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.

Rhein

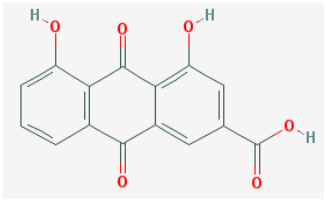
Evidence Summary

Offers minor benefits in neuroprotection, blood glucose regulation, reduction of joint pain, and reducing atherosclerosis by preventing oxidative stress and inflammation. Use may be limited by laxative activity.

Neuroprotective Benefit: May help protect the brain against inflammatory and oxidative stress damage, but benefits limited by poor bioavailability and BBB penetration.

Aging and related health concerns: Offers minor benefits in the reduction of joint pain, cholesterol, and blood glucose levels.

Safety: Acts as a laxative, which can lead to gastrointestinal side effects, such as diarrhea. May also increase risk for bleeding and loss of potassium.

Availability: OTC (rhubarb root), Rx (Diacerin)	**Dose**: Diacerein 50 mg 2x daily (oral) Rhubarb 20-50 mg/kg daily	**Chemical formula:** $C_{15}H_8O_6$ **MW**: 284.223 g/mol  Source: Pubchem
Half-life: 4-10 hours	**BBB**: Rhein is not penetrant (metabolite penetrant).	
Clinical trials: Meta-analyses of RCTs indicate minor benefits for patients with osteoarthritis, Type 2 diabetes, and atherosclerosis.	**Observational studies**: Use of traditional Chinese medicines containing rhein associated with possible reduction in dementia risk.	

What is it? Rhein, also known as cassic acid, is an anthraquinone contained in rhubarb root, and the active component of the traditional Chinese medicine, Da Huang. Rhein is also found in the roots of the common traditional Chinese medicinal herb, *Polygonum multiflorum* Thunb. Although it has low oral bioavailability [1], rhein can be detected in the blood after consumption of rhubarb root extract from the Chinese medicinal rhubarb, *rheum palmatum* [2]. While rhubarb contains other compounds with potentially anti-inflammatory and antioxidant activity, only rhein is detectable in the blood in appreciable quantities. Rhein is not found in appreciable quantities in the English variety of rhubarb commonly found in the US, *rheum rhaponticum*. Rhubarb (Da Huang) has traditionally been used as a laxative. Rhein is also available by prescription in a doubly acetylated slow-acting prodrug formulation called diacerein (diacetylrhein) for the treatment of osteoarthritis and joint pain. In animal studies, rhein has been shown to protect against kidney damage through the preservation of Klotho expression by preventing/reversing Klotho promoter silencing (DNA methylation).

Neuroprotective Benefit: May help protect the brain against inflammatory and oxidative stress damage, but benefits limited by poor bioavailability and BBB penetration.

Types of evidence:

- 2 clinical studies (In China using traditional Chinese medicine, questionable design)
- 2 observational studies (In China involving use of traditional Chinese medicine)
- Several laboratory studies

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

Dementia Prevention: **Possible minor benefit/unclear**

Two observational studies using patient data extracted from the Taiwanese National Health Insurance Research Database indicate that people who used Da Huang had a lower risk of developing dementia than those who did not take use any form of traditional Chinese medicine. In a study of hypertensive patients (ages 20-90) comparing 52,365 users of traditional Chinese medicine with 91, 017 non-user controls, there was a significantly reduced risk of dementia with overall traditional Chinese medicine use [adjusted Hazard ratio (HR): 0.76, 95% Confidence Interval (CI) (0.74 to 0.81)], and a slightly reduced risk specifically with Da Huang use [HR: 0.92, 95% CI (0.70 to 1.20)] [3]. A reduced risk for dementia was also found in a smaller study of 2,804 migraine patients with overall traditional Chinese medicine use [adjusted HR: 0.65, 95% CI (0.46 to 0.95)] and Da Huang use [HR: 0.36, 95% CI (0.14 to 0.93)][4]. However, these associations should be considered weak because the studies were unable to account for potential confounding factors, such as alcohol and tobacco use, physical activity, diet, social network, or education.

