

COMPOSITIONS AND METHODS FOR THE TREATMENT OF “PLAQUES AND TANGLES” IN HUMANS AND ANIMALS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 62/126,026 filed February 27, 2015, and U.S. Provisional Application 62/170,822, filed June 4, 2015, herein incorporated by reference in their entireties.

TECHNICAL FIELD

[0002] The invention relates to blended compositions of plant extracts of *Uncaria tomentosa* and Oolong tea and methods for treating “plaques and tangles” that accumulate in the aging brain in amyloidosis and tauopathies in humans and animals (i.e. such as aged dogs and cats). In addition, the invention relates to the development of a nutraceutical blended composition consisting of a combination of a plant extract of *Uncaria tomentosa* and a specific Oolong tea, to prevent and treat traumatic brain injury (TBI), concussions (as observed in most athletes and the military/soldiers), single and repeated blows to the head, and chronic traumatic encephalopathy (CTE).

BACKGROUND

AMYLOIDOSIS AND THE ACCUMULATION OF BETA-AMYLOID “PLAQUES IN THE BRAIN OF A VARIETY OF DISORDERS

[0003] Alzheimer’s disease is characterized by the accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein or A β , in a fibrillary form, existing as extracellular amyloid plaques and as amyloid within the walls of cerebral blood vessels. Fibrillar A β amyloid deposition in Alzheimer’s disease is believed to be detrimental to the patient and eventually leads to toxicity and neuronal cell death, a characteristic hallmark of Alzheimer’s disease. Accumulating evidence implicates amyloid, and more specifically, the formation, deposition, accumulation and/or persistence of A β fibrils, as a major causative factor of Alzheimer’s disease pathogenesis. In addition, besides Alzheimer’s disease, a number of other amyloid diseases involve formation, deposition, accumulation and persistence of A β fibrils, including Down’s syndrome, disorders involving congophilic angiopathy, such as but not limited to, hereditary cerebral hemorrhage of the Dutch type, inclusion body myositis, dementia

pugilistica, cerebral β -amyloid angiopathy, dementia associated with progressive supranuclear palsy, dementia associated with cortical basal degeneration and mild cognitive impairment.

[0004] The amyloid diseases (amyloidosis) are classified according to the type of amyloid protein as well as the underlying disease. Amyloid diseases have a number of common characteristics including each amyloid consisting of a unique type of amyloid protein. The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease, Down's syndrome, Canine Dysfunction syndrome (CDS), Canine Cognitive Dysfunction (CCD), as seen in aged animals such as dogs and cats, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, dementia pugilistica, inclusion body myositis (Askanas et al., *Ann. Neurol.* 43:521-560, 1993) and mild cognitive impairment (where the specific amyloid is referred to as beta-amyloid protein or A β), the amyloid associated with chronic inflammation, various forms of malignancy and Familial Mediterranean Fever (where the specific amyloid is referred to as AA amyloid or inflammation-associated amyloidosis), the amyloid associated with multiple myeloma and other B-cell dyscrasias (where the specific amyloid is referred to as AL amyloid), the amyloid associated with type 2 diabetes (where the specific amyloid is referred to as amylin or islet amyloid polypeptide or IAPP), the amyloid associated with the prion diseases including Creutzfeld-Jakob disease, Gerstmann-Strausler syndrome, kuru and animal scrapie (where the specific amyloid is referred to as PrP amyloid), the amyloid associated with long-term hemodialysis and carpal tunnel syndrome (where the specific amyloid is referred to as α 2-microglobulin amyloid), the amyloid associated with senile cardiac amyloidosis and Familial Amyloidotic Polyneuropathy (where the specific amyloid is referred to as transthyretin or prealbumin), and the amyloid associated with endocrine tumors such as medullary carcinoma of the thyroid (where the specific amyloid is referred to as variants of procalcitonin). In addition, the α -synuclein protein which forms amyloid-like fibrils, and is Congo red and Thioflavin S positive (specific stains used to detect amyloid fibrillary deposits), is found as part of Lewy bodies in the brains of patients with Parkinson's disease, Lewy body disease (Lewy in *Handbuch der Neurologie*, M. Lewandowski, ed., Springer, Berlin pp. 920-933, 1912; Pollanen et al, *J. Neuropath. Exp. Neurol.* 52:183-191, 1993; Spillantini et al, *Proc. Natl. Acad. Sci. USA* 95:6469-6473, 1998; Arai et al, *Neurosc. Lett.* 259:83-86, 1999), multiple system atrophy (Wakabayashi et al, *Acta Neuropath.* 96:445-452, 1998), dementia with Lewy bodies, and the Lewy body variant of Alzheimer's disease. For purposes of this disclosure, Parkinson's disease, due to the fact that fibrils develop in the brains of patients with this disease (which are Congo red and Thioflavin S positive, and which contain predominant beta-pleated

