# PALM OIL TOCOTRIENOLS AND ITS POTENTIAL AGAINST ALZHEIMER'S DISEASE AND BRAIN ISCHEMIA

**Ibrahim Musa*[1],** Huzwah Khaza'ai[1], Mohd Sokhini Abdul Mutalib[2]

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
[2]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**E-mail of Corresponding Author:** ibrahimm_83@yahoo.com

**Abstract**
Many neurodegenerative disorders and syndromes are associated with an excessive generation of reactive oxygen species (ROS) and oxidative stress. In the past few years, the pathophysiological role of ROS has been intensively studied in *in-vitro* and *in vivo* models of chronic neurodegenerative disorders such as Alzheimer's disease (AD) and stroke. In AD, oxidative neuronal cell dysfunction and cell death caused by protofibrils and aggregates of the AD-associated amyloid β protein (Aβ) may causally contribute to pathogenesis and progression. ROS and reactive nitrogen species also take part in the complex cascade of events and the detrimental effects occuring during ischemia and reperfusion in stroke. Lately tocotrienols have gained increasing attention as its therapeutic potential against neurodegenerative disorders was investigated. Compared to tocopherols, recent evidences have shown that tocotrienols are more potent antioxidant. In this review, the potential of tocotrienols to provide protection against selected neurodegenerative disorders is discussed.
**Keywords:** vitamin E, tocotrienol, antioxidant, oxidative stress

## 1. Introduction

A lot of research efforts into the elucidation of the mechanisms of neurodegeneration in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and stroke has led to an increased understanding of the basic mechanisms of nerve cell death. In general, cell death can follow two basic pathways; apoptosis and necrosis. Apoptosis which is also known as programmed cell death, is an active form of cell degeneration and is executed by enzymes such as caspases. On the other hand, necrosis is frequently occurs during acute insults and is characterized by rapid cell lysis. Both process of cellular degeneration are frequently associated with an excessive generation of free radicals and other cellular events which later lead to oxidative stress[1,2,3]. By definition, the term oxidative stress refers to the excessive oxidation of cellular biomolecules that leads to cellular damage, which is carried out by reactive oxygen species (ROS) including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$), and metabolites thereof[4]. Numerous types of radicals with diverse lifetimes, reactivities, and oxidative potencies exist, but some of the most biologically detrimental ones are formed, for instance, during the reduction of molecular oxygen to water in mitochondria.

The high reactivity of certain ROS causes oxidative damage. Oxidation may affect the DNA, which may lead to nucleotide dimerization and ultimately to errors during the replication process. Lastly, they frequently affect membrane lipids, or more specifically, the unsaturated carbohydrate side chains of phospholipids that are preferred sites for oxidation, leading to membrane dysfunction and cell lysis[5]. ROS are regular byproducts of cellular physiology. When generated in excess, for example in the mitochondria or after peractivation of intracellular oxidases, ROS can have destructive effects. In general, however, many ROS also exert vital functions that range from host defense to neuronal signal transduction. In order to maintain the balance between ROS generation and detoxification, as well as to prevent ROS from accumulates, several cellular defense systems exist. These comprise of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT)and glutathione peroxidase (GPx), and non-enzymatically actine compounds, for example; ascorbate, vitamin C, glutathione (GSH) and vitamin E. Mitochondrial dysfunction, various endogenous oxidase systems (eg. Cyclooxygenases) and other enzymes activated by exogenous triggers, which include neurotoxins, may cause overproduction and

accumulation of ROS which may lead to oxidative stress[6].

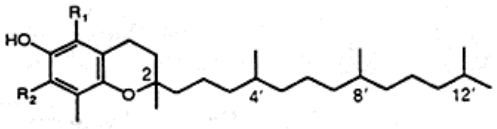
The evidence that suggest oxidative stress is tightly associated with the neurodegenerative disorders is increasing. These disorders include Alzheimer's disease (AD), Parkinson's disease (PD), stroke, traumatic brain injury (TBI) and amyotrophic lateral sclerosis (ALS). Although the specific sources of the accumulating oxidants and the affected target structures may differ between the particular pathologies in the nervous system, general features can be defined that include:

1. Increased levels of oxidatively altered metabolites are found in the post mortem tissue in neurodegenerative disease,
2. Compensatory defense reactions (oxidative stress response) can be seen in the affected nerve cells,
3. Disturbances of mitochondrial metabolism are observed which may account for an increased leakage of ROS originating from the reactions of the respiratory chain.

