Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

PB125

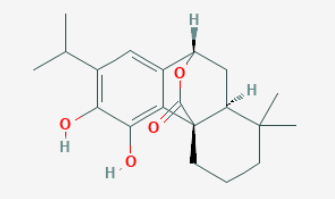
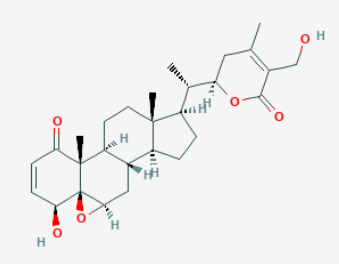
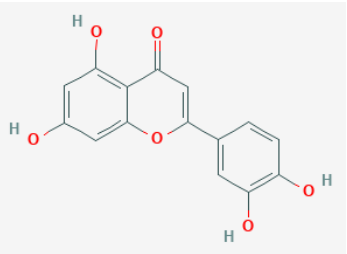
Evidence Summary

In vitro studies suggest PB125 has antioxidant, anti-inflammatory, cholesterol-lowering, and homocysteine-lowering effects, but no peer-reviewed evidence from in vivo models exist yet.

Neuroprotective Benefit: Although some evidence exists for rosemary, ashwagandha, and luteolin, PB125 has not been tested in any in vivo models for neuroprotection.

Aging and related health concerns: *In vitro* studies suggest PB125 activates Nrf2 antioxidant pathways, increases LDL clearance, decreases homocysteine levels, and reduces proinflammatory cytokines. It is being tested in the NIA-ITP for lifespan extension.

Safety: Rosemary, ashwagandha, and luteolin are generally considered as safe when ingested in usual doses. No in vivo studies have examined the safety profile of the PB125 combination.

Availability: available as a supplement	**Dose**: Effective dose for humans has not been established. A capsule of PB125® contains 68 mg of rosemary leaf extract, 23 mg of ashwagandha root extract, and 9 mg of luteolin.	**Chemical formula:** numerous active ingredients are included, such as carnosol, withaferin A, and luteolin. **MW**:  Carnosol—330.4 (PubChem)  Withaferin A—470.6 (PubChem)  Luteolin—286.24 (PubChem)
Half life: not documented	**BBB**: not documented	
Clinical trials: No peer-reviewed publications exist to date.	**Observational studies**: none	

What is it? PB125® is a supplement sold by Pathways Bioscience and is composed of a combination of compounds found in rosemary (*Rosmarinus officinalis*), ashwagandha (*Withania somnifera*), and *Sophora japonica* (Hybertson et al., 2019). The main active ingredients are carnosol, withaferin A, and luteolin. The rationale for selection of these compounds was based on their individual effects on the nuclear factor erythroid-related factor 2 (Nrf2), a transcription factor that regulates the gene expression of a wide variety of cytoprotective phase II detoxification enzymes and antioxidant enzymes through its binding to the antioxidant-responsive element (ARE) in the promoter region of these genes. These ingredients have been shown to exert synergistic effects in activating Nrf2 when used in combination (Hybertson et al., 2019). Rosemary is purported to have anti-inflammatory, antioxidant, and antimicrobial benefits. Ashwagandha has been utilized for anxiety, chronic inflammation, depression, fatigue, stress, and other conditions. Luteolin is a bioflavanoid flavone commonly consumed in the diet through multiple food sources (e.g., celery, broccoli, green pepper, parsley, etc.) and is frequently used as a dietary supplement for antioxidant and anti-inflammatory effects.

PB125 is one of the therapies being tested in the National Institute on Aging Interventions Testing Program (NIA ITP; see list of compounds here) for evaluating lifespan extension in mice. The NIA ITP was designed to test compounds such as PB125 that are purported to delay the onset of age-related diseases.

Neuroprotective Benefit: ***Rated C for potential and D for evidence.*** Although some evidence exists for rosemary, ashwagandha, and luteolin, PB125 has not been tested in any *in vivo* models for neuroprotection.

Types of evidence:

- 1 laboratory study

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function: None.

Human research to suggest benefits to patients with dementia: None.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:
No studies have examined whether PB125 is protective against cognitive decline or dementia in humans.

