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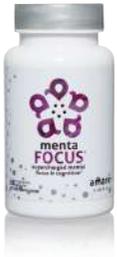
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Supercharge your brain with targeted phytonutrients to enhance clarity, cognition, and creativity. Proprietary blend of patented research-supported plant extracts provide wide-ranging benefits for brain health and function.\*

## KEY INGREDIENTS

**Pomegranate Extract** (*Wonderful Variety, punica granatum*) (whole fruit) (supplying 65% polyphenols) - Enhances overall brain activation, indicating significantly higher brain blood flow including bilateral activation of the hippocampus (area of the brain involved in memory). For many years, researchers have been studying the potential benefits of bioactive compounds from pomegranate fruit to improve overall brain function, and perhaps to even delay Alzheimer's and Parkinson's. A number of studies have found a unique pomegranate compound called "punicalagin" to reduce inflammation throughout the body, including in the brain (neuro-inflammation), and particularly in specific brain cells known as microglia. Inflammation in microglia leads to destruction of other brain cells which can make symptoms worse for people with Alzheimer's or dementia. Researchers are exploring a compound in the pomegranate fruit that may slow the progression of Alzheimer's and Parkinson's disease.\*

**Asian Apple Polyphenols** (*Applephenon*®) - Carefully extracted from specially selected wild green unripe apple fruits. The fruit is sourced and harvested from the region of Central Asia where apples originated and were cultivated thousands of years ago. This extract has powerful antioxidant properties with an optimized profile of procyanidins, members of the proanthocyanidin class of flavonoids. Foods rich in procyanidins have high oxygen radical absorbance capacity. Recent research shows that plant polyphenols also influence and modulate gut microbiota. Polyphenols appear to have a prebiotic effect by protecting and nourishing beneficial gut bacteria.\*

**French Grape Seed Polyphenols** (*Enovita*®) - Contains flavonoids which are considered to have numerous biological properties, including but not limited to antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, cardioprotective, neuroprotective, and hepatoprotective activities (liver-protective). Enovita is a proprietary proanthocyanidins (OPCs) rich extract made exclusively by water-extraction of grape seeds from white wine production. New studies show gastric protectant abilities.\*

**New Zealand Pine Bark Polyphenols** (*Enzogenol*™) – is produced using proprietary water extraction methods from selected pinus radiata bark from trees grown in the pristine, unpolluted environment of New Zealand's sustainable forest plantations. Extremely high in OPCs with antibacterial, antiviral, anticarcinogenic, anti-aging, anti-inflammatory and anti-allergic properties.\*

## Two Brains Support Blend (Rosemary, Holy Basil, Oregano, Clove, Sage)

**Rosemary** – traditional medicinal uses for rosemary have involved improving memory, particularly for its “stimulating” effect on the mind. It also is used for “cleansing” the body, where its antioxidant/anti-inflammatory compounds may help control the growth of many pathogenic bacteria without killing the good microflora (beneficial bacteria and yeast) in your body.\*

**Holy Basil** - known as an “adaptogenic” herb that helps the body to better “adapt” to stress by modulating the production of stress hormones such as cortisol (which is highly toxic to brain neurons and can lead to brain fog).\*

**Oregano** - has extremely high levels of free-radical-fighting antioxidants, higher concentrations than found in antioxidant superstars such as blueberries, based on polyphenol/flavonoid profiles.\*

**Clove** - the wide-range of brain-health benefits of cloves (memory, learning, mood) may be attributed to its anti-inflammatory effects.\*

**Sage** – has been used since the Middle Ages for “quickenning the nerves and mind” – effects which have been supported by modern scientific studies of memory, concentration, and general brain performance.\*

## CLINICAL STUDIES

### **BMC Complement Altern Med. 2017 Jun 27;17(1):339.**

*Effect of pomegranate extracts on brain antioxidant markers and cholinesterase activity in high fat-high fructose diet induced obesity in rat model.*

Amri Z, Ghorbel A, Turki M, Akroun FM, Ayadi F, Elfeki A, Hammami M.

#### **Abstract**

##### BACKGROUND:

To investigate beneficial effects of Pomegranate seeds oil (PSO), leaves (PL), juice (PJ) and (PP) on brain cholinesterase activity, brain oxidative stress and lipid profile in high-fat-high fructose diet (HFD) induced-obese rat.

##### METHODS:

In vitro and in vivo cholinesterase activity, brain oxidative status, body and brain weight and plasma lipid profile were measured in control rats, HFD-fed rats and HFD-fed rats treated by PSO, PL, PJ and PP.

##### RESULTS:

In vitro study showed that PSO, PL, PP, PJ inhibited cholinesterase activity in dose dependent manner. PL extract displayed the highest inhibitory activity by IC50 of 151.85 mg/ml. For in vivo study, HFD regime induced a significant increase of cholinesterase activity in brain by 17.4% as compared to normal rats. However, the administration of PSO, PL, PJ and PP to HFD-rats decreased cholinesterase activity in brain respectively by 15.48%, 6.4%, 20% and 18.7% as compared to untreated HFD-rats. Moreover, HFD regime caused significant increase in brain stress, brain and body weight, and lipid profile disorders in blood. Furthermore, PSO, PL, PJ and PP modulated lipid profile in blood and prevented accumulation

of lipid in brain and body evidenced by the decrease of their weights as compared to untreated HFD-rats. In addition administration of these extract protected brain from stress oxidant, evidenced by the decrease of malondialdehyde (MDA) and Protein carbonylation (PC) levels and the increase in superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels.

**CONCLUSION:**

These findings highlight the neuroprotective effects of pomegranate extracts and one of mechanisms is the inhibition of cholinesterase and the stimulation of antioxidant capacity.

**Nutr Neurosci. 2017 Apr 9:1-8.**

*Punicalagin reduces H2O2-induced cytotoxicity and apoptosis in PC12 cells by modulating the levels of reactive oxygen species.*

Clementi ME, Pani G, Sampaolese B, Tringali G.

**Abstract****BACKGROUND:**

Oxidative stress has long been linked to neuronal cell death in many neurodegenerative diseases. Antioxidant conventional supplements are poorly effective in preventing neuronal damage caused by oxidative stress due to their inability to cross the blood brain barrier. Hence the use of molecules extracted from plants and fruits such as phenolics, flavonoids, and terpenoids compounds constitute a new wave of antioxidant therapies to defend against free radicals.

**OBJECTIVE:**

In this study we examined the effects of punicalagin, a ellagitannin isolated from the pomegranate juice, on a rat adrenal pheochromocytoma cell line, treated with hydrogen peroxide, evaluating the viability, oxidation potential, mitochondrial function, and eventual apoptosis.

**METHODS:**

This study was performed on PC12 cells pretreated with punicalagin (0.5, 1, 5, 10 e 20 µM) 24 hours before of the damage by hydrogen peroxide (H2O2). H2O2 concentration (300 µM) used in our study was determined by preliminary experiments of time course. The cell viability and ROS production were evaluated by MTS assay and cytofluorometry assays, respectively. Subsequently, the number of apoptotic-positive cells and mitochondrial transmembrane potential, were measured by flow cytometry, in the same experimental paradigm. Finally, the expression of Bax and enzymatic activity of Caspase 3, some of the principle actors of programmed cell death, were investigated by semiquantitative PCR and utilizing a colorimetric assay kit, respectively.

**RESULTS:**

We found that pretreatment with punicalagin protected the cells from H2O2-induced damage. In particular, the protective effect seemed to be correlated with a control both in radical oxygen species production and in mitochondrial functions. In fact the cells treated with H2O2 showed an altered mitochondrial membrane integrity while the pretreatment with punicalagin retained both the cellular viability and the mitochondrial membrane potential similar to the control. Furthermore, the punicalagin, modulated the apoptotic cascade triggered reducing Bax gene expression and Caspase 3 activity.

**DISCUSSION:**

Results of the present study demonstrated a neuroprotective effect of punicalagin on H2O2-induced PC12 cell death, including mitochondria damage and expression of apoptotic gene Bax; therefore we hypothesize a possible prevent role for this molecule in neurodegenerative diseases related to oxidative stress.

**Neuropharmacology. 2017 May 1;117:21-32.**

*Inhibitory effect of punicalagin on lipopolysaccharide-induced neuroinflammation, oxidative stress and memory impairment via inhibition of nuclear factor-kappaB.*

Kim YE, Hwang CJ, Lee HP, Kim CS, Son DJ, Ham YW, Hellström M, Han SB, Kim HS, Park EK, Hong JT.