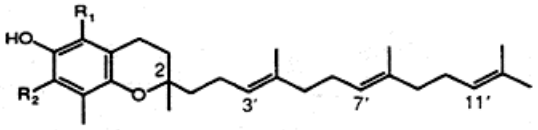
The brain appears to be particularly vulnerable to oxidations. Neuronal brain tissue is characterized by:

1. High energy requirements and high oxygen consumption,
2. An enrichment in peroxidizable fatty acids,
3. High levels of transition metals,
4. A relative deficit of antioxidant defenses compared with other tissues.

**2. Vitamin E family: Tocopherol and Tocotrienol:** In 1922, Herbert Evans and Katherine Bishop, two prominent researchers from Berkeley, first isolated fat-soluble vitamin E from green leafy vegetables and described it as a fertility factor. Deficiency of this vitamin is now known to cause severe degenerative diseases such as ataxia, Duchenne muscular dystrophy-like muscle degeneration, and infertility[7]. Vitamin E is present in most edible oils to various extents, including those extracted from wheat germ oil, wheat, rice bran, barley, oats, coconut and palm. While alpha-tocopherol was the first vitamin E analogue to be recognized, eight chemically distinct analogues are now known, consisting of alpha (α), beta (β), gamma (γ) and delta (δ)-tocopherols (TP), and α-, β-, γ-, and δ-tocotrienols (T3). Tocopherols are the saturated forms of vitamin E, whereas the tocotrienols are unsaturated and possess as isoprenoid side chain (Figure 1). Tocopherols consist of a chromanol ring and a 15-carbon tail. The presence of three trans double bonds in the tail distinguishes tocopherols from tocotrienols. The isomeric forms of toctrienol are distinguished by the number and location of methyl groups in the chromanol rings. Palm oil represents one of the most abundant natural sources of tocotrienols[8].




Tocopherol




Tocotrienol

| | α | β | γ | δ |
|---|---|---|---|---|
| **R1** | CH3 | CH3 | H | H |
| **R2** | CH3 | H | CH3 | H |

**Figure 1. Chemical structure of vitamin E analogs. The term vitamin E includes four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ).**

Palm tocotrienol-rich fraction (TRF) is an extract of palm oil and consists of 25% α-tocopherol and 75% tocotrienols. TRF has been shown to possess potent antioxidant[9,10], anti-inflammatory[11], anticancer[12, 13,14], neuroprotection[15,16] and cholesterol-lowering[17,18,19] activities. Previously, most studies have been focused on the antioxidant property of tocopherol. However, its passage through the blood brain barrier (BBB) is limited. Compared to tocotrienol, its unsaturated side chain allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver[20]. In this review, the potential of tocotrienol to provide protection against selected neurodegenerative disorders is discussed.

**3. Tocotrienol and Alzheimer's disease (AD)**

AD is characterized symptomatically by progressive cognitive and memory decline, speech loss and personality changes. It is histopathologically characterized by:

1. Synaptic alterations,
2. Nerve cell loss in certain brain regions,

3. Formation of protofibrils of amyloid β protein (Aβ) and extracellular deposition of amorphous Aβ,
4. The intracellular aggregation of hyperphosphorylated tau protein

A commonly held hypothesis regarding the development of AD is that it is due to altered processing of the amyloid precursor protein (APP), which leads to excessive β-amyloid (Aβ) formation or aggregation[21]. Several genes are known to cause AD in a small percentage of patients. All these genes, for example, APP gene on chromosome 21, presenilin 1 gene on chromosome 14, and presenilin 2 gene on chromosome 1, are associated with an increase in β-amyloid formation[22]. The apolipoprotein (apo) E-4 genotype is a significant risk factor for AD. Similar to the other genes that cause AD, the APOE*E4 allele is associated with increased amyloid deposition. Aβ aggregates then trigger the activation of microglia, inducing the production of oxidants, cytokines and prostanoids, that work in various ways to: (1) boost the production of Aβ by neurons and astrocytes; (2) kill neurons, either directly or by increasing their sensitivity to excitotoxicity; (3) disrupt neuron structure and function by promoting excessive phosphorylation of tau, giving rise to the characteristic neurofibrillary tangles; (4) disrupt the protective function of astrocytes[23].