In an *in vitro* study (HepG2 hepatocellular carcinoma cells), PB125 treatment induced expression of low density lipoprotein receptor-related protein 10 (LRP10) by 2.5-fold (Hybertson et al., 2019). LRP10 is a functional amyloid precursor protein (APP) receptor involved in APP trafficking and processing, inducing the accumulation of mature APP in the Golgi, reducing its presence at the cell surface, and its processing into Aβ. Knockdown of LRP10 expression increases Aβ production, and expression of LRP10 is significantly lower in the post-mortem brain tissues of AD patients (Brodeur et al., 2012). Thus, PB125 could indirectly decrease Aβ production through increased LRP10 expression. However, no *in vivo* studies currently exist that probes whether PB125 improves Alzheimer's pathology in animal models.

PB125 treatment also upregulated C9orf72 by 3.1-fold (Hybertson et al., 2019). C9orf72 mutations are associated with amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration. C9orf72 deletion or knockdown in two animal models leads to motor neuron degeneration (Lall and Baloh, 2017), suggesting that PB125-induced enhancement of C9orf72 expression might be neuroprotective in certain neurodegenerative conditions.

While not tested in *in vivo* models yet, PB125's activation of Nrf2 would likely upregulate endogenous antioxidant pathways and may protect against oxidative stress-related neuronal/mitochondrial injury. The rosemary extract contains carnosic acid, which may be converted to carnosol (Hybertson et al., 2019). Carnosol activates the Nrf2 pathway as an electrophilic compound reacting more specifically with cysteine-151 of Keap1, an inhibitor of Nrf2, and releasing Nrf2.

Ashwagandha (*Withania somnifera*) contains withaferin A, which activates Nrf2 in a Keap1-independent manner via the PTEN/PI3K/Akt pathway, a mechanism distinct from carnosic acid. Withaferin A was the top-scoring compound for gene expression similarity to metformin and also displayed significant pathway- and gene-level similarity to rapamycin measured by pathway activation and deep learning (Hybertson et al., 2019).

Luteolin activates Nrf2 by a PI3K-dependent mechanism, and chosen as the third ingredient for PB125 primarily for its ability to affect Nrf2 activity by additional mechanisms distinct from those of carnosol or withaferin A (Hybertson et al., 2019). Luteolin doubles Nrf2 mRNA production by relieving epigenetic silencing of the Nrf2 promoter through reduction of CpG methylation on the Nrf2 promoter region (Zuo et al., 2018). Luteolin also inhibits GSK3B (Bustanji et al., 2009), which is responsible for activation of the Fyn kinase that phosphorylates nuclear Nrf2 at tyrosine 586, causing Nrf2 to be ejected from the nucleus, effectively shutting down Nrf2-dependent transcription.

APOE4 interactions: Unknown.

Aging and related health concerns: ***Rated A for potential and C for evidence.*** *In vitro* studies suggest PB125 activates Nrf2 antioxidant pathways, increases LDL clearance, decreases homocysteine levels, and reduces proinflammatory cytokines. It is being tested in the NIA-ITP for lifespan extension.

Types of evidence:

- 1 small open-label study
- 3 laboratory studies

Antioxidant effects in humans: On the website of Pathways Bioscience, the manufacturer of PB125, results from an open-label study are noted, though this study is not published or peer-reviewed. Nine human subjects with elevated plasma levels (greater than 1.5 µM, ranging from 1.5 to 3.5 µM) of thiobarbituric acid reactive substances (TBARS) were given PB125 supplement (dosage not noted). After supplementation, all subjects showed TBARS levels decline below 1.5 µM. TBARS is produced when polyunsaturated fatty acids are oxidized and is used as a measure of oxidative stress.

Antioxidant/Nrf2 effects in cell culture: PB125 is composed of three ingredients, rosemary (*Rosmarinus officinalis*), ashwagandha (*Withania somnifera*), and *Sophora japonica*, with specified levels of carnosol/carnosic acid, withaferin A, and luteolin, respectively (Hybertson et al., 2019). Each ingredient contributes to the activation of the Nrf2 pathway in unique ways, which leads to upregulation of cytoprotective genes and protection of cells against oxidative stress. The rationale for activating Nrf2 is that older rats and people exhibit lower nuclear Nrf2 levels compared to younger counterparts, with decreased antioxidant defenses and repair mechanisms (Zhou et al., 2018).