sheet secondary structure). is now regarded as a disease that also displays the characteristics of an amyloid-disease.

AMYLOID AS A THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE

[0005] Alzheimer's disease is characterized by the deposition and accumulation of a 39-43 amino acid peptide termed the beta-amyloid protein, A β or β /A4 (Glenner and Wong, Biochem. Biophys. Res. Comm. 120:885-890, 1984; Masters et al, Proc. Natl. Acad. Sci. USA 82:4245-4249, 1985; Husby et al, Bull. WHO 71:105-108, 1993). A β is derived by protease cleavage from larger precursor proteins termed β -amyloid precursor proteins (APPs) of which there are several alternatively spliced variants. The most abundant forms of the APPs include proteins consisting of 696, 751 and 770 amino acids (Tanzi et al, Nature 31:528-530, 1998).

[0006] The small A β peptide is a major component that makes up the amyloid deposits or "plaques" in the brains of patients with Alzheimer's disease. In addition, Alzheimer's disease is characterized by the presence of numerous neurofibrillary "tangles", consisting of paired helical filaments which abnormally accumulate in the neuronal cytoplasm (Grundke-Iqbal et al, Proc. Natl. Acad. Sci. USA 83:4913-4917, 1986; Kosik et al, Proc. Natl. Acad. Sci. USA 83:4044-4048, 1986; Lee et al, Science 251:675-678, 1991). The pathological hallmark of Alzheimer's disease is therefore the presence of both "plaques" and "tangles", with amyloid being deposited in the central core of the plaques. The other major type of lesion found in the Alzheimer's disease brain is the accumulation of amyloid in the walls of blood vessels, both within the brain parenchyma and in the walls of meningeal vessels that lie outside the brain. The amyloid deposits localized to the walls of blood vessels are referred to as cerebrovascular amyloid or congophilic angiopathy (Mandybur, J. Neuropath. Exp. Neurol. 45:79-90, 1986; Pardridge et al., J. Neurochem. 49:1394-1401, 1987).

[0007] For many years there has been an ongoing scientific debate as to the importance of "amyloid" in Alzheimer's disease, and whether the "plaques" and "tangles" characteristic of this disease were a cause or merely a consequence of the disease. Within the last few years, studies now indicate that amyloid is indeed a causative factor for Alzheimer's disease and should not be regarded as merely an innocent bystander. The Alzheimer's A β protein in cell culture has been shown to cause degeneration of nerve cells within short periods of time (Pike et al, Br. Res. 563:311-314, 1991; J. Neurochem. 64:253-265, 1995). Studies show that it is the fibrillary structure (consisting of a predominant β -pleated sheet secondary structure) characteristic of all

amyloids that is responsible for the neurotoxic effects. A β has also been found to be neurotoxic in slice cultures of hippocampus (Harrigan et al, Neurobiol. Aging 16:779-789, 1995) and to induce nerve cell death in transgenic mice (Games et al, Nature 373:523-527, 1995; Hsiao et al, Science 272:99-102, 1996). Injection of the Alzheimer's A β into rat brain also causes memory impairment and neuronal dysfunction (Flood et al, Proc. Natl. Acad. Sci. USA 88:3363-3366, 1991; Br. Res. 663:271-276, 1994).