It has been demonstrated that treatment of neuronal cells with TRF is able to reduce neurotoxicity caused by the exposure to Aβ protein[24]. In this study, neuronal cells were divided into 4 groups; untreated cells served as control, treated with 10 µM Aβ peptide for 24 hours, 5 µg/ml TRF for 24 hours followed by 10 µM Aβ peptide for 24 hours (pre-treatment) or 10 µM Aβ peptide for 24 hours followed by 5µg/ml TRF for 24 hours (post-treatment). From this study, results showed that when neurons were exposed to 10 µM Aβ peptide, percentage of DNA damage is significantly higher compared to control. Treatment with TRF significantly lowers the DNA damage. Pre- and post-treatment with 5 µg/ml TRF also significantly increased the number of viable cells, which demonstrated that TRG was able to prevent cell death induced by the Aβ peptide. This observation is further supported through fluorescence staining, where cells that treated with TRF still able to retain their membrane integrity.

Aβ can induce ROS production through multiple pathways. One way is by binding metal-ions ($Fe^{2+}$, $Cu^{2+}$), which then can drive the production of $OH^{\cdot}$ from peroxides through the Fenton reaction. Initial lysis of neurons occurring in AD tissue also can leads to the release of excitatory amino acids such as glutamate, which subsequently induces oxidative processes either though binding to NMDA receptor or through its competetition for the neuronal cystine antiporter system leading to a depletion of intracellular redox-shuttle glutathione, GSH[25,26]. Aβhas been reported to produce $H_2O_2$ through the metal ion reduction pathway[27]. In the past few years, more studies have been conducted to observe the neuroprotective effects of tocotrienol against peroxides. Tocomin 50% which contains α-tocopherol, and α-, γ-, and δ-tocotrienols significantly inhibited $H_2O_2$-induced neuronal death using primary cultures of rat striatal neurons, in contrast toα-tocopherol, it failed to reduce $H_2O_2$-induced neuronal death[28]. In this study, both the LDH release assay and MTT assay revaled that treatment of striatal cultures with various concentrations (10-100 µM) of $H_2O_2$ for 24 hours resulted in a concentration-dependent reduction in cell viability. Then the cultures were exposed to 30 µM $H_2O_2$ in the presence or absence of Tocomin 50% (0.1-10 µM) for 24 hours. As expected, $H_2O_2$ caused neuronal death, however, Tocomin 50% produced a concentration-dependent suppression of $H_2O_2$-induced neurotoxocity, with significant protection at concentrations of 1 and 10 µM. This study also demonstrated that, in terms of neuroprotective efficacy in rat striatal cultures, vitamin E analogs ranked as follows: α-tocotrienol > γ-tocotrienol > δ-tocotrienol. According to indices of protein oxidation and lipid peroxidation, however, γ-tocotrienol is most effective followed by α-tocotrienol, δ-tocotrienol and α-tocopherol[29,30].

The suggestion that tocotrienols can protect nerve cell from hydrogen peroxide toxicity is further supported by other study. In a recent study, the death of neurons that have been exposed to various concentrations of $H_2O_2$ were prevented when treated with 5 µM of vitamin E isoforms[31]. The preventive effects of α- and γ-tocotrienols were significantly stronger than α-tocopherol. To detect early events of cell degeneration, neurite formation was checked with phase-contrast microscopy. Treatment with $H_2O_2$ induced abnormal morphology and significantly induced neurite beading, which was prevented by treatment with α- and γ-tocotrienols. This study also focused the effect of vitamin E treatment towards the changes of

collapsin response mediator proteins (CRMPs). These proteins belong to a family of cytoplasmic proteins in the brain and plays a crucial role in neurite polarity and axon guidance[32,33,34]. Changes in CRMP-2 level have been implicated in various neurodegenerative disorders, including AD, ischemia and Wallerian degeneration[35,36]. Administration of α- or γ-tocotrienol significantly attenuated the induction of unusual CRMP-2 protein in $H_2O_2$-treated neurons.

The effects of $H_2O_2$ toxicity towards viability and apoptosis of astrocytes also has been assessed by the MTS, LDH release assays (viability) and ssDNA assay (apoptosis) by using primary culture of astrocytes. Cell viability decreased by 50% when exposed to 100 µM of $H_2O_2$, and at more than 200 µM $H_2O_2$, almost all cells were dead. Pre-treatment with 1-10 µM γ-tocotrienol protected cells against $H_2O_2$-induced cell loss significantly[37]. α-Tocotrienol and γ-tocotrienol in µM concentration have also been reported to be more effective than α-tocopherol in protecting against glutamate-induced cell death in HT4 neuron cell cultures[20]. More interestingly, 100 nM of α-tocotrienol has been showed to be able to protect HT4 hippocampal neurons[16,38] and primary cortical neurons against glutamate-induced cell death. There are several other studies have verified that tocotrienols were more effective antioxidants than α-tocopherol. For example, α-tocotrienol exhibited a higher level of antioxidant activity against lipid oxidation in rat liver microsomal membranes than α-tocopherol[10], and greater peroxyl radical-scavenging activity in liposomes[20]. TRF has been reported to be more effective than α-tocopherol in protecting rat brain mitochondria and rat liver microsomes against oxidative damage[29,30].