**Abstract**

Neuroinflammation is significant in the pathogenesis and development of Alzheimer's disease (AD). Previously, we showed lipopolysaccharide (LPS)-induced neuroinflammation caused memory impairment. We investigated the possible preventive effects of punicalagin (PUN), a component of pomegranate, on memory deficiency caused by LPS, along with the fundamental mechanisms. LPS-treated cultured astrocytes and microglial BV-2 cells were investigated for anti-neuroinflammatory effects of PUN. PUN (1.5 mg/kg) ameliorates LPS (250 µg/kg daily 7 times)-induced memory impairment as well as prevents the LPS-induced expression of inflammatory proteins. In in vitro study, we also found that PUN (1 µg/ml) inhibited the LPS-(10, 20 and 50 µM) induced expression of iNOS and Cox-2 as well as the production of ROS, NO, TNF-α and IL-1β. PUN also suppress activation of NF-κB via inhibition of IκB degradation as well as p50 and p65 translocation into the nucleus in LPS treated mouse brain and cultured astrocytes and microglial BV-2 cells. Consistent with the inhibitory effect on neuro inflammation, PUN inhibited LPS-induced Aβ1-42 generation through down-regulation of APP and BACE1 expression in in vivo and in vitro study. Moreover, PUN directly binds to NF-κB subunit p50 evidenced by a docking model and pull down assay. These results suggest that PUN inhibits LPS-induced memory impairment via anti-inflammatory and anti-amylogenic mechanisms through inhibition of NF-κB activation.

**PLoS One. 2016 Nov 9;11(11).**

*Neuroprotective Effects of Pomegranate Peel Extract after Chronic Infusion with Amyloid-β Peptide in Mice.*

Morzelle MC, Salgado JM, Telles M, Mourelle D, Bachiega P, Buck HS, Viel TA.

**Abstract**

Alzheimer's disease is a chronic and degenerative condition that had no treatment until recently. The current therapeutic strategies reduce progression of the disease but are expensive and commonly cause side effects that are uncomfortable for treated patients. Functional foods to prevent and/or treat many conditions, including neurodegenerative diseases, represent a promising field of study currently gaining attention. To this end, here we demonstrate the effects of pomegranate (*Punica granatum*) peel extract (PPE) regarding spatial memory, biomarkers of neuroplasticity, oxidative stress and inflammation in a mouse model of neurodegeneration. Male C57Bl/6 mice were chronically infused for 35 days with amyloid-β peptide 1-42 (Aβ) or vehicle (control) using mini-osmotic pumps. Another group, also infused with Aβ, was treated with PPE (p.o.- βA+PPE, 800 mg/kg/day). Spatial memory was evaluated in the Barnes maze. Animals treated with PPE and in the control group exhibited a reduction in failure to find the escape box, a finding that was not observed in the Aβ group. The consumption of PPE reduced amyloid plaque density, increased the expression of neurotrophin BDNF and reduced the activity of acetylcholinesterase enzyme. A reduction in lipid peroxidation and in the concentration of the pro-inflammatory cytokine TNF-α was also observed in the PPE group. No hepatic lesions were observed in animals treated with PPE. In conclusion, administration of pomegranate peel extract has neuroprotective effects involving multiple mechanisms to prevent establishment and progression of the neurodegenerative process induced by infusion with amyloid-β peptide in mice.

**Oncotarget. 2016 Oct 4;7(40):64589-64604.**

*Consumption of pomegranates improves synaptic function in a transgenic mice model of Alzheimer's disease.*  
Braidy N, Essa MM, Poljak A, Selvaraju S, Al-Adawi S, Manivasagam T, Thenmozhi AJ, Ooi L, Sachdev P, Guillemin GJ.

**Abstract**

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by extracellular plaques containing abnormal Amyloid Beta (A $\beta$ ) aggregates, intracellular neurofibrillary tangles containing hyperphosphorylated tau protein, microglia-dominated neuroinflammation, and impairments in synaptic plasticity underlying cognitive deficits. Therapeutic strategies for the treatment of AD are currently limited. In this study, we investigated the effects of dietary supplementation of 4% pomegranate extract to a standard chow diet on neuroinflammation, and synaptic plasticity in APPsw/Tg2576 mice brain. Treatment with a custom mixed diet (pellets) containing 4% pomegranate for 15 months ameliorated the loss of synaptic structure proteins, namely PSD-95, Munc18-1, and SNAP25, synaptophysin, phosphorylation of Calcium/Calmodulin Dependent Protein Kinase II $\alpha$  (p-CaMKII $\alpha$ /CaMKII $\alpha$ ), and phosphorylation of Cyclic AMP-Response Element Binding Protein (pCREB/CREB), inhibited neuroinflammatory activity, and enhanced autophagy, and activation of the phosphoinositide-3-kinase-Akt-mammalian target of rapamycin signaling pathway. These neuroprotective effects were associated with reduced  $\beta$ -site cleavage of Amyloid Precursor Protein in APPsw/Tg2576 mice. Therefore, long-term supplementation with pomegranates can attenuate AD pathology by reducing inflammation, and altering APP-dependent processes.

**Curr Alzheimer Res. 2014;11(9):834-43.**

*Pomegranate extract modulates processing of amyloid- $\beta$  precursor protein in an aged Alzheimer's disease animal model.*

Ahmed AH, Subaiea GM, Eid A, Li L, Seeram NP, Zawia NH.

**Abstract**

Accumulating research supports the neuroprotective effects of pomegranate (*Punica granatum*) juice and extracts against Alzheimer's disease (AD) but there is limited data available in animal models. Here we investigated the effects of a standardized pomegranate extract (PE) on AD pathology in an aged transgenic AD animal model (R1.40). The mice (age 24-30 months) received either PE (at 100 and 200 mg/kg) or a control solution daily for three weeks, and were evaluated in the Morris water maze and the Y-maze for improvements in spatial long-term and working memory functions. Cortical amyloid- $\beta$  precursor protein (APP) and amyloid- $\beta$  (A $\beta$ ) levels, along with other relevant biomarkers for AD, were measured in brain tissues. PE did not improve cognitive performance of the mice, but altered levels and ratio of the A $\beta$ 42 and A $\beta$ 40 peptides which would favor a diminution in AD pathogenesis. Further analysis revealed that this reversal could be the product of the modification of  $\gamma$ -secretase enzyme activity, the enzyme involved in the generation of these A $\beta$  isoforms. Our findings support a specific anti-amyloidogenic mechanism of a pomegranate extract in this aged AD animal model.

**Mol Nutr Food Res. 2014 Sep;58(9):1843-51.**

***Punicalagin inhibits neuroinflammation in LPS-activated rat primary microglia.***

**Olajide OA, Kumar A, Velagapudi R, Okorji UP, Fiebich BL.**

**Abstract**

**SCOPE:**

In this study, the effects of punicalagin on neuroinflammation in LPS-activated microglia were investigated.

**METHODS AND RESULTS:**

The ability of punicalagin to reduce the production of TNF- $\alpha$ , IL-6 and prostaglandin E2 was measured in culture medium using enzyme immunoassay. TNF- $\alpha$  and IL-6 gene expression in mouse hippocampal slices was measured with PCR. cyclooxygenase-2 and microsomal prostaglandin E synthase 1 protein and mRNA were evaluated with Western blotting and PCR, respectively. Further experiments to investigate effects of punicalagin on protein expressions of inflammatory targets were also determined with Western blotting. Pretreatment of rat primary microglia with punicalagin (5-40  $\mu$ M) prior to LPS (10 ng/mL) stimulation produced a significant ( $p < 0.05$ ) inhibition of TNF- $\alpha$ , IL-6 and prostaglandin E2 production. Punicalagin completely abolished TNF- $\alpha$  and IL-6 gene expression in LPS-stimulated hippocampal slices. Protein and mRNA expressions of cyclooxygenase-2 and microsomal prostaglandin E synthase 1 were also reduced by punicalagin pretreatment. Results show that punicalagin interferes with NF- $\kappa$ B signalling through attenuation of NF- $\kappa$ B-driven luciferase expression, as well as inhibition of I $\kappa$ B phosphorylation and nuclear translocation of p65 subunit in the microglia.

**CONCLUSION:**

These results suggest that punicalagin inhibits neuroinflammation in LPS-activated microglia through interference with NF- $\kappa$ B signalling, suggesting its potential as a nutritional preventive strategy in neurodegenerative disorders.