Human research to suggest benefits to patients with dementia: **Possible minor benefit/unclear**

There are two clinical studies performed in China indicating that the use of traditional Chinese medicines which contain rhein improved memory performance in patients with memory impairment or Alzheimer's disease (AD). However, only the abstracts are available in English, therefore it was not possible to evaluate whether these were well-designed studies, or if the inclusion criteria used to select the patients in the studies was appropriate. One study of patients with memory impairment comparing the use of the compound Tong Jiang Oral Liquid with or without Da Huang, claims that the inclusion of Da Huang led to improved performance on a memory ability test ($P<0.05$) [5]. The other study of 209 AD patients compared use of Polygonum multiforum extract with general Chinese herb use and piracetam use. They claim that scores on the Mini-Mental State Exam (MMSE) and Ability of Daily Living Scale improved in all groups, and the group using Polygonum improved the most ($P<0.01$) [6]. Their claim that the total effective rate was 93.33% in the compound Polygonum multiflorum extract treatment, compared to 73.33% with Chinese herbs and 68.97% with Piracetam, leads one to believe that their threshold for 'efficacy' was quite low and unlikely to be clinically meaningful.

Mechanisms of action for neuroprotection identified from laboratory and clinical research:

Animals studies indicate that rhein exerts its neuroprotective effects primarily through its antioxidant and anti-inflammatory activities. Rhein is highly lipophilic and is readily absorbed in the blood and distributed in tissues throughout the body [1], however, it has minimal capacity to penetrate the blood brain barrier (BBB), and is not present in the cerebrospinal fluid (CSF) of healthy individuals [7]. Yet, when the BBB is disrupted during brain trauma, rhein can readily enter brain tissues [7]. It is possible that rhein may still be beneficial in the absence of CNS injury, because its phase 1 metabolite, anthrone, can cross the BBB [1] and has more potent antioxidant activity than its parent compound, rhein [8].

Alzheimer's disease and Inflammation-associated cognitive impairment:

Potential benefit (rodents)

Rhein-huprine hybrids were developed with the rationale that combining the anti-tau and A□-aggregating effects of rhein with the anticholinesterase inhibitory effects of huprine would be effective in reducing AD pathology and preserving cognitive function. Studies in APP/PS1 AD mouse models indicate that these compounds (2mg/kg i.p. 3X per week for 4 weeks) are BBB penetrant, can reduce A□ and tau aggregation, protect against Aβ-associated synaptic dysfunction, reduce neuroinflammation (reactive gliosis), and partially recover cognitive function on a memory flexibility task [9; 10]. Notably, the beneficial effects were only seen with the (+) enantiomer of these compounds [10].

In 4-month-old senescence-accelerated (SAMP8) mice, treatment with a water-soluble form of rhein (rhein lysinate 50 mg/kg in drinking water for 6 months), **prevented age-associated inflammation and oxidative stress** damage normally present in these mice. Rhein treatment reduced levels of Aβ40 and Aβ42, inflammation (TNFα, IL-6), and markers of oxidative stress (ROS and malondialdehyde (MDA)[11].

Rhein treatment (120 mg/kg orally daily for 6 weeks) was able to prevent high-fat-diet induced obesity and associated cognitive impairment in mice as measured by a memory recognition task [12]. These effects appear to have been mediated by rhein's anti-inflammatory activity, and modulation of the microbiome. Rhein prevented the high-fat diet induced decrease in brain derived neurotrophic factor (BDNF), induction of the TLR4/My88/JNK/NF-κB pro-inflammatory signaling pathway, and macrophage infiltration and microbiota alterations in the colon.

As with other anti-oxidants and anti-inflammatory agents, rhein is likely to be most effective for prevention of cellular damage, or mitigating cell stressors very early after onset of pathology.