In an *in vitro* study (HepG2 hepatocellular carcinoma cells), the 3 different components of PB125 each exhibited Nrf2 activation, but worked synergistically, such that much greater activation was observed than the sum of each individual activities (Hybertson et al., 2019). The most optimal ratio for activating Nrf2 was rosemary extract, ashwagandha extract, and luteolin mixed at 15:5:2. Even though the molar concentration of withaferin A is much less than that of carnosic acid, its contribution to Nrf2 activation is at about 60% that of carnosic acid. With synergism, Nrf2 activation by PB125 is about 4.6 times that of carnosic acid alone.

PB125 treatment (16 µg/mL, 24 hours) in HepG2 cells significantly upregulated canonical Nrf2-regulated genes HMOX1 by 2.6-fold, GCLM by 5.4-fold, and SLC7A11 by 4.4-fold (Hybertson et al., 2019).

Effects on cholesterol pathways in cell culture: PB125 downregulated DKK1 (negative regulator of Wnt signaling) by -9.5-fold, FABP1 (metabolic gene increased in obesity) by -6.5-fold, flavin containing monooxygenase 5 (FMO5) by -3.3-fold, HMG CoA reductase by -2.8-fold, liver-expressed antimicrobial peptide 2 (LEAP2) by -5.8-fold, or PCSK9 by -4.4-fold (Hybertson et al., 2019). HMG CoA reductase catalyzes the rate-limiting step in the biosynthesis of cholesterol and is the target of statins, suggesting that PB125, if efficacious in *in vivo* models, could lower cholesterol. PCSK9 can bind to the LDL receptor (LDLR; removes cholesterol for excretion), causing it to be degraded rather than recycled, with the effect of slowing cholesterol removal. PB125's downregulatory effect on PCSK9 could increase cholesterol removal. Thus, PB125 may have a dual action on cholesterol lowering, by slowing cholesterol synthesis through downregulation of HMG CoA reductase, while also increasing clearance by downregulating PCSK9. These *in vitro* findings need *in vivo* and clinical validation.

Effects on metabolic pathways in cell culture: PB125 treatment in cell culture upregulated 3 enzymes involved in methionine/homocysteine metabolism: betaine-homocysteine S-methyltransferase (BHMT), cystathionine beta-synthase (CBS), and methionine adenosyltransferase 1 alpha (MAT1A) (Hybertson et al., 2019). MAT1A is essential for the formation of S-adenosylmethionine, the methyl donor required by numerous metabolic pathways throughout the body. BHMT can re-methylate homocysteine back to methionine to continue the cycle. CBS is essential for catabolizing the excess homocysteine produced by the cycle. CBS detoxifies homocysteine and produces hydrogen sulfide, which activates Nrf2. Together, PB125 may decrease homocysteine levels, though this needs to be validated in *in vivo* settings.

Other effects in cell culture: The group of genes with statistically significant expression changes induced by PB125 that are likely due to other, non-Nrf2-related effects of PB125 also includes genes of interest due to their potential health benefits when upregulated (such as VGF, PLAU, IL4R, CBS, and NOS3) or downregulated (such as APOL1, IFIT1, YPEL3. and CYP1A1) (Hybertson et al., 2019).

Anti-inflammatory and anti-COVID19 effects in cell culture: Nrf2 activity declines with age, making the elderly more susceptible to different age-related diseases as well as to viral infections. In cell culture (human liver-derived HepG2 cells), PB125® downregulated ACE2 mRNA by -3.5-fold and TMPRSS2 mRNA

by -2.8-fold (McCord et al., 2020, bioRxiv, not peer-reviewed). These findings are relevant for COVID-19 because ACE2 is a surface receptor and TMPRSS2 activates the spike protein for the virus to enter host cells. PB125 also upregulated PAI-1, a potent TMPRSS2 inhibitor, by 17.8-fold. Together, these findings suggest that PB125 has the potential to diminish the ability of COVID-19 to bind to a host cell and to obtain spike protein activation, which are required for host cell entry.