**Life Sci. 2014 Aug 21;110(2):61-9.**

***Pomegranate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats.***

**Ahmed MA, El Morsy EM, Ahmed AA.**

**Abstract**

**AIM:**

Interruption to blood flow causes ischemia and infarction of brain tissues with consequent neuronal damage and brain dysfunction. Pomegranate extract is well tolerated, and safely consumed all over the world. Interestingly, pomegranate extract has shown remarkable antioxidant and anti-inflammatory effects in experimental models. Many investigators consider natural extracts as novel therapies for neurodegenerative disorders. Therefore, this study was carried out to investigate the protective effects of standardized pomegranate extract against cerebral ischemia/reperfusion-induced brain injury in rats.

**MAIN METHODS:**

Adult male albino rats were randomly divided into sham-operated control group, ischemia/reperfusion (I/R) group, and two other groups that received standardized pomegranate extract at two dose levels (250, 500 mg/kg) for 15 days prior to ischemia/reperfusion (PMG250+I/R, and PMG500+I/R groups).

After I/R or sham operation, all rats were sacrificed and brains were harvested for subsequent biochemical analysis.

**KEY FINDINGS:**

Results showed reduction in brain contents of MDA (malondialdehyde), and NO (nitric oxide), in addition to enhancement of SOD (superoxide dismutase), GPX (glutathione peroxidase), and GRD (glutathione reductase) activities in rats treated with pomegranate extract prior to cerebral I/R. Moreover, pomegranate extract decreased brain levels of NF- $\kappa$ B p65 (nuclear factor kappa B p65), TNF- $\alpha$  (tumor necrosis factor-alpha), caspase-3 and increased brain levels of IL-10 (interleukin-10), and cerebral ATP (adenosine triphosphate) production. Comet assay showed less brain DNA (deoxyribonucleic acid) damage in rats protected with pomegranate extract.

**SIGNIFICANCE:**

The present study showed, for the first time, that pre-administration of pomegranate extract to rats, can offer a significant dose-dependent neuroprotective activity against cerebral I/R brain injury and DNA damage via antioxidant, anti-inflammatory, anti-apoptotic and ATP-replenishing effects.

**Evid Based Complement Alternat Med. 2013;2013:946298.**

*Pomegranate juice augments memory and fMRI activity in middle-aged and older adults with mild memory complaints.*

Bookheimer SY, Renner BA, Ekstrom A, Li Z, Henning SM, Brown JA, Jones M, Moody T, Small GW.

**Abstract**

Despite increasing emphasis on the potential of dietary antioxidants in preventing memory loss and on diet as a precursor of neurological health, rigorous studies investigating the cognitive effects of foods and their components are rare. Recent animal studies have reported memory and other cognitive benefits of polyphenols, found abundantly in pomegranate juice. We performed a preliminary, placebo-controlled randomized trial of pomegranate juice in older subjects with age-associated memory complaints using memory testing and functional brain activation (fMRI) as outcome measures. Thirty-two subjects (28 completers) were randomly assigned to drink 8 ounces of either pomegranate juice or a flavor-matched placebo drink for 4 weeks. Subjects received memory testing, fMRI scans during cognitive tasks, and blood draws for peripheral biomarkers before and after the intervention. Investigators and subjects were all blind to group membership. After 4 weeks, only the pomegranate group showed a significant improvement in the Buschke selective reminding test of verbal memory and a significant increase in plasma trolox-equivalent antioxidant capacity (TEAC) and urolithin A-glucuronide. Furthermore, compared to the placebo group, the pomegranate group had increased fMRI activity during verbal and visual memory tasks. While preliminary, these results suggest a role for pomegranate juice in augmenting memory function through task-related increases in functional brain activity.

**J Nutr. 2013 May;143(5):597-605.**

*Pomegranate polyphenols and extract inhibit nuclear factor of activated T-cell activity and microglial activation in vitro and in a transgenic mouse model of Alzheimer disease.*

Rojanathammanee L, Puig KL, Combs CK.

## Abstract

Alzheimer disease (AD) brain is characterized by extracellular plaques of amyloid  $\beta$  ( $A\beta$ ) peptide with reactive microglia. This study aimed to determine whether a dietary intervention could attenuate microgliosis. Memory was assessed in 12-mo-old male amyloid precursor protein/presenilin 1 (APP/PS1) transgenic mice via Barnes maze testing followed by division into either a control-fed group provided free access to normal chow and water or a treatment group provided free access to normal chow and drinking water supplemented with pomegranate extract (6.25 mL/L) for 3 mo followed by repeat Barnes maze testing for both groups. Three months of pomegranate feeding decreased the path length to escape of mice compared with their initial 12-mo values ( $P < 0.05$ ) and their control-fed counterparts ( $P < 0.05$ ). Brains of the 3-mo study pomegranate-fed mice had lower tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) concentrations ( $P < 0.05$ ) and lower nuclear factor of activated T-cell (NFAT) transcriptional activity ( $P < 0.05$ ) compared with controls. Brains of the 3-mo pomegranate or control mice were also compared with an additional control group of 12-mo-old mice for histologic analysis. Immunocytochemistry showed that pomegranate- but not control-fed mice had attenuated microgliosis ( $P < 0.05$ ) and  $A\beta$  plaque deposition ( $P < 0.05$ ) compared with 12-mo-old mice. An additional behavioral study again used 12-mo-old male APP/PS1 mice tested by T-maze followed by division into a control group provided with free access to normal chow and sugar supplemented drinking water or a treatment group provided with normal chow and pomegranate extract-supplemented drinking water (6.25 mL/L) for 1 mo followed by repeat T-maze testing in both groups. One month of pomegranate feeding increased spontaneous alternations versus control-fed mice ( $P < 0.05$ ). Cell culture experiments verified that 2 polyphenol components of pomegranate extract, punicalagin and ellagic acid, attenuated NFAT activity in a reporter cell line ( $P < 0.05$ ) and decreased  $A\beta$ -stimulated TNF- $\alpha$  secretion by murine microglia ( $P < 0.05$ ). These data indicate that dietary pomegranate produces brain anti-inflammatory effects that may attenuate AD progression.

## Altern Med Rev. 2008 Jun;13(2):128-44.

*Therapeutic applications of pomegranate (Punica granatum L.): a review.*

Jurenka JS.

## Abstract

The pomegranate, *Punica granatum L.*, is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity.

**J Nutr Health Aging. 2004;8(6):492-6.**

*Dietary supplementation with apple juice concentrate alleviates the compensatory increase in glutathione synthase transcription and activity that accompanies dietary- and genetically-induced oxidative stress.*

Tchantchou F, Graves M, Ortiz D, Rogers E, Shea TB.

**Abstract**

Increased oxidative stress, which can arise from dietary, environmental and/or genetic sources, contributes to the decline in cognitive performance during normal aging and in neurodegenerative conditions such as Alzheimer's disease. Supplementation with fruits and vegetables that are high in antioxidant potential can compensate for dietary and/or genetic deficiencies that promote increased oxidative stress. We have recently demonstrated that apple juice concentrate (AJC) prevents the increase in oxidative damage to brain tissue and decline in cognitive performance observed when transgenic mice lacking apolipoprotein E (ApoE<sup>-/-</sup>) are maintained on a vitamin-deficient diet and challenged with excess iron (included in the diet as a pro-oxidant). However, the mechanism by which AJC provided neuroprotection was not conclusively determined. Herein, we demonstrate that supplementation with AJC also prevents the compensatory increases in glutathione synthase transcription and activity that otherwise accompany maintenance of ApoE<sup>-/-</sup> mice on this vitamin-free diet in the presence of iron. Inclusion of the equivalent composition and concentration of sugars of AJC did not prevent these increases. These findings provide further evidence that the antioxidant potential of AJC can compensate for dietary and genetic deficiencies that otherwise promote neurodegeneration.

**J Alzheimers Dis. 2006 Aug;9(3):287-91.**

*Apple juice concentrate maintains acetylcholine levels following dietary compromise.*

Chan A, Graves V, Shea TB.