<u>Traumatic brain injury/Stroke:</u> Potential benefit (rodents)

Rhein was detectable in the CSF of patients (n=9, age 18-65) with severe traumatic brain injury (TBI) following nastrogastric administration of rhubarb extract (500 mg/kg) [7]. CSF levels of rhein peaked 1 hour after administration and were approximately 17% of the levels found in plasma. Similarly, in rats, the absorption rate was increased, and the clearance rate decreased in the context of cerebral ischemic injury [13].

In rats with TBI, rhein (from rhubarb extract or purified rhein 12 mg/kg) was found to mitigate damage to the BBB, reduce associated brain edema, and reduce markers of oxidative stress [14; 15]. Rhein treatment reduced MDA levels and NADPH oxidase induced production of ROS, while increasing antioxidants such as superoxide dismutase (SOD) and glutathione.

A meta-analysis of 12 RCTs involving the use of rhubarb and other rhizome based Chinese medicinal herbs (some of which contain rhein), found that rhizome therapies improved the clinical effective rate in patients with acute ischemic stroke when compared with Western conventional medicine controls (n = 788, Relative risk (RR) :1.27, 95% CI (1.18 to 1.37), z = 6.31, p < 0.01), and in a subset of 5 trials rhizome-based prescriptions improved neurological deficits compared with controls (*n* = 420, Weighted mean difference (WMD): −3.36, 95% CI (−6.10 to −0.62), z = 2.40, p = 0.02) [16]. However, the RCTs included in this analysis have many methodological weaknesses, including the lack of a placebo control, which makes it unclear whether rhubarb offers any clinically meaningful benefits for stroke patients.

Rhein treatment (50 or 100 mg/kg rhein orally) for 3 days after cerebral ischemic injury (MCAO) reduced the area of infarction and improved neurological function scores to a similar degree as nimodipine, which is currently used for preventing subarachnoid hemorrhage [17]. This was accompanied by a decrease in markers of apoptosis (caspase-3 and 9) and increased antioxidant activity (SOD, catalase, glutathione).

Overall, rhein was beneficial in mitigating, but not fully preventing or reversing damage, as the treated animals still had significant neurological impairments and pathology compared to sham controls.

<u>APOE4 interactions</u>:

Unknown

Aging and related health concerns: Offers minor benefits in the reduction of joint pain, cholesterol, and blood glucose levels.

Types of evidence:

- 4 meta-analyses (Diacerein use in osteoarthritis (n=31, n=6, n=10 studies), Type-2 Diabetes n=4 studies).
- 6 clinical trials (Diacerein for Type 2 Diabetes, Kidney disease, Congestive heart failure) (Rhubarb for atherosclerosis and radiation-induced toxicity)
- Numerous laboratory studies

Osteoarthritis: Benefit (minor)

Diacerin is used for the treatment of osteoarthritis associated joint pain primarily in Europe and the Middle East. A Cochrane systematic review of 10 clinical trials involving 2,210 patients found that diacerin use (50 mg 2x daily) had **a small beneficial effect on overall pain** (measured on a 100 mm visual analogue scale) at 3 to 36 months, equivalent to a 9% (95% CI -16% to -2%) pain reduction compared to placebo [18]. However, the evidence was regarded as low-quality and unlikely to be clinically meaningful. Two other meta-analyses also provide evidence for minor benefit of **questionable clinical significance**. A meta-analysis of 6 studies involving 1,533 patients found that the combined efficacy effect size (estimated using Hedges's standardized mean difference) was −0.24 (95% CI 0.39 to −0.08, P=0.003], favoring diacerein [19]. An analysis of 31 studies comparing the use of diacerin, glucosamine and NSAIDs for osteoarthritic knee pain found that diacerin clinically improved visual analog scores [−2.23 95% CI (−2.82 to −1.64)], function [−6.64 95% CI (−10.50 to −2.78)], and stiffness [−0.68 (−1.20 to −0.16)] on osteoarthritic index scales, compared to placebo [20]. Diacerin was found to have similar efficacy to glucosamine, but was associated with more side effects. Although diacerin is effective for alleviating joint pain, the benefits are relatively minor and the gastrointestinal side effects make it a less attractive option than other available treatments.