**4. Tocotrienols and Stroke (Brain ischemia)**

Free radicals have also been linked to a variety of neurological disorders and brain injuries accompanied by acute nerve cell death[4,39]. In focal and global cerebral ischemia, cerebral blood and oxygen flow is reduced in brain regions that are normally supplied by the occluded vessels. Brain damage associated with stroke is dependent on the severity and the duration of the interrupted blood supply to the area. Superoxide, $O_2^{\cdot -}$ and nitric oxide, NO. are generally believed to play major roles in the development of cerebral ischaemic damage[40]. Reperfusion leads to an abrupt supply of glucose and oxygen to previously deprived neurons, and this disrupts mitochondrial function by uncoupling oxidative phosphorylation and bringing about inhibition of complex I. These processes increase superoxide generation. In addition, disturbed intracellular homeostasis, including increased calcium levels, activates nitric oxide synthase, NOS to produce nitric oxide. The high concentration of superoxide and nitric oxide thus form peroxynitrie, ONOO-, which can directly induce damage or converted to other strong oxidants.

In addition to the direct oxidative damage to cellular macromolecules, ROS may also induce secondary events that mediate ischemia-related cellular changes. As major intracellular oxidative stress-sensitive targets, mitochondria, intracellular signaling cascades and transcription factors have been identified. ROS generated inside the mitochondria during ischemia/reperfusion cause the release of cytochrome c, which then interacts with the factors Apaf-1 and caspase-9, ultimately leading to the activation of apoptosis-associated caspases[4]. Downstream activation of caspase 3 leads to DNA-fragmentation and apoptosis. Mitochondria, however, are well known to be involved in necrotic pathways, and there is strong evidence that it depends on the severity of the ischemic insult, the particular cell type affected, and the nature of the intracellular signaling status, whether necrosis, apoptosis or intermediate forms of cell death occur. It has been demonstrated that if mitochondrial ATP production is completely stopped during ischemia, necrosis occurs[41,42]. The pathogenetic role of the excitatory amino acid L-glutamate in ischemic damage is of particular interest. Glutamate in an excitatory neurotransmitter that is present throughout the central nervous system (CNS). Elevated levels of extracellular glutamate however, are neurotoxic[26]. Glutamate toxicity is a major contributor to pathological cell death within the nervous system. Two forms of glutamate toxicity are present; receptor-initiated excitotoxicity[43] and non-receptor mediated toxicity[44]. The physiological consequences of extracellular glutamate are mediated by three classes of membrane proteins within the CNS. These are ionotropic glutamate receptors, metabotropic glutamate receptors and the cystine/glutamate antiporter. Glutamate acts post-synaptically on three families of ionotropic receptors, named after their preferred agonists, N-methyl-D-aspartate (NMDA), α-amino-5-methyl-4-isoxazolepropionic acid (AMPA) and

kainite. These receptors all incorporate ion channels that are permeable to cations, although the relative permeability to $Na^+$ and $Ca^{2+}$ varies according to the family and subunit composition of the receptor. In addition, glutamate can activates a family of G-protein receptors, referred as metabotropic glutamate receptors[45]. Both families of receptors are situated on various aspects of an excitatory synapse, including the pre-synaptic terminal, the post-synaptic terminal and astrocytes that sheath the synapse[46] (Engelman and MacDermott, 2004). Another target for extracellular glutamate in the central nervous system (CNS) is through the inhibition of the glutamate/cystine antiporter $X_c^-$, which is responsible for the transportation of cystine that is required for the synthesis of GSH.

