direct inhibitory activity on BACE1. It was reported in one *in vitro* study that quercetin and rutin at a concentration of 100 µm can inhibit the activity of BACE1 up to 11.85 percent and 50.67 percent, respectively [40].

13.2 Inhibition of NFTs Formation

Hyperphosphorylation of tau protein that leads to the formation of NFTs is a vital regulator of AD as discussed in the above chapters. Studies till now cannot conclude whether Aβ can form NFTs or not. Various recent studies show that quercetin can directly reduce the hyperphosphorylation of Tau. It can reverse the MAPK and PI3/Akt/GSK3β pathways in the HT22 cells from mice models which are related to hyperphosphorylation of tau [26].

13.3 Inhibition of AchE Activity

Acetylcholinesterase enzyme (AchE) is responsible for degradation of the acetylcholine which is an important neurotransmitter. The high amount of AchE means lower H acetylcholine and hence low cognitive functions. This is known as the cholinergic hypothesis of AD which describes the role of AchE in preventing cholinergic transmission**.** Quercetin can make either H- a bond or hydrophobic interaction with AchE and hence can terminate its activity [26]. Quercetin at a dose of 50mg/kg body weight can stop the activity of AchE in the cerebral cortex and hippocampus. Another study suggests that ingesting quercetin before 1 hour of administration of scopolamine can prevent the memory impairment caused due to the action of scopolamine [41]. Quercetin is found to be much superior to the conventional AchE inhibitor drugs hence it needs further studies [42].

14. CONCLUSION

Quercetin is a notable flavonol with pharmacological effects and promising therapeutic potential. It has the potential to combat neurodegeneration. It can protect neuronal cells by attenuating oxidative stress and neuroinflammation. The anti-AD properties of quercetin include the prevention of Aβ aggregation and tau hyperphosphorylation. It also restores the Acetylcholine levels through the inhibition of hydrolysis of acetylcholine by the AchE enzyme. The ERK1/2, Nrf-2, PI3K/Akt, JNK, and MAPK are the pathways that describe the mechanism of action for

the antioxidant potentiality of quercetin. In particular, quercetin's anti – Alzheimer activity has witnessed various mechanisms of action both direct and indirect at the cellular and molecular levels.

However, its application in the pharmacology field is limited by its low absorption into the body based on its poor solubility, low bioavailability, poor permeability, and instability. But some modifications can change its bioavailability and antioxidant effects. The modification methods of quercetin are usually divided into two types- the derivation of quercetin or recombination with active groups. These previous changes the structure of quercetin and improves its solubility through derivation, whereas the latter produces a synergistic impact supported by the properties of active teams and quercetin-like metal complexes of quercetin [24]. Being a potent lead compound as an antioxidant, structural manipulations on quercetin can provide a drug candidate/ a drug that limits the progression of the disease. Therefore, further preclinical and clinical studies need to be done so as improve quercetin molecule and pharmacological properties.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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