In primary human pulmonary artery endothelial cells exposed to an endotoxin (LPS), PB125 markedly downregulated 36 genes encoding cytokines, such as IL-1β, IL-6, TNF-α, the cell adhesion molecules intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), and E-selectin, and a group of IFN-γ-induced genes (McCord et al., 2020, bioRxiv, not peer-reviewed). LPS-induced IL-6 release was reduced by 61% in the cells pretreated with PB125. The mRNA levels of IL-1β and IL-6 were inhibited by 61% and 44%, respectively, with PB125 pretreatment. The proinflammatory cytokine-induced adhesion molecules, ICAM1, VCAM1, and E-selectin were suppressed by an average of 78%. The mRNA level of TNF was reduced by 33%, but a group of five TNF-induced proteins (TNFAIPs) were repressed on average by 70%. Also, a family of five interferon-inducible genes were downregulated by an average of 63%. Plasmin can trigger proinflammatory release of cytokines and PB125 downregulated plasminogen mRNA expression by -1.9-fold. Many of the cytokines downregulated by PB125 have been implicated in the "cytokine storm" observed in COVID-19. It remains possible that activation of Nrf2 with PB125 or other agents may decrease the intensity of the cytokine storm, but these initial findings require validation in *in vivo* and clinical settings.

Safety: ***Rated B for potential and D for evidence.*** Rosemary, ashwagandha, and luteolin are generally considered as safe when ingested in usual doses. No *in vivo* studies have examined the safety profile of the PB125 combination.

Types of evidence:

- 1 laboratory study

In an *in vitro* study (HepG2 hepatocellular carcinoma cells), PB125 treatment at 5 µg/mL was not cytotoxic as determined by cell viability (measured by the CCK8 cell proliferation assay) and did not induce cellular injury (measured by release of LDH into the culture media)(Hybertson et al., 2019). When the cells were challenged with an oxidant, cumene hydroperoxide (25 µM), for 6 hours, pretreatment with 5 µg/mL PB125 protected against oxidative-stress-induced loss of cell viability and cell damage.

Drug interactions: Unknown. No studies have examined drug interactions for PB125.

Sources and dosing: PB125® is a supplement sold by Pathways Bioscience and is composed of a combination of compounds found in rosemary (*Rosmarinus officinalis*), ashwagandha (*Withania somnifera*), and luteolin (Hybertson et al., 2019). The main active ingredients are carnosol, withaferin A, and luteolin. A single capsule of PB125® contains 68 mg of rosemary leaf extract, 23 mg of ashwagandha root extract, and 9 mg of luteolin. The effective dose for humans has not been established.

In the published *in vitro* studies, rosemary extract from *Rosmarinus officinalis* (standardized to 6% carnosol; 15% carnosic acid) was obtained from Flavex (Rehlingen, Germany), ashwagandha extract from *Withania somnifera* (standardized to 2% withaferin A) was obtained from Verdure Sciences (Noblesville, IN, USA), and luteolin (standardized to 98% luteolin, from *Sophora japonica*) was obtained from Jiaherb (Pine Brook, NJ, USA)(Hybertson et al., 2019). PB125 solutions were made by mixing the rosemary, ashwagandha, and luteolin powders at a 15:5:2 ratio by mass, then extracted at 50 mg of mixed powder per mL in ethanol overnight and the supernatant isolated.

Research underway: Based on ClinicalTrials.gov, there is currently one double-blind randomized clinical trial testing PB125 (100 mg on days 1-5 of bed rest) or MITO-AO (another anti-oxidant supplement; 160 mg on day 1 and 40 mg on days 2-5) in preventing vascular and skeletal muscle dysfunction during disuse (NCT04351113).

PB125 is one of the therapies being tested in the NIA-ITP Program (see list of compounds here). The NIA ITP was designed to test compounds such as PB125 that are purported to extend lifespan and/or delay onset of age-related diseases. This collaborative program uses 1) parallel studies in male and female mice at 3 different sites, 2) genetically heterogeneous mice to guard against conclusions based on a single inbred genotype, and 3) enough samples to provide statistical power.

Search terms:

Pubmed, Google:

- PB125, PB-125

Websites visited for PB125:

- Clinicaltrials.gov (1)
- Examine.com (0)
- DrugAge (0)
- Geroprotectors (0)
- Drugs.com (0)
- WebMD.com (0)
- PubChem (Carnosol; Withaferin A; Luteolin)
- DrugBank.ca (0)
- Labdoor.com (0)
- Cafepharma (0)
- Pharmapro.com (0)