[0008] Probably, the most convincing evidence that A β amyloid is directly involved in the pathogenesis of Alzheimer's disease comes from genetic studies. It was discovered that the production of A β can result from mutations in the gene encoding its precursor, β -amyloid precursor protein (Van Broeckhoven et al, Science 248:1120-1122, 1990; Murrell et al, Science 254:97-99, 1991; Haass et al, Nature Med. 1: 1291-1296, 1995). The identification of mutations in the beta-amyloid precursor protein gene that cause early onset familial Alzheimer's disease is the strongest argument that amyloid is central to the pathogenic process underlying this diseases. Several reported disease-causing mutations have been discovered which demonstrate the importance of A β in causing familial Alzheimer's disease (reviewed in Hardy, Nature Genet. 1:223-234, 1992). All of these studies suggest that providing a therapy, drug or supplement to reduce, eliminate and/or prevent fibrillary A β formation, deposition, accumulation and/or persistence in the brains of humans and animals, well serve as an effective therapeutic.

THE ACCUMULATION OF "PLAQUES AND TANGLES" IN THE AGING HUMAN AND ANIMAL BRAIN

[0009] The human brain is the most complex organ in the universe. It weighs only 3 pounds, or about 2% of body weight. Yet it uses 20-30% of the calories consumed, 20% of the oxygen breathed, and 25% of the blood flow in the body; it consists of 85% water (Daniel G. Amen, M.D., *12 prescriptions for creating a brain healthy life*. Source: www.amenclinics.com/cybcyb/12-prescriptions-for-creating-a-brain-healthy-life/). There are approximately 100 billion nerve cells (i.e. neurons) in the brain, and up to a quadrillion connections called synapses (ibid). The human brain as it ages, loses about 85,000 cortical neurons per day, or about one every second (Deepak Chopra, M.D. and Rudolph Tanzi, Ph.D. *Super Brain. Unleashing the Explosive Power of Your Mind to Maximize Health, Happiness, and Spiritual Well Being*. See <http://www.chopra.com/super-brain-by-deepak-chopra-rudolph-tanzi>.) As the brain ages, starting in the 20's, there is a slow but deliberate accumulation of two

neurotoxic proteins. The first is the brain accumulation of an insoluble (aggregated) specific neurotoxic protein known as the “beta-amyloid protein” or A β . Beta-amyloid protein deposits in the form of “plaques” (looking like “meatballs” in the brain under a microscope), have been shown to be instrumental in killing healthy neurons that lead to a decline in hippocampus-dependent memory and cognition. Dr. Alan Snow and co-inventors developed patented methods to produce “plaques in a test-tube” (identical to what is seen in the human brain) and used these methods to screen for and identify natural “plaque-reducing” nutraceutical ingredients (US Patent No. 7,148,001, which is incorporated herein by reference in its entirety).

[0010] The second neurotoxic protein that accumulates in the aging brain is known as the “tau protein” and forms twisted paired helical filaments known as “tangles.” Neurofibrillary tangles accumulate inside neurons that causes them to die, and look like “dried spaghetti strands” under a microscope. Dr. Snow’s laboratories developed proprietary methods to form “tangles” in a test-tube, and then used these assays to identify “tangle-inhibiting” nutraceutical ingredients (see examples below). Thus, in the aging brain, both “plaques and tangles” accumulate, causing neurons to die; connections between nerve cells (called synapses) to disintegrate; and memory and cognition to progressively decline. Compounds or agents able to disaggregate and reduce the accumulation of “plaques and tangles” have been shown to lead to memory improvement and a reduction in memory decline (Karlinski et al. Suppression of amyloid deposition leads to long-term reductions in Alzheimer’s pathologies in Tg2576 mice. *J. Neurosci.* 29:4964-4971, 2009; Vellas et al. Long-term follow-up of patients immunized with AN1792: Reduced functional decline in antibody responders. *Current Alz. Res.* 6:144-151, 2009; Morgan et al. A β peptide vaccination prevents memory loss in an animal model of Alzheimer’s disease. *Nature* 408:982-985, 2000; Chen et al. A learning deficit related to age and β -amyloid plaques in a mouse model of Alzheimer’s disease. *Nature* 408:975-979, 2000; Janus et al. A β peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer’s disease. *Nature* 408:979-985, 2000; Schenk et al. Immunization with amyloid- β attenuates Alzheimer-disease like pathology in PDAPP mouse. *Nature* 400:173-177, 1999; Yanamandra et al. Anti-tau antibodies that block tau aggregate seeding in vitro markedly decreases pathology and improves cognition in vivo. *Neuron* 80:402-414, 2013; Dumont et al. Bezafibrate administration improves behavioral deficits and tau pathology in P3015 mice. *Human Molecular Genetics* 21:5091-5105, 2012; Oddo et al. Reduction of soluble A β and tau, but not soluble A β alone, ameliorates cognitive decline in transgenic mice with plaques