Type 2 Diabetes: Potential benefit

A meta-analysis of 4 RCTs testing the safety and efficacy of diacerin (100 mg per day) in patients with Type 2 diabetes found that it was **beneficial in regulating blood glucose**. Diacerein significantly reduced fasting glycemia [WMD: -0.66, 95% CI (-1.16 to -0.16)] and glycated hemoglobin A1c (HbA1c) [WMD: -0.85, 95% CI (-1.44 to -0.26)] [21]. HbA1c levels serve as a measure of blood glucose levels for the past 2-3 months. In 3 of the 4 studies, diacerin was used as a supplement to the patients' existing anti-diabetic drug regimen, suggesting it may have additive effects with other therapies. In a separate 12-week study

(ReBEC number RBR-29j956) with patients (n=72) taking dicaerin (50 mg/day) in addition to their normal anti-diabetic therapy, diacerin led to a decrease in HbA1c levels compared to placebo [−0.98, 95% CI (−2.02 to 0.05), P = 0.06], which was attributed to a positive effect on insulin secretion [22]. Metrics related to fasting glucose, inflammation, or renal function were not significantly affected by diacerin in this study. The long-term value of diacerin supplementation is unclear, as the longest trial was only 48 weeks, and the greatest benefits were seen in the shortest duration trials.

Based on rodent models, the beneficial effects on blood glucose levels may be related to its anti-inflammatory activity and an improvement of insulin signaling in the liver and adipose tissue. In a mouse model of high-fat diet induced diabetes, diacerin treatment (20 mg/kg for 10 days) improved endoplasmic reticulum stress, decreased macrophage infiltration in adipose tissue, and reduced expression and activity of proinflammatory mediators (IL-6, TNFα, IL-1β, iNOS, JNK and IKKβ phosphorylation) [23].

Kidney Disease: Potential benefit/Unclear

An RCT (ReBeC U1111-1156-0255) of diacerin use (50 mg 2x daily) for 3 months in Type 2 diabetic patients (n=81) with kidney disease found that diacerin improved metabolic control by 74%, and reduced systolic and diastolic blood pressure relative to placebo [24]. However, it had no effect on glomerular filtration rate, making it unclear whether there was any real improvement in renal function. A small study of 12 elderly patients with congestive heart failure taking diacerin for 5 days also did not show any evidence of a significant effect on renal function [25], but because diacerin is a slow-acting drug, this study may have been too short to detect any possible benefits.

Animal models suggest that rhein can be protective in the context of acute kidney injury, and the lack of efficacy in human trials may be due to the low bioavailability of rhein used in these formulations. In LPS-induced inflammatory acute kidney injury, rhein pretreatment (120 mg/kg) 1 day prior to LPS, attenuated inflammation and renal damage in a Klotho-dependent manner [26]. Rhein facilitates the induction of Klotho by preventing its epigenetic silencing. In the context of kidney injury, the Klotho promoter becomes hypermethylated due to aberrant activity of DNA methyltransferases 1 and 3a [27]. Rhein treatment corrected DNA methyltransferase levels and prevented Klotho silencing. The upregulation of Klotho promoted the degradation of TLR4, and inhibition of its associated pro-inflammatory signaling cascade [26].

The renal protective effects of rhein may be enhanced by using novel formulations or encapsulations with improved bioavailability and pharmacokinetics. Rhein-loaded polyethyleneglycol-co-polycaprolactone-co-polyethylenimine nanoparticles (PPPCy5-RH-NP) had fast cellular uptake, but did

not show burst release properties [28]. The rhein-nanoparticles were able to localize to the kidney and exhibited improved measures of kidney function (increased creatine clearance and reduced serum urea nitrogen) in a model of streptozocin-induced diabetic nephropathy. The protection involved inhibition of the TGF-β1 signaling pathway.