**Abstract**

Oxidative stress contributes to age-related cognitive decline. In some instances, consumption of fruits and vegetables rich in antioxidant can provide superior protection than supplementation with purified antioxidants. Our prior studies have shown that supplementation with apple juice concentrate (AJC) alleviates oxidative damage and cognitive decline in adult (9-12 months) mice lacking ApoE (as a model of increased oxidative stress) and in normal aged (2-2.5 years) mice when challenged with a vitamin-deficient, oxidative stress-promoting diet. Here, we demonstrate that AJC, administered in drinking water, maintains acetylcholine levels that otherwise decline when adult and aged mice are maintained on the above deficient diet. Normal mice aged either 9-10 months or 2-2.5 years and ApoE<sup>-/-</sup> mice aged 9-10 months were maintained for 1 month on a complete diet or a diet lacking folate and vitamin E and containing iron as a pro-oxidant, and additional groups received 0.5% AJC ad libitum in drinking water. Spectrophotometric assay of acetylcholine levels revealed a significant decline in homogenates of combined frontal cortex and hippocampus for all mice maintained on the deficient diet, and a prevention of this decline in mice maintained on the deficient diet when supplemented with AJC. These findings provide a likely mechanism by which consumption of antioxidant-rich foods such as apples can prevent the decline in cognitive performance that accompanies dietary and genetic deficiencies and aging.

**J Nutr Health Aging. 2004;8(2):92-7.**

*Apple juice prevents oxidative stress and impaired cognitive performance caused by genetic and dietary deficiencies in mice.*

Rogers EJ, Milhalik S, Orthiz D, Shea TB.

**Abstract**

Increased oxidative stress contributes to the decline in cognitive performance during normal aging and in neurodegenerative conditions such as Alzheimer's disease. Dietary supplementation with fruits and vegetables that are high in antioxidant potential have in some cases compensated for dietary and/or genetic deficiencies that promote increased oxidative stress. Herein, we demonstrate that apple juice concentrate, administered ad libitum in drinking water, can compensate for the increased reactive oxygen species and decline in cognitive performance in maze trials observed when normal and transgenic mice lacking apolipoprotein E are deprived of folate and vitamin E. In addition, we demonstrate that this protective effect is not derived from the sugar content of the concentrate.

**J Agric Food Chem. 2008 Jul 9;56(13):4855-73.**

*Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms.*

Singh M, Arseneault M, Sanderson T, Murthy V, Ramassamy C.

**Abstract**

Polyphenols are the most abundant antioxidants in diet. Indeed, fruits, vegetables, beverages (tea, wine, juices), plants, and some herbs are loaded with powerful antioxidant polyphenols. Despite their wide distribution, research on human health benefits truly began in the mid-1990s (Scalbert, A.; Johnson, I. T.; Saltmarsh, M. Am. J. Clin. Nutr. 2005, 81, S15S-217S). Phenolic compounds have been receiving increasing interest from consumers and manufacturers because numerous epidemiological studies have suggested associations between consumption of polyphenol-rich foods or beverages and the prevention of certain chronic diseases such as cancers and cardiovascular diseases (Manach, C.; Mazur, A.; Scalbert, A. Curr. Opin. Lipidol. 2005, 16, 77-84; Duthie, S. J. Mol. Nutr. Food Res. 2007, 51, 665-674). Furthermore, in the past 10 years, research on the neuroprotective effects of dietary polyphenols has developed considerably. These compounds are able to protect neuronal cells in various in vivo and in vitro models through different intracellular targets (Ramassamy, C. Eur. J. Pharmacol. 2006, 545, 51-64). However, it is not at all clear whether these compounds reach the brain in sufficient concentrations and in a biologically active form to exert beneficial effects. On the other hand, it has become clear that the mechanisms of action of these polyphenols go beyond their antioxidant activity and the attenuation of oxidative stress. Therefore, there is a need for more research on their intracellular and molecular targets as special pathways underlying distinct polyphenol-induced neuroprotection. The focus of this review is aimed at presenting the role of some polyphenols from fruits, vegetables, and beverages in neuroprotection and particularly in Alzheimer's disease and the research challenges in this area.

**J Alzheimers Dis. 2005 Dec;8(3):283-7.*****Apple juice concentrate prevents oxidative damage and impaired maze performance in aged mice.*****Tchantchou F, Chan A, Kifle L, Ortiz D, Shea TB.****Abstract**

Oxidative stress contributes to age-related cognitive decline. In some instances, consumption of fruits and vegetables rich in antioxidant can provide superior protection than supplementation with purified antioxidants. Our prior studies have shown that supplementation with apple juice concentrate (AJC) alleviates oxidative damage and cognitive decline in a transgenic murine model compromised in endogenous antioxidant potential when challenged with a vitamin-deficient, oxidative stress-promoting diet. Herein, we demonstrate that AJC, administered in drinking water, is neuroprotective in normal, aged mice. Normal mice aged either 9-10 months or 2-2.5 years were maintained for 1 month on a complete diet or a diet lacking folate and vitamin E and containing iron as a pro-oxidant, after which oxidative damage was assayed by thiobarbituric acid-reactive substances and cognitive decline as assayed by performance in a standard Y-maze. Mice 9-12 months of age were unaffected by the deficient diet, while older mice demonstrated statistically-increased oxidative damage and poorer performance in a Y maze test. Supplementation with AJC prevented these neurodegenerative effects. These data are consistent with normal aged individuals being susceptible to neurodegeneration following dietary compromise such as folate deficiency, and a hastened onset of neurodegeneration in those individuals harboring a genetic risk factor such as ApoE deficiency. These findings also support the efficacy of antioxidant supplementation, including consumption of antioxidant-rich foods such as apples, in preventing the decline in cognitive performance that accompanies normal aging.

**Curr Med Chem. 2011;18(8):1195-212.*****Neuroprotective actions of flavonoids.*****Gutierrez-Merino C1, Lopez-Sanchez C, Lagoa R, Samhan-Arias AK, Bueno C, Garcia-Martinez V.****Abstract**

The experimental evidences accumulated during last years point out a relevant role of oxidative stress in neurodegeneration. As anti-cellular oxidative stress agents flavonoids can act either as direct chemical antioxidants, the classic view of flavonoids as antioxidants, or as modulators of enzymes and metabolic and signaling pathways leading to an overshoot of reactive oxygen species (ROS) formation, a more recently emerging concept. Flavonoids, a large family of natural antioxidants, undergo a significant hepatic metabolism leading to flavonoid-derived metabolites that are also bioactive as antioxidant agents. The development of more efficient flavonoid's based anti-oxidative stress therapies should also take into account their bioavailability in the brain using alternate administration protocols, and also that the major ROS triggering the cellular oxidative stress are not the same for all neurodegenerative insults and diseases. On these grounds, we have reviewed the reports on neuroprotection by different classes of flavonoids on cellular cultures and model animals. In addition, as they are now becoming valuable pharmacological drugs, due to their low toxicity, the reported adverse effects of flavonoids in model experimental animals and humans are briefly discussed.

**Brain Res. 2014 Mar 25;1555:60-77.**

*Neuroprotective effects of anthocyanin- and proanthocyanidin-rich extracts in cellular models of Parkinson's disease.*

Strathearn KE, Yousef GG, Grace MH, Roy SL, Tambe MA, Ferruzzi MG, Wu QL, Simon JE, Lila MA, Rochet JC.

**Abstract**

Neuropathological evidence indicates that dopaminergic cell death in Parkinson's disease (PD) involves impairment of mitochondrial complex I, oxidative stress, microglial activation, and the formation of Lewy bodies. Epidemiological findings suggest that the consumption of berries rich in anthocyanins and proanthocyanidins may reduce PD risk. In this study, we investigated whether extracts rich in anthocyanins, proanthocyanidins, or other polyphenols suppress the neurotoxic effects of rotenone in a primary cell culture model of PD. Dopaminergic cell death elicited by rotenone was suppressed by extracts prepared from blueberries, grape seed, hibiscus, blackcurrant, and Chinese mulberry. Extracts rich in anthocyanins and proanthocyanidins exhibited greater neuroprotective activity than extracts rich in other polyphenols, and a number of individual anthocyanins interfered with rotenone neurotoxicity. The blueberry and grape seed extracts rescued rotenone-induced defects in mitochondrial respiration in a dopaminergic cell line, and a purple basal extract attenuated nitrite release from microglial cells stimulated by lipopolysaccharide. These findings suggest that anthocyanin- and proanthocyanidin-rich botanical extracts may alleviate neurodegeneration in PD via enhancement of mitochondrial function.

**Mol Nutr Food Res. 2013 Dec;57(12):2091-102.**

*Role of standardized grape polyphenol preparation as a novel treatment to improve synaptic plasticity through attenuation of features of metabolic syndrome in a mouse model.*

Wang J, Tang C, Ferruzzi MG, Gong B, Song BJ, Janle EM, Chen TY, Cooper B, Varghese M, Cheng A, Freire D, Bilski A, Roman J, Nguyen T, Ho L, Talcott ST, Simon JE, Wu Q, Pasinetti GM.