The major tocotrienol-sensitive signaling pathways which are known to be involved in glutamate-induced neurodegeneration include c-Src and 12-lipoxygenase[16,38,47,48]. The c-Src and its structurally related members of the Src family are non-receptor tyrosine kinases that reside within the cell associated with cell membranes and appear to transduce signals from transmembrane receptors to the cell interior. Treatment of HT4 neuronal cells with elevated levels of extracellular glutamate resulted in more than 95% loss of cell viability within a duration of 12 hours, and this glutamate-induced cell death was prevented when pre-treated with nanomolar concentration of α-tocotrienol[16]. In this study, it was shown that, on a concentration basis, α-tocotrienol was more effective than α-tocopherol in protecting HT4 cells against glutamate-induced cytotoxicity. A dose-dependent study of α-tocotrienol and α-tocopherol showed that at a concentration of 50 nM α-tocotrienol, but not α-tocopherol, partially protected the cells against glutamate-induced death. At 250 nM α-tocotrienol, but not α-tocopherol, provided complete protection against loss of cell viability. Comparison of the two analogues of tocotrienol, α- and γ-, showed that α-tocotrienol was more effective than γ-tocotrienol in protecting against glutamate.

Vitamin E and its analogs are known to be potent inhibitors of 5-LOX [49], Vitamin E is also known to inhibit 15-LOX acitivity by specifically complexing with the enzyme protein[50]. A central role of inducible 12-LOX has been proposed in the execution of glutamate-induced neuronal death[57,58]. It has been reported that 12-LOX is subjected to rapid tyrosine phosphorylation in neuronal cells challenged with glutamate or GSH-lowering agents. Such phosphorylation is rapid and coincides with the c-Src activation. Previous studies have reported that rapid c-Src activation plays a central role in executing neurodegeneration[16,38]. It was also demonstrated in the other report that Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke[51]. It was also supported by other observation that Src family kinase-inhibitor PP2 reduces focal ischemic brain injury[52]. Further investigation has identified the 12-lipoxygenase (12-LOX) as a key tocotrienol-sensitive mediator of neurodegeneration [38]. This study showed that neurons isolated from 12-LOX-deficient mice are resistant to glutamate-induced cell death, which established that the 12-LOX indeed represents a critical checkpoint in glutamate-induced neurodegeneration. Further observation on *in vivo* model revealed that tocotrienol-supplemented rats showed more protection against stroke-induced injury compared to to controls[48]. Such protection was associated with lower c-Src activation and 12-LOX phosphorylation at the stroke site.

Neurons and the brain are rich in arachidonic acid (AA). Massive amounts of AA are released from the membranes in response to brain ischemia or trauma[53]. Subsequent work has established that AA and its metabolites may be neurotoxic. There are three major pathways of AA metabolsim lipoxygenases, cyclooxygenases and cytochrome P450. In the lipoxygenase pathway, metabolites of 12-LOX seem to be the major metabolite of AA in the brain[54] as well as in cultured cortical neurons[55] (Ishizaki and Murota, 1991). Lipoxygenases, mainly 5-, 12-, and 15-LOX, are named after their ability to insert molecular oxygen at the 5, 12, or 15-carbon atom of AA forming a distinct hydroperoxy-eicosatetraenoic (HPETE) acid[56] . 12-LOX produces 12(S)-HPETE which is further metabolized into 4 distinct products; an alcohol [12(S)-HETE], a ketone (12-keto-eicosatetraenoic acid), or two epoxy alcohols (hepoxilin A3 and B3). Using immature cortical neurons and HT cells, it has been shown that a decrease in intracellular concentration of GSH, [GSH]i triggers the activation of neuronal 12-LOX, which leads to the production of peroxides, the influx of $Ca^{2+}$, and ultimately cell death[57,58]. The 12-LOX metabolite 12-HPETE proved to be capable of causing cell death[59]. Highly reactive oxygen radicals are produced during the conversion of HPETEs to HETEs[60], contributing to the overall burden of oxidative stress following stroke. Under conditions of

GSH depletion, as in acute focal stroke, 12-LOX-derived 12-HPETE triggers nitric oxide (NO)-induced neural cell death[61]. 12-HETE has been shown to increase mitochondrial NO production, induce cytochrome c release, and subsequently cause mitochondrial dysfunction[62]. It has been proved that α-tocotrienol prevents glutamate-induced cell death in active c-Src overexpressing cells [16](Sen et al., 2000). Furthermore, glutamate-induced c-Src activity is completely blocked by nanomolar amounts of α-tocotrienol. Taken together, the powerful effects of palm oil-derived α-tocotrienol on c-Src and 12-LOX suggest that it is a potent inhibitor of 12-LOX-mediated AA metabolism and neurodegeneration.