Atherosclerosis: Potential benefit

In an RCT of patients with atherosclerosis (n=83), the use of rhubarb as a supplement to standard therapy for 6 months showed significant decreases in serum total cholesterol and low-density lipoprotein cholesterol (LDL-c), as well as an increase in flow-mediated dilation compared to the control group. The improvement in dilation was correlated with the reduction in cholesterol [29].

In rabbits with balloon catheter injury and high-cholesterol diet induced atherosclerosis, daily rhubarb (50 mg/kg) was compared to simvastatin (5 mg/kg) [30]. While both were able to reduce total cholesterol, LDL-c, carotid thickness (IMT), and inflammatory mediators (TLR2, TLR4, NF-kB) compared to controls, simvastatin was more effective than rhubarb in reducing plaque rupture (35.7% vs 42.9%, compared to 80% ruptures in untreated control, $P<0.05$).

Rhubarb may be beneficial in reducing atherosclerotic plaque formation when used in combination with other cholesterol-lowering therapies.

Cancer: Potential benefit (novel formulations in rodents)

Rhein has been demonstrated to have anti-tumorigenic activity in cell culture and rodent xenograft models, and may be beneficial in helping overcome chemo-resistance and reduce radiation-associated side effects as a combination therapy. Many of these studies have used novel formulations of rhein that have improved bioavailability and efficacy.

In an RCT of lung cancer patients undergoing radiation therapy (n=74), rhubarb extract treatment (20 mg/kg daily for 6 weeks) was demonstrated to reduce radiation-induced lung toxicity [31]. At 6 weeks, the incidence of lung toxicity was 32.4% (95% CI 18 to 50%) versus 56.7% (95% CI 39 to 73%) for placebo, and 27.0% (95% CI 14 to 44%) versus 52.8% (95% CI 35 to 70%) at 6 months post-treatment ($P<0.05$). This was accompanied by an increase in lung capacity and decrease in inflammation (TGF-β1 and IL-6), and suggests that the anti-inflammatory actions of rhubarb may **help prevent radiation-induced damage to healthy tissue**. It is not known, whether the anti-tumorigenic properties of the radiation treatment were augmented by the rhubarb supplementation.

In carcinoma cells, rhein was shown to dose-dependently decrease mitochondrial metabolic rates and suppress expression of fibrotic and tumorigenic mediators, especially sonic hedgehog signaling and Akt phosphorylation [32]. Rhein was also found to target AlkB enzymes, which repair methylated DNA damage, in U87 cancer cells, and may be useful in helping overcome tumor resistance to methylating anticancer drugs [33]. In tumor xenograft models, rhein was found to enhance anti-tumorigenic properties when used in combination therapy. In a breast cancer xenograft model, liposomes loaded with diacerin attached with synthetic stable somatostatin had better tumor cell engagement and suppressed tumor proliferation better than free diacerin [34]. Anti-proliferative effects were mediated by the targeting of IL-6/MAP/Akt signaling. Similarly, in a doxorubin-resistant ovarian cancer xenograft model, rhein co-loaded with doxorubin in nanoparticles had enhanced tumor targeting capacity and cytotoxicity [35].

The best anti-tumor response was demonstrated in a study using the rhein-analog, AQ-101 (20 mg/kg daily for 3 weeks) in an acute lymphoblastic leukemia (ALL) xenograft model [36]. AQ-101 was able to inhibit ALL development, with all of the mice surviving to end of study (150 days), while the untreated controls all died within 50 days.

Modified rhein (conjugated with gadolinium) is also being developed as a novel MRI contrast agent to detect necrotic cancer tissue [37]. In breast carcinoma bearing rats, the conjugated rhein produced a contrast ratio between necrotic and viable tumor of 1.63 ± 0.11 by 3 hours after administration.