**Abstract****SCOPE:**

Metabolic syndrome has become an epidemic and poses tremendous burden on the health system. People with metabolic syndrome are more likely to experience cognitive decline. As obesity and sedentary lifestyles become more common, the development of early prevention strategies is critical. In this study, we explore the potential beneficial effects of a combinatory polyphenol preparation composed of grape seed extract, Concord purple grape juice extract, and resveratrol, referred to as standardized grape polyphenol preparation (SGP), on peripheral as well as brain dysfunction induced by metabolic syndrome.

**METHODS AND RESULTS:**

We found dietary fat content had minimal effect on absorption of metabolites of major polyphenols derived from SGP. Using a diet-induced animal model of metabolic syndrome (DIM), we found that brain functional connectivity and synaptic plasticity are compromised in the DIM mice. Treatment with SGP not only prevented peripheral metabolic abnormality but also improved brain synaptic plasticity.

**CONCLUSION:**

Our study demonstrated that SGP, comprised of multiple bioavailable and bioactive components targeting a wide range of metabolic syndrome related pathological features, provides greater global protection against peripheral and central nervous system dysfunctions and can be potentially developed

as a novel prevention/treatment for improving brain connectivity and synaptic plasticity important for learning and memory.

### **Exp Gerontol. 2011 Nov;46(11):958-64.**

***Grape seed proanthocyanidin lowers brain oxidative stress in adult and middle-aged rats.***

**Asha Devi S, Sagar Chandrasekar BK, Manjula KR, Ishii N.**

#### **Abstract**

There is growing concern over the increasing instances of decline in cognitive abilities with aging in humans. The present study evaluated the benefits of the natural antioxidant, grape seed proanthocyanidin extract (GSPE) in treating the effects of age-related oxidative stress (OS) and accumulation of lipofuscin (LF) on the cognitive ability in rats. Female Wistar rats of 3- and 12-months of age received a daily oral supplement of GSPE until they attained 6- and 15-months of age. During this period, rats were tested for their cognitive ability. At the end of this period, blood glucose and markers of OS were assessed in the hippocampus. GSPE lowered blood glucose, lipid peroxidation, hydrogen peroxide level, and increased protein sulphhydryl (P-SH) content in the hippocampus. In addition, GSPE significantly improved cognitive performance in the two age groups. These results demonstrate that the extent of OS-related LF accumulation is reducible by GSPE. They also suggest a critical role for GSPE as a neuroprotectant in the hippocampus and in preventing cognitive loss with aging.

### **Expert Rev Proteomics. 2010 Aug;7(4):579-89.**

***Polyphenolic compounds for treating neurodegenerative disorders involving protein misfolding.***

**Ho L, Pasinetti GM.**

#### **Abstract**

A diverse group of neurodegenerative diseases are characterized by progressive, age-dependent intracellular formation of misfolded protein aggregates. These include Alzheimer's disease, Huntington's disease, Parkinson's disease and a number of tau-mediated disorders. There is no effective treatment for any of these disorders; currently approved interventions are designed to treat disease symptoms and generally lead to modest modulation of clinical symptoms. None are known to mitigate underlying neuropathologic mechanisms and, thus, it is not unexpected that existing treatments appear ineffective in modulating disease progression. We note that these neurodegenerative disorders all share a common mechanistic theme in that depositions of misfolded protein in the brain is a key molecular feature underlying disease onset and/or progression. While previous studies have identified a number of drugs and nutraceuticals capable of interfering with the formation and/or stability of misfolded protein aggregates, none have been demonstrated to be effective in vivo for treating any of the neurodegenerative disorders. We hereby review accumulating evidence that a select nutraceutical grape-seed polyphenolic extract (GSPE) is effective in vitro and in vivo in mitigating certain misfolded protein-mediated neuropathologic and clinical phenotypes. We will also review evidence implicating bioavailability of GSPE components in the brain and the tolerability as well as safety of GSPE in animal models and in humans. Collectively, available information supports continued development of the GSPE for treating a variety of neurodegenerative disorders involving misfolded protein-mediated neuropathologic mechanisms.

**Neurotox Res. 2009 Jan;15(1):3-14.**

*Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse.*

Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, Pollard A, Fenech M, Zhou XF.

**Abstract**

Polyphenols extracted from grape seeds are able to inhibit amyloid-beta (Abeta) aggregation, reduce Abeta production and protect against Abeta neurotoxicity in vitro. We aimed to investigate the therapeutic effects of a polyphenol-rich grape seed extract (GSE) in Alzheimer's disease (AD) mice. APP(Swe)/PS1dE9 transgenic mice were fed with normal AIN-93G diet (control diet), AIN-93G diet with 0.07% curcumin or diet with 2% GSE beginning at 3 months of age for 9 months. Total phenolic content of GSE was 592.5 mg/g dry weight, including gallic acid (49 mg/g), catechin (41 mg/g), epicatechin (66 mg/g) and proanthocyanidins (436.6 mg catechin equivalents/g). Long-term feeding of GSE diet was well tolerated without fatality, behavioural abnormality, changes in food consumption, body weight or liver function. The Abeta levels in the brain and serum of the mice fed with GSE were reduced by 33% and 44%, respectively, compared with the Alzheimer's mice fed with the control diet. Amyloid plaques and microgliosis in the brain of Alzheimer's mice fed with GSE were also reduced by 49% and 70%, respectively. Curcumin also significantly reduced brain Abeta burden and microglia activation. Conclusively, polyphenol-rich GSE prevents the Abeta deposition and attenuates the inflammation in the brain of a transgenic mouse model, and this thus is promising in delaying development of AD.

**Med Sci Monit. 2006 Apr;12(4):BR124-9. Epub 2006 Mar 28.**

*Grape seed proanthocyanidin extract (GSPE) and antioxidant defense in the brain of adult rats.*

Devi A1, Jolitha AB, Ishii N.

**Abstract****BACKGROUND:**

Proanthocyanidin (PA) is a naturally occurring antioxidant from grape seed extract. The present study aims at assessing the neuroprotective effects of grape seed proanthocyanidin (GSPE) on the cerebral cortex (CC), cerebellum (CB), and hippocampus (HC) in the adult rat brain.

**MATERIAL/METHODS:**

GSPE was orally administered at 25, 50, and 75 mg per kg body weight daily and for a total period of 9 weeks. Antioxidant enzymes (AOEs), superoxide dismutase (SOD), and catalase (CAT) were analyzed along with malondialdehyde (MDA) and protein carbonyl content (PCC) as markers of lipid peroxidation (LPO) and protein oxidation (PO). The cholinergic system was studied by analyzing choline acetyl transferase (ChAT) and acetylcholine esterase (AChE) activities along with acetylcholine content (ACh).

**RESULTS:**

The results obtained revealed an increased SOD activity in the 75-mg PA-supplemented animals, with a substantial decrease in MDA and PCC. The cholinergic neurotransmittary system analysis showed increased ChAT activity indicative of increased ACh content in the supplemented animals and the increase was more in the 75-mg PA group with a concomitant and moderate decrease in AChE activity. Regional changes were more with reference to HC.

**CONCLUSIONS:**

Our study shows that PA intake in moderately low quantity is effective in up-regulating the antioxidant

defense mechanism by attenuating LPO and PO. Changes in the cholinergic system, however, indicate an increase in the ACh concentration with a moderate reduction in AChE activity, suggesting further that PA may have a potent role in enhancing cognition in older rats.

### **Brain Res Bull. 2006 Feb 15;68(6):469-73.**

***Modulatory role of grape seed extract on age-related oxidative DNA damage in central nervous system of rats.***

Balu M, Sangeetha P, Murali G, Panneerselvam C.

#### **Abstract**

Aging is the accumulation of diverse deleterious changes in the cells and tissues leading to increased risk of diseases. Oxidative stress is considered as a major risk factor and contributes to age related increase in DNA oxidation and DNA protein cross-links in central nervous system during aging. In the present study, we have evaluated the salubrious role of grape seed extract on accumulation of oxidative DNA damage products such as 8-OHdG and DNA protein cross-links in aged rats. Male albino rats of Wistar strain were divided into four groups: Group I, young control rats; Group II, young rats treated with grape seed extract (100 mg/kg b.wt.) for 30 days; Group III, aged control rats; Group IV, aged rats supplemented with grape seed extract (100 mg/kg b.wt.) for 30 days. Our results, thus, revealed that grape seed extract has inhibiting effect on the accumulation of age-related oxidative DNA damages in spinal cord and in various brain regions such as cerebral cortex, striatum and hippocampus.