**Conclusion**

Oxidative damage to cellular molecules and structures are related to the pathogeneses of neurodegenerative disorders. AD and stroke are among the most prominent examples. Previously, most works related to vitamin E are mainly focused on the antioxidative ability of α-tocopherol. However, its passage through the blood brain barrier is limited. Compared to tocotrienol, its unsaturated side chain allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver. Other than its neuroprotective property, tocotrienols has also demonstrated therapeutic potential in the treatment of cancer and hypercholesterolemia. Based on these increasing evidence, these isoforms of vitamin E remain as the most potent antioxidant available that could play a major role in the fight against neurodegenerative disorders.

**Acknowledgement**

This work was supported by the Malaysian Ministry of Science and Technology under research grant FRGS0106-26.

**References**

1. Behl C. Apoptosis and Alzheimer's disease. *Journal of Neural Transmission* 2000; 107:1325-1344.
2. Bradesen DE. Apoptosis: Overview and Signal Transduction Pathways. *Journal of Neurotrauma* 2000; 17:801-810.
3. Maher P, Schubert D. Signaling by reactive oxygen species in the nervous system. *Cellular and Molecular Life Sciences* 2000; 57:1287-1305.
4. Behl C, Moosmann B. Oxidative nerve cell death in Alzheimer's disease and Stroke: Antioxidants as neuroprotective compounds. *The Journal of Biological Chemistry* 2005; 383:521-536.
5. Sies H. Review: Strategies of antioxidant defense. *European Journal of Biochemistry* 1993; 215:213-219.
6. Sies H. Biochemistry of oxidative stress. *Angewandte Chemie International Edition in English* 1986; 25:1058-1071.
7. Aggarwal BB, Sundaram C, Prasad S, Kannappan R. Tocotrienols, the vitamin E of 21st century: Its potential against cancer and other chronic diseases. *Biochemical Pharmacology* 2012; 80:1613-1631.
8. Elson CE. Tropical oils: Nutritional and scientific issues. *Critical Reviews in Food Science and Nutrition* 1992; 31:79-102.
9. Maniam S, Mohamed N, Shuid AN, Soelaiman IN. Palm tocotrienol exerted better antioxidant activites in bone than alpha-tocopherol. *Basic and Clinical Pharmacology and Toxicology* 2008; 103:56-60.
10. Serbinova B, Kagan Y, Han D, Packer L. Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radical Biology and Medicine* 1991; 10:263-275.
11. Wu SJ, Liu PL, Ng LT. Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. *Molecular Nutrition and Food Research* 2008;, 52:921-929.
12. Wu SJ, Ng LT. Antioxidant and antihepatoma activities of palm oil extract. *Journal of Food Lipids* 2007; 14:122-137.
13. Takahashi K, Loo G. Disruption of mitochondria during tocotrienol-induced apoptosis in MDA-MB-231 human breast cancer cells. *Biochemical Pharmacology* 2004; 67:315-324.
14. Goh SW, Hew NF, Norhanom AW, Yadav M. Inhibition of tumour promotion by various palm oil tocotrienols. *International Journal of Cancer* 1994; 57:529-531.
15. Osakada F, Hashino A, Kume T, Katsuki H, Kaneko S, Akaike A. α-Tocotrienol provides the most potent neuroprotection among vitamin E analogs on cultured striatal neurons. *Neuropharmacology* 2004 47:904-915.
16. Sen CK, Khanna S, Roy S, Packer L. Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells. *Journal of Biological Chemistry* 2000; 275:13049-13055.
17. Minhajuddin M, Beg ZH, Iqbal J. Hypolipidemic and antioxidant properties of

tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food and Chemical Toxicology* 2005; 43:747-753.
18. Abdul Mutalib MS, Khaza'ai H, Klaus WJW. Palm tocotrienol rich fraction (TRF) is a more effective inhibitor of LDL oxidation and endothelial cell lipid peroxidation than α-tocopherol *in-vitro*. *Food Research International* 2003; 36:405-413.
19. Qureshi AA, Bradlow BA, Brace L, Manganello J, Peterson DM, Pearce BC, Wright JJK, Gapor A, Elson CE. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 1995; 30:171-177.
20. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L. Structural and dynamic membrane properties of α-tocopherol and α-tocotrienol: Implication to the molecular mechanism of their antioxidant potency. *Biochemistry* 1993; 32:10692-10699.
21. Selkoe DJ. Cell biology of the amyloid β-protein precursor and the mechanism of Alzheimer's disease. *Annual Review of Cell Biology* 1994; 10:373-403.
22. Grundman M. Vitamin E and Alzheimer disease: the basis for additional clinical trials. *American Journal of Clinical Nutrition* 2000; 71:630S-636S.
23. McCarty MF. Toward prevention of Alzheimer's disease – Potential nutraceutical strategies for suppressing the production of amylod beta peptides. *Medical Hypothesis* 2006; 67:682-697.
24. Musalmah M, Rusdiah RJ, Noor Aini AH. Induction of DNA damage and cell death by beta amyloid peptide and its modification by tocotrienol rich fraction (TRF). *Medicine and Health* 2009; 4:8-15.
25. Schubert D, Piasecki D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *The Journal of Neuroscience* 2001; 21:7455-7462.
26. Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. *Science* 1993; 262:689-695.
27. Brera B, Serrano A, de Ceballos ML. β-Amyloid peptides are cytotoxic to astrocytes in culture: a role for oxidative stress. *Neurobiology of Disease* 2000; 7:395-405.
28. Osakada F, Hashino A, Kume T, Katsuki H, Kaneko S, Akaike A. Neuroprotective effects of α-tocopherol on oxidative stress in rat striatal cultures. *European Journal of Pharmacology* 2003; 465:15-22.
29. Kamat JP, Sarma HD, Devasayagam TPA, Nesaretnam K, Basiron Y. Tocotrienols from palm oil as effective inhibitors of protein oxidation and lipid peroxidation in rat liver microsomes. *Molecular and Cellular Biochemistry* 1997; 170:131-138.
30. Kamat JP, Devasayagam TP. Tocotrienols from palm oil as potent inhibitors of lipid peroxidation and protein oxidation in rat brain mitochondria. *Neuroscience Letter* 1995; 195:179-182.
31. Fukui K, Takatsu H, Koike T, Urano S. Hydrogen peroxide induces neurite degeneration: Prevention by tocotrienols. *Free Radical Research* 2011; 45:681-691.
32. Yuasa-Kawada J, Suzuki R, Kano F, Ohkawara T, Murata M, Noda M. Axonal morphologenesis controlled by antagonistic roles of two CRMP subtypes in microtubule organization. *European Journal of Neuroscience* 2003; 17:2329-2343.
33. Fukata Y, Itoh T, Kimura T, Manager C, Nishimura T, Shiromizu T, Watanabe H, Inagaki N, Iwamatsu A, Hotani H, Kaibuchi K. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nature Cell Biology* 2002; 4:583-591.
34. Wang LH, Strimatter SM. A family of rat CRMP genes is differentially-expressed in the nervous system. *Journal of Neuroscience* 1996; 16:6197-6207.
35. Petratos S, Li QX, George AJ, Hou X, Kerr ML, Unabia SE, Hatzinisirou I, Maksel D, Aguilar MI, Small DH. The β-amyloid protein of Alzheimer's disease increases neuronal CRMP-2 phosphorylation by a Rho-GTP mechanism. *Brain* 2008; 131:90-108.
36. Cole AR, Noble W, van Aalten L, Plattner F, Meimaridou R, Hogan D, Taylor M, LaFrancois J, Gunn-Moore F, Verkhratsky A, Oddo S, LaFerla F, Giese KP, Dineley KT, Duff K, Richardson JC, Yan SD, Hanger DP, Allan SM, Sutherland C. Collapsin response mediator protein-2 hyperphosphorylation is an early event in Alzheimer's disease progression. *Journal of Neurochemistry* 2007; 103: 1132-1144.
37. Mazlan M, Sue Mian TS, Mat Top G, Wan Ngah WZ. Comparative effects of α-tocopherol and γ-tocotrienol against hydrogen peroxide induced apoptosis on primary-cultured astrocytes. *Journal of the Neurological Sciences* 2006; 243:5-12.
38. Khanna S, Roy S, Ryu H, Bahadduri P, Swaan PW, Ratan RR, Sen CK. Molecular basis of vitamin E action: Tocotrienol modulates 12-lipoxygenase, a key mediator of glutamate-induced neurodegeneration. *The Journal of Biological Chemistry* 2003; 278:43508-43515.