Safety: Acts as a laxative, which can lead to gastrointestinal side effects, such as diarrhea. May also increase risk for bleeding and loss of potassium.

Types of evidence:

- 4 meta-analyses (Diacerein use in osteoarthritis (n=31, n=6, n=10 studies), Type-2 Diabetes n=4 studies).
- 7 clinical trials (Diacerein for Type 2 Diabetes, Kidney disease, Congestive heart failure) (Rhubarb for atherosclerosis, radiation-induced toxicity, and pharmacokinetics)
- Numerous laboratory studies

In clinical trials, diacerein use was associated with **increased incidence of gastrointestinal events**, primarily diarrhea, which is related to the ability of rhein to act as a laxative. In a meta-analysis of diacerein use in patients with osteoarthritis, diacerein use was associated with an increased risk for adverse events compared to placebo [Risk ratio (RR): 3.52, 95% CI (2.42 to 5.11)], but there were no

significant differences in withdrawal due to adverse events [18]. The risk for diarrhea was RR: 3.51 (95% CI 2.55 to 4.83; P<0.0001) in patients with osteoarthritis [19] and RR: 2.50 (95% CI 1.10 to 5.65) in patients with Type 2 diabetes [21]. Additionally, in diabetic patients, diacerein use was associated with an increased incidence of dark urine [22; 24]. Due to the risk of diarrhea, the EMA issued new guidelines in 2014 recommending that diacerein not be used in people over age 65, or those with a history of liver disease, due to increases in liver enzymes in some patients. There is limited reporting of adverse events in clinical trials involving rhubarb extract, but are generally limited to gastrointestinal events. Drugs.com warns that rhubarb could increase the toxicity of cardiac glycosides and interfere with antiarrhythmics and diuretics due to loss of potassium. Due to the possibility for vitamin and ionic imbalances associated with diarrhea, rhubarb could also increase bleeding risk with warfarin use.

Sources and dosing:

Diacerein is marketed under a variety of brand labels and is available by prescription primarily in Europe, India, and the Middle East, but is not available in the USA. For osteoarthritis, the dosing is 50 mg 2x daily, but the recommended starting dose is 50 mg per day to assess tolerability.

Rhein is also available in oral form (pills and powders) as rhubarb root extract (Da Huang). In clinical trials showing efficacy rhubarb extract was used at 20-50 mg/kg, however, there is no information from reputable sources about the best quality rhubarb supplements. Since rhein is only found in high concentrations in Chinese rhubarb (*rheum palmatum)*, only rhubarb extracted from the root of these varieties would offer the benefits of rhein.

Research underway:

According to Clinicaltrials.gov, there are 5 active/recruiting trials for diacerein and 4 trials for traditional Chinese medicine that incorporates rhubarb.

The trials with rhubarb are for improvement of kidney or gastrointestinal function. The diacerein trials are for osteoarthritis, thrymbocytopenia, and a topical formulation for Epidermolysis Bullosa (a blistering condition). A previous Phase 2 trial for Epidermolysis Bullosa was terminated in October 2018 because an independent data monitoring committee suggested that the study would not meet statistical objectives.

Search terms:

Pubmed, Google: Rhein or Cassic acid or Rhubarb or Da Huang or Diacerein +

dementia, Alzheimer's disease, neurodegeneration, cognitive, brain, aging, lifespan, cardiovascular, kidney, klotho, diabetes, atherosclerosis, arthritis, inflammation, pharmacokinetics, bioavailability, safety, meta-analysis, clinical trials

Websites visited for Rhein:

- Clinicaltrials.gov (diacerein), (rhubarb)
- Drugs.com (diacerein), (rhubarb)
- WebMD.com (rhubarb)
- PubChem (rhein)
- DrugBank.ca (rhein)
- Practo.com (diacerein)

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