### **Int J Dev Neurosci. 2005 Oct;23(6):501-7.**

***Age-related oxidative protein damages in central nervous system of rats: modulatory role of grape seed extract.***

Balu M, Sangeetha P, Murali G, Panneerselvam C.

#### **Abstract**

Oxidative stress has been shown to play a major role in aging and in neurodegenerative disorders. Protein modification is one of the important consequences of oxidative stress. In the present study, we evaluated the role of grape seed extract on memory, reactive oxygen species production, protein carbonyls (PCO), and thiol status in discrete regions of central nervous system of young and aged rats. Male albino rats of Wistar strain were divided into four groups: Group I--control young rats, Group II--young rats treated with grape seed extract (100 mg/kg BW) for 30 days, Group III--aged control rats and Group IV--aged rats supplemented with grape seed extract (100 mg/kg BW) for 30 days. Memory loss was observed in the aged rats. Age associated increase in reactive oxygen species production and protein oxidation was observed in the spinal cord; cerebral cortex, striatum and the hippocampus regions of aged rats (Group III). The levels of total thiol, non-protein thiol, protein thiols were found to be significantly decreased in spinal cord and all the brain regions studied in aged rats when compared to young rats. Supplementation of aged rats with grape seed extract showed increased memory performance and declined reactive oxygen species production, decreased protein carbonyl levels and improved thiol levels. These findings demonstrated that grape seed extract enhanced the antioxidant status and decreased the incidence of free radical induced protein oxidation in aged rats thereby protecting the central nervous system from the reactive oxygen species.

**Eur J Neurol. 2013 Aug;20(8):1135-44.**

*Enzogenol for cognitive functioning in traumatic brain injury: a pilot placebo-controlled RCT.*

Theadom A, Mahon S, Barker-Collo S, McPherson K, Rush E, Vandal AC, Feigin VL.

**Abstract**

**BACKGROUND AND PURPOSE:**

Enzogenol, a flavonoid-rich extract from *Pinus radiata* bark with antioxidant and anti-inflammatory properties has been shown to improve working memory in healthy adults. In traumatic brain injury (TBI), oxidation and inflammation have been linked to poorer cognitive outcomes. Hence, this phase II, randomized controlled trial investigated safety, compliance and efficacy of Enzogenol for improving cognitive functioning in people following mild TBI.

**METHODS:**

Sixty adults, who sustained a mild TBI, 3-12 months prior to recruitment, and who were experiencing persistent cognitive difficulties [Cognitive Failures Questionnaire (CFQ) score > 38], were randomized to receive Enzogenol (1000 mg/day) or matching placebo for 6 weeks. Subsequently, all participants received Enzogenol for a further 6 weeks, followed by placebo for 4 weeks. Compliance, side-effects, cognitive failures, working and episodic memory, post-concussive symptoms and mood were assessed at baseline, 6, 12 and 16 weeks. Simultaneous estimation of treatment effect and breakpoint was effected, with confidence intervals (CIs) obtained through a treatment-placebo balance-preserving bootstrap procedure.

**RESULTS:**

Enzogenol was found to be safe and well tolerated. Trend and breakpoint analyses showed a significant reduction in cognitive failures after 6 weeks [mean CFQ score, 95% CI, Enzogenol versus placebo -6.9 (-10.8 to -4.1)]. Improvements in the frequency of self-reported cognitive failures were estimated to continue until week 11 before stabilizing. Other outcome measures showed some positive trends but no significant treatment effects.

**CONCLUSIONS:**

Enzogenol supplementation is safe and well tolerated in people after mild TBI, and may improve cognitive functioning in this patient population. This study provides Class IIB evidence that Enzogenol is well tolerated and may reduce self-perceived cognitive failures in patients 3-12 months post-mild TBI.

**Phytother Res. 2008 Sep;22(9):1168-74.**

*Improved cognitive performance after dietary supplementation with a *Pinus radiata* bark extract formulation.*

Pipingas A, Silberstein RB, Vitetta L, Rooy CV, Harris EV, Young JM, Frampton CM, Sali A, Nastasi J.

**Abstract**

Dietary interventions may have the potential to counter age-related cognitive decline. Studies have demonstrated an improvement in age-related cognitive impairment in animals after supplementation with plant extracts containing flavonoids but there are few human studies. This double-blind, controlled study examined the effects on cognitive performance of a 5 week supplementation with Enzogenol *Pinus radiata* bark extract containing flavonoids, in 42 males aged 50-65 years, with a body mass index >25. Participants were supplemented for 5 weeks either with Enzogenol plus vitamin C, or with vitamin C only. A battery of computerized cognitive tests was administered, and cardiovascular and haematological

parameters were assessed prior to and following supplementation. The speed of response for the spatial working memory and immediate recognition tasks improved after supplementation with Enzogenol plus vitamin C, whereas vitamin C alone showed no improvements. A trend in a reduction of systolic blood pressure was observed with Enzogenol plus vitamin C, but not with vitamin C alone. The blood safety parameters were unchanged. The findings suggest a beneficial effect of supplementation with Enzogenol on cognition in older individuals. Larger studies are needed to ascertain its potential as a preventive treatment for age-related cognitive decline.

### **Iran J Pharm Res. 2016 Fall;15(4):875-883.**

*The Neuroprotective Effect of Rosemary (Rosmarinus officinalis L.) Hydro-alcoholic Extract on Cerebral Ischemic Tolerance in Experimental Stroke.*

Seyedemadi P, Rahnema M, Bigdeli MR, Oryan S, Rafati H.

#### **Abstract**

The prevention of BBB breakdown and the subsequent vasogenic edema are important parts of the medical management of ischemic stroke. The purpose of this study was to investigate the ischemic tolerance effect of *Rosmarinus officinalis* leaf hydro-alcoholic extract (RHE). Five groups of animals were designed: sham (underwent surgery without MCAO) and MCAO groups, the MCAO groups were pretreated orally by gavages with RHE (50, 75, and 100 mg/Kg/day), daily for 30 days. Two hours after the last dose, serum lipid levels were determined and then the rats were subjected to 60 min of middle cerebral artery occlusion followed by 24 h of reperfusion. Subsequently, brain infarct size, brain edema and Evans Blue dye extravasations were measured and neurological deficits were scored. Dietary RHE could significantly reduce cortical and sub-cortical infarct volumes ( $211.55 \pm 24.88$  mm<sup>3</sup> vs.  $40.59 \pm 10.04$  mm<sup>3</sup> vs.  $29.96 \pm 12.19$  mm<sup>3</sup> vs.  $6.58 \pm 3.2$  mm<sup>3</sup>), neurologic deficit scores, cerebral edema ( $82.34 \pm 0.42\%$  vs.  $79.92 \pm 0.49\%$  vs.  $79.45 \pm 0.26\%$  vs.  $79.30 \pm 0.19\%$ ), blood-brain barrier (BBB) permeability ( $7.73 \pm 0.4$  µg/g tissue vs.  $4.1 \pm 0.23$  µg/g tissue vs.  $3.58 \pm 0.3$  µg/g tissue vs.  $3.38 \pm 0.25$  µg/g tissue) in doses of 50, 75 and 100 mg/Kg/day as compared with the control group in the transient model of focal cerebral ischemia. Although pretreatment with RHE plays an important role in the generation of tolerance against cerebral I/R injury, further studies are needed to clarify the mechanism of the ischemic tolerance.

### **Neurosci Lett. 2016 May 27;622:95-101.**

*Rosemary extract improves cognitive deficits in a rats model of repetitive mild traumatic brain injury associated with reduction of astrocytosis and neuronal degeneration in hippocampus.*

Song H, Xu L, Zhang R, Cao Z, Zhang H, Yang L, Guo Z, Qu Y, Yu J.

#### **Abstract**

In this study, we investigated whether Rosemary extract (RE) improved cognitive deficits in repetitive mild Traumatic brain injury (rmTBI) rats and its potential mechanisms. The present results showed that rmTBI caused cognitive deficits, such as increased latency to find platform and decreased time spent in target quadrant in Morris water maze (MWM). These behavioral alterations were accompanying with the increased neuronal degeneration and glial fibrillary acidic protein (GFAP)-positive cells, increased Reactive oxygen species (ROS) generation, decreased activity of Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT), elevated protein level of IL-1β, IL-6 and TNF-α in hippocampus.

Treatment with RE prevented these changes above. Our findings confirmed the effect of rosemary extract on improvement of cognitive deficits and suggested its mechanisms might be mediated by anti-oxidative and anti-inflammatory. Therefore, rosemary extract may be a potential treatment to improve cognitive deficits in rmTBI patients.