39. Chan PH. Reactive oxygen radicals in signaling and damage in the ischemic brain. *Journal of Cerebral Blood Flow and Metabolism* 2001; 21:2-14.
40. White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *Journal of the Neurological Sciences* 2000; 179:1-33.
41. Reed JC. Apoptosis-regulating proteins as targets for drug discovery. *TRENDS in Molecular Medicine* 2001; 7:314-319.
42. Fujimura M, Morita-Fujimura Y, Noshita N, Sugawara T, Kawase M, Chan PH. The cytosolic antioxidant copper/zinc-superoxide dismutase prevents the early release of mitochondrial cytochrome c in ischemic brain after transient focal cerebral ischemia in mice. *The Journal of Neuroscience* 2000; 20:2817-2824.
43. Finiels F, Gimenez y Ribotta M, Barkats M, Samolyk M-L, Robert JJ, Privat A, Revah F Mallet J. Specific and efficient gene transfer strategy offers new potentialities for the treatment of motor neuron diseases. *NeuroReport* 1995; 6: 2473-2478.
44. Froissard P, Monrocq H, Duval D. Role of glutathione metabolism in the glutamate-induced programmed cell death on neuronal-like PC-12 cells. *European Journal of Pharmacology* 1997; 326:93-99.
45. Nakanishi S, Masu M. Molecular diversity and functions of glutamate receptors. *Annual Review of Biophysics and Biomolecular Structure* 1994; 23:319-348.
46. Engelman HS, MacDermott AB. Presynaptic ionotropic receptors and control of transmitter release. *Nature Reviews Neuroscience* 2004; 5:135-145.
47. Khanna S, Roy S, Parinandi NL, Maurer M, Sen CK. Characterization of the potent neuroprotective properties of the natural vitamin E alpha-tocotrienol. *Journal of Neurochemistry* 2006; 98:1474–86.
48. Khanna S, Roy S, Slivka A, Craft TKS, Chaki S, Rink C, Notestine MA, DeVries AC, Parinandi NL, Sen CK. Neuroprotective properties of the natural vitamin E α-tocotrienol. *Stroke* 2005; 36:2258-2264.
49. Reddanna P, Rao MK, Reddy CC. Inhibition of 5-lipoxygenase by vitamin E. *FEBS Letters* 1985; 193:39-43.
50. Grossman S, Waksman EG. New aspects of the inhibition of soybean lipoxygenase by α-tocopherol: Evidence for the existence of a specific complex. *International Journal of Biochemistry* 1984; 16:281-289.
51. Paul R, Zhang ZG, Eliceiri BP, Jiang Q, Boccia AD, Zhang RL, Chopp M, Cheresh DA. Src deficiency or blockade of Src activity in mice provides cerebral protection following stroke. *Nature Medicine* 2001; 7:222-227
52. Lennmyr FE, Ericsson E, Gerwins P, Akterin S, Ahlström H, Terént A. Src family kinase-inhibitor PP2 reduces focal ischemic brain injury. *Acta Neurologica Scandinavia* 2004; 110:175-179.
53. Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: The other half of the natural vitamin E family. *Molecular Aspects of Medicine* 2007; 28:692-728.
54. Carlen PL, Gurevich N, Zhang L, Wu PH, Reynaud D, Pace-Asciak CR. Formation and electrophysiological actions of the arachidonic acid metabolites, hepoxilins, at nanomolar concentrations in rat hippocampal slices. *Neuroscience* 1994; 58:493-502.
55. Ishizaki Y, Murota S. Arachidonic acid metabolism in cultured astrocytes: presence of 12-lipoxygenase activity in the intact cells. *Neuroscience Letters* 1991; 131:149-152.
56. Yamamoto S. Mammalian lipoxygenases: molecular structures and functions. *Biochimica et Biophysica Acta* 1992; 1128:117-131.
57. Tan S, Schubert D, Maher P. Oxytosis: a noverl form of programmed cell death. *Current Topics in Medicinal Chemistry* 2001; 1:497-506.
58. Li Y, Maher P, Schubert D. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. *Neuron* 1997; 19:453–463.
59. Gu J, Liu Y, Wen Y, Natarajan R, Lanting L, Nadler JL. Evidence that increased 12-lipoxygenase activity induces apoptosis in fibroblasts. *Journal of Cellular Physiology* 2001; 186:357-365.
60. Katsuki H, Okuda S. Arachidonic acid as a neurotoxic and neurotrophic substance. *Progress in Neurobiology* 1995; 46:607-636.
61. Canals S, Casarejos MJ, de Bernardo S, Rodriguez-Martins E, Mena MA. Nitric oxide triggers the toxicity due to glutathione depletion in midbrain cultures through 12-lipoxygenase. *The Journal of Biological Chemistry* 2003; 24:21542-21549.
62. Nazarewicz RR, Zenebe WJ, Parihar A, Parihar MS, Vaccaro M, Rink C, Sen CK, Ghafourifar P. 12(S)-hydroperoxyeicosatetraenoic acid (12-HETE) increases mitochondrial nitric oxide by increasing intramitochondrial calcium. *Archives of Biochemistry and Biophysics* 2007; 468:114-120.