## **Evid Based Complement Alternat Med. 2016;2016:2680409.**

*The Therapeutic Potential of Rosemary (Rosmarinus officinalis) Diterpenes for Alzheimer's Disease.*

Habtemariam S.

### **Abstract**

Rosemary (*Rosmarinus officinalis* L.) is one of the most economically important species of the family Lamiaceae. Native to the Mediterranean region, the plant is now widely distributed all over the world mainly due to its culinary, medicinal, and commercial uses including in the fragrance and food industries. Among the most important group of compounds isolated from the plant are the abietane-type phenolic diterpenes that account for most of the antioxidant and many pharmacological activities of the plant. Rosemary diterpenes have also been shown in recent years to inhibit neuronal cell death induced by a variety of agents both in vitro and in vivo. The therapeutic potential of these compounds for Alzheimer's disease (AD) is reviewed in this communication by giving special attention to the chemistry of the compounds along with the various pharmacological targets of the disease. The multifunctional nature of the compounds from the general antioxidant-mediated neuronal protection to other specific mechanisms including brain inflammation and amyloid beta (A $\beta$ ) formation, polymerisation, and pathologies is discussed.

## **Adv Exp Med Biol. 2015;863:95-116.**

*Brain Food for Alzheimer-Free Ageing: Focus on Herbal Medicines.*

Hügel HM.

### **Abstract**

Healthy brain aging and the problems of dementia and Alzheimer's disease (AD) are a global concern. Beyond 60 years of age, most, if not everyone, will experience a decline in cognitive skills, memory capacity and changes in brain structure. Longevity eventually leads to an accumulation of amyloid plaques and/or tau tangles, including some vascular dementia damage. Therefore, lifestyle choices are paramount to leading either a brain-derived or a brain-deprived life. The focus of this review is to critically examine the evidence, impact, influence and mechanisms of natural products as chemopreventive agents which induce therapeutic outcomes that modulate the aggregation process of beta-amyloid (A $\beta$ ), providing measurable cognitive benefits in the aging process. Plants can be considered as chemical factories that manufacture huge numbers of diverse bioactive substances, many of which have the potential to provide substantial neuroprotective benefits. Medicinal herbs and health food supplements have been widely used in Asia since over 2,000 years. The phytochemicals utilized in traditional Chinese medicine have demonstrated safety profiles for human consumption. Many herbs with anti-amyloidogenic activity, including those containing polyphenolic constituents such as green tea, turmeric, *Salvia miltiorrhiza*, and *Panax ginseng*, are presented. Also covered in this review are extracts from kitchen spices including cinnamon, ginger, rosemary, sage, *salvia* herbs, Chinese celery and many others some of which are

commonly used in herbal combinations and represent highly promising therapeutic natural compounds against AD. A number of clinical trials conducted on herbs to counter dementia and AD are discussed.

### **Chem Biol Interact. 2015 Jul 25;237:47-57.**

***Rosemary tea consumption results to anxiolytic- and anti-depressant-like behavior of adult male mice and inhibits all cerebral area and liver cholinesterase activity; phytochemical investigation and in silico studies.***

**Ferlemi AV, Katsikoudi A, Kontogianni VG, Kellici TF, Iatrou G, Lamari FN, Tzakos AG, Margarity M.**

#### **Abstract**

Our aim was to investigate the possible effects of regular drinking of *Rosmarinus officinalis* L. leaf infusion on behavior and on AChE activity of mice. Rosemary tea (2% w/w) phytochemical profile was investigated through LC/DAD/ESI-MS(n). Adult male mice were randomly divided into two groups: "Rosemary-treated" that received orally the rosemary tea for 4 weeks and "control" that received drinking water. The effects of regular drinking of rosemary tea on behavioral parameters were assessed by passive avoidance, elevated plus maze and forced swimming tests. Moreover, its effects on cerebral and liver cholinesterase (ChE) isoforms activity were examined colorimetrically. Phytochemical analysis revealed the presence of diterpenes, flavonoids and hydroxycinnamic derivatives in rosemary tea; the major compounds were quantitatively determined. Its consumption rigorously affected anxiety/fear and depression-like behavior of mice, though memory/learning was unaffected. ChE isoforms activity was significantly decreased in brain and liver of "rosemary treated" mice. In order to explain the tissue ChE inhibition, principal component analysis, pharmacophore alignment and molecular docking were used to explore a possible relationship between main identified compounds of rosemary tea, i.e. rosmarinic acid, luteolin-7-O-glucuronide, caffeic acid and known AChE inhibitors. Results revealed potential common pharmacophores of the phenolic components with the inhibitors. Our findings suggest that rosemary tea administration exerts anxiolytic and antidepressant effects on mice and inhibits ChE activity; its main phytochemicals may function in a similar way as inhibitors.

### **Fitoterapia. 2013 Dec;91:261-71.**

***Rosmarinus officinalis* L. leaf extract improves memory impairment and affects acetylcholinesterase and butyrylcholinesterase activities in rat brain.**

**Ozarowski M, Mikolajczak PL, Bogacz A, Gryszczynska A, Kujawska M, Jodynys-Liebert J, Piasecka A, Napieczynska H, Szulc M, Kujawski R, Bartkowiak-Wieczorek J, Cichocka J, Bobkiewicz-Kozlowska T, Czerny B, Mrozikiewicz PM.**

#### **Abstract**

*Rosmarinus officinalis* L. leaf as part of a diet and medication can be a valuable proposal for the prevention and treatment of dementia. The aim of the study was to assess the effects of subchronic (28-fold) administration of a plant extract (RE) (200 mg/kg, p.o.) on behavioral and cognitive responses of rats linked with acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity and their mRNA expression level in the hippocampus and frontal cortex. The passive avoidance test results showed that RE improved long-term memory in scopolamine-induced rats. The extract inhibited the AChE activity and showed a stimulatory effect on BuChE in both parts of rat brain. Moreover, RE produced a lower mRNA BuChE expression in the cortex and simultaneously an increase in the hippocampus. The study suggests

that RE led to improved long-term memory in rats, which can be partially explained by its inhibition of AChE activity in rat brain.

### **Phytother Res. 2016 May;30(5):805-14.**

*Anti-stress Activity of Ocimum sanctum: Possible Effects on Hypothalamic-Pituitary-Adrenal Axis.*

Jothie Richard E, Illuri R, Bethapudi B, Anandhakumar S, Bhaskar A, Chinampudur Velusami C, Mundkinajeddu D, Agarwal A.

#### **Abstract**

The present study investigated anti-stress potential of *Ocimum sanctum* in chronic variable stress (CVS) paradigm. Further, the possible mechanism of anti-stress was explored in vitro using cell and cell-free assays. Rats were administered *O. sanctum* followed by CVS regimen for a period of 16 days. On days 4, 8, 12, and 16, body weight and immobility time in forced swim test were measured. In addition, the possible inhibitory effect of *O. sanctum* and ursolic acid on cortisol release and CRHR1 receptor activity were studied in cell-based assays, while inhibitory effects on 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) and catechol-O-methyltransferase (COMT) were studied in cell-free assays. CVS group demonstrated less body weight gain and higher immobility time than *O. sanctum* administered groups, while oral administration of *O. sanctum* significantly increased body weight gain and decreased the immobility time. Further, *O. sanctum* and its constituents inhibited cortisol release and exhibited a significant CRHR1 receptor antagonist activity. Also, they had specific inhibitory activity towards 11 $\beta$ -HSD1 and COMT activity. Thus, *O. sanctum* was found to be effective in the management of stress effects, and anti-stress activity could be due to inhibition of cortisol release, blocking CRHR1 receptor, and inhibiting 11 $\beta$ -HSD1 and COMT activities.

### **Indian J Med Res. 2012 Apr;135(4):548-54.**

*Restraint stress-induced central monoaminergic & oxidative changes in rats & their prevention by novel Ocimum sanctum compounds.*

Ahmad A, Rasheed N, Chand K, Maurya R, Banu N, Palit G.

#### **Abstract**

##### **BACKGROUND & OBJECTIVES:**

*Ocimum sanctum* (OS) is known to possess various therapeutic properties. We have earlier isolated and characterized three OS compounds; Ocimarin, Ocimumoside A and Ocimumoside B. However, their role in modulating stress-induced central changes is unexplored. Thus, the present study was aimed to investigate the effect of these OS compounds on restraint stress (RS)-induced changes in the monoaminergic and antioxidant systems in the frontal cortex, striatum and hippocampus of rats.

##### **METHODS:**

RS was produced by immobilizing (restraining) the Sprague Dawley rats for a period of 2.5 h inside cylindrical steel tubes. The monoamine levels and the in vivo antioxidant status in brain regions were evaluated by HPLC-EC and spectrophotometric assays, respectively.

##### **RESULTS:**

RS significantly increased the dopamine levels in the frontal cortex and decreased in the striatum and hippocampus, and accompanied with selective increase of dopamine metabolites compared to the NS

control group. The serotonin and its metabolite levels were significantly increased, while noradrenaline levels were decreased by RS in the three brain regions studied. The activities of superoxide dismutase and glutathione peroxidase in the frontal cortex and striatum were significantly increased by RS with decreased glutathione levels and increased lipid peroxidation. Pre-treatment with Ocimumoside A and B (40 mg/kg po) for a period of 3 days prevented the RS-induced changes with an efficacy similar to that of standard anti-stress (Panax quinquefolium; 100 mg/kg po) and antioxidant (Melatonin; 20 mg/kg ip) drugs, while, Ocimarin failed to modulate these changes. OS compounds per se had no effect on these parameters.

#### INTERPRETATION & CONCLUSIONS:

The present findings showed the anti-stress potential of Ocimumoside A and B in relation to their simultaneous modulatory effects on the central monoaminergic and antioxidant systems implicating their therapeutic importance in stress-related disorders. Further studies are required to understand the mechanism of action of these compounds.

### **Neurol Sci. 2012 Dec;33(6):1239-47.**

*Ocimum sanctum attenuates oxidative damage and neurological deficits following focal cerebral ischemia/reperfusion injury in rats.*

Ahmad A, Khan MM, Raza SS, Javed H, Ashafaq M, Islam F, Safhi MM, Islam F.

#### **Abstract**

Stroke is an enormous public health problem with an imperative need for more effective therapy. Free radicals have been reported to play a role in the expansion of ischemic brain lesions, and the effect of free radical scavengers is still under debate. The present study investigated the neuroprotective effect of Ocimum sanctum (OS) to reduce brain injury after middle cerebral artery occlusion (MCAO). Male Wistar rats were subjected to MCAO for 2 h and reperfused for 22 h. The administration of OS (200 mg/kg bwt., orally) once daily for 15 days before MCAO showed marked reduction in infarct size, reduced the neurological deficits, and suppressed neuronal loss in MCAO rats. A significantly depleted activity of antioxidant enzymes and content of glutathione in MCAO group were protected significantly in MCAO group pretreated with OS. Conversely, the elevated level of thiobarbituric acid-reactive substances (TBARS) in MCAO group was attenuated significantly in OS-pretreated group when compared with MCAO group. Consequently, OS pretreatment may reduce the deterioration caused by free radicals, and thus may be used to prevent subsequent behavioral, biochemical and histopathological changes that transpire during cerebral ischemia. This finding reflects that supplementation of OS intuitively by reasonable and understandable treatment effectively ameliorates the cerebral ischemia-induced oxidative damage.

### **J Med Food. 2011 Sep;14(9):912-9.**

*Ocimum sanctum Linn. leaf extracts inhibit acetylcholinesterase and improve cognition in rats with experimentally induced dementia.*

Giridharan VV, Thandavarayan RA, Mani V, Ashok Dundapa T, Watanabe K, Konishi T.

#### **Abstract**

Cognitive disorders such as dementia, attention deficits, and Alzheimer's disease (AD) have been well

investigated. However, effective interventions for the promotion and progression of AD are unavailable to date. The present work was undertaken to investigate the effects of the aqueous (300 and 500 mg/kg) and alcoholic (300 and 500 mg/kg) extracts of *Ocimum sanctum* Linn. leaves as an antidementic and anticholinesterase agent and also as an immunostimulant in rats. Maximal electroshock, atropine, and cyclosporine were used to induce dementia. The passive avoidance task was used for assessing memory. Acetylcholinesterase (AChE) activity was estimated in different parts of the brain, and immune status was studied using dinitrochlorobenzene (DNCB) skin sensitivity tests. In all the three models both aqueous and alcoholic *O. sanctum* extracts decreased the time taken to reach the shock-free zone and the number of mistakes and significantly decreased the AChE activity in rats. *O. sanctum* treatment significantly increased the induration in the DNCB skin test. Therefore, *O. sanctum* was shown to be useful for the management of experimentally induced cognitive dysfunctions in rats.

### **J Pharmacol Sci. 2005 Aug;98(4):354-60.**

***Noise-stress-induced brain neurotransmitter changes and the effect of *Ocimum sanctum* (Linn) treatment in albino rats.***

**Ravindran R, Rathinasamy SD, Samson J, Senthilvelan M.**

#### **Abstract**

In this modern world, stress and pollution are unavoidable phenomena affecting the body system at various levels. A large number of people are exposed to potentially hazardous noise levels in daily modern life, such as noise from work environments, urban traffic, and household appliances. A variety of studies have suggested an association between noise exposure and the occurrence of disorders involving extra-auditory organs such as disorders of the nervous, endocrine, and cardiovascular systems. In this study, Wistar strain albino rats were subjected to 100 dB broadband white noise, 4 h daily for 15 days. The high-pressure liquid chromatographic estimation of norepinephrine, epinephrine, dopamine, and serotonin in discrete regions of the rat brain indicates that noise stress can alter the brain biogenic amines after 15 days of stress exposure. *Ocimum sanctum* (OS), a medicinal herb that is widely claimed to possess antistressor activity and used extensively in the Indian system of medicine for a variety of disorders, was chosen for this study. Administration of the 70% ethanolic extract of OS had a normalizing action on discrete regions of brain and controlled the alteration in neurotransmitter levels due to noise stress, emphasizing the antistressor potential of this plant.

### **Drugs R D. 2017 Mar;17(1):53-64.**

***Salvia (Sage): A Review of its Potential Cognitive-Enhancing and Protective Effects.***

**Lopresti AL.**

#### **Abstract**

Genus *Salvia*, commonly known as sage, is the largest genus in the Lamiaceae family. It comprises many species traditionally used as brain-enhancing tonics. In vitro and animal studies have confirmed that several *Salvia* species contain a large array of active compounds that may enhance cognitive activity and protect against neurodegenerative disease. In this review, the active constituents in plants belonging to the genus *Salvia* are summarized, and their influence on pharmacodynamics pertinent to cognitive activity

are detailed. In particular, the effects of plants belonging to the genus *Salvia* and their constituents on cognitive skills including memory, attention and learning are detailed. Their potential effects in dementia, including Alzheimer's disease, are also examined. Completed human trials are summarized, and factors influencing the potency of *Salvia* plants are covered. Finally, directions for future research are proposed to enhance our understanding of the potential health benefits of *Salvia* plants.

## **Psychopharmacology (Berl). 2008 May;198(1):127-39.**

***An extract of Salvia (sage) with anticholinesterase properties improves memory and attention in healthy older volunteers.***

**Scholey AB, Tildesley NT, Ballard CG, Wesnes KA, Tasker A, Perry EK, Kennedy DO.**

### **Abstract**

#### **RATIONALE:**

Species of *Salvia* (sage) have a long-standing reputation in European medical herbalism, including for memory enhancement. In recent controlled trials, administration of sage extracts with established cholinergic properties improved cognitive function in young adults.

#### **OBJECTIVES:**

This randomized, placebo-controlled, double-blind, balanced, five-period crossover study investigated the acute effects on cognitive performance of a standardized extract of *Salvia officinalis* in older adults.

#### **MATERIALS AND METHODS:**

Twenty volunteers (>65 years of age, mean = 72.95) received four active doses of extract (167, 333, 666 and 1332 mg) and a placebo with a 7-day wash-out period between visits. Assessment involved completion of the Cognitive Drug Research computerized assessment battery. On study days, treatments were administered immediately following a baseline assessment with further assessment at 1, 2.5, 4 and 6 h post treatment.

#### **RESULTS:**

Compared with the placebo condition (which exhibited the characteristic performance decline over the day), the 333-mg dose was associated with significant enhancement of secondary memory performance at all testing times. The same measure benefited to a lesser extent from other doses. There also were significant improvements to accuracy of attention following the 333-mg dose. In vitro analysis confirmed cholinesterase inhibiting properties for the extract.

#### **CONCLUSIONS:**

The overall pattern of results is consistent with a dose-related benefit to processes involved in efficient stimulus processing and/or memory consolidation rather than retrieval or working memory efficiency. These findings extend those of the memory-enhancing effects of *Salvia* extracts in younger populations and warrant further investigation in larger series, in other populations and with different dosing regimes.