and tangles. J. Biol. Chem. 281:39413-39423, 2006; Santacruz et al. Tau suppression in a neurodegenerative mouse model improves memory function. Science 309:476-481, 2005.)

[0011] The only difference between an aging brain that could lead to age-associated memory impairment (AAMI), then to mild-cognitive impairment (MCI), and potentially to Alzheimer's disease, and a brain that does not, is the number of "plaques and tangles" in the brain. Alzheimer's diseased brains are loaded with tens to hundreds of thousands of "plaques and tangles," per square millimeter, causing a marked increase in the death of neurons, leading to a loss of synapses (connections between neurons), and concurrent memory loss and cognitive decline.

[0012] Therefore, beta-amyloid and tau are two key proteins in the aging brain that accumulate as insoluble "**plaques and tangles**" that have been shown to be directly linked to memory loss and cognitive decline. There is currently no pharmaceutical drug that has been approved for reducing and removing both beta-amyloid protein "plaques" and tau protein-containing "tangles" in the brain.

THE ACCUMULATION OF "PLAQUES" IN THE AGING DOG AND CAT BRAIN

[0013] Dogs and cats also accumulate "plaques" (and, to a lesser extent, "tangles") in their brains as they age that are believed to contribute to memory decline and cognitive impairment. The same beta-amyloid protein (i.e. "plaques") and tau protein ("tangles") that accumulate in the human brain also accumulate in aged dogs (Papoianou et al, Immunohistochemical investigation of the brain of aged dogs. I. Detection of neurofibrillary tangles and of 4-hydroxynonenal protein, an oxidative damage product, in senile plaques. Amyloid 8:11-21, 2001; Uchida et al, Amyloid angiopathy with cerebral hemorrhage and senile plaque in aged dogs. Nihon Juigaku Zasshi 52: 605-11, 1990) and cats (Gunn-Moore et al, Cognitive dysfunction and the neurobiology of ageing in cats. J Small Anim. Pract. 48: 546-53, 2007; Nakamura et al. Senile plaques in very aged cats. Acta Neuropath. 91: 437-9, 1996).

[0014] Canine Cognitive Dysfunction (CCD) (also known as Cognitive Dysfunction Syndrome or CDS) is a disease prevalent in dogs (and cats) that exhibit symptoms of dementia or Alzheimer's disease as seen in humans. CCD creates pathological changes in the brain that slow the mental functioning of dogs (and cats) resulting in loss of memory, motor function and learned behaviors from training early in life. In the dog's and cat's brain, the culprit is again is the beta-amyloid protein or A β that forms "plaques" in the brain. As the dog ages, more and

more “plaques” accumulate and nerve cells die. Although the initial symptoms of the disorders are mild, they gradually worsen over time, also known as “cognitive decline”. Amyloid “plaques” occur in aged dogs at about five to seven years of age, and in cats of about ten years of age (which is proportional to their average lifespan of 15-20 years). In fact, clinical signs of cognitive dysfunction syndrome are found in 50% of dogs over the age of 11, and by the age of 15, 68% of dogs display at least one sign. Dogs will often find themselves confused in familiar places of the home, spending long periods of time in one area of the home, not responding to calls or commands, and experiencing abnormal sleeping patterns.

[0015] Beta-amyloid protein containing “plaques” also have been identified in the brains of other higher mammals including monkeys, bears, camels, and horses. (Nakamura et al, Histopathological studies of senile plaques and cerebral amyloidosis in cynomolgus monkeys. *J Med Primatol.* 27: 244–52, 1998; Capucchio et al. studies. *J Comp Pathol* 142: 61–73, 2010; Nakamura et al, Senile plaques in an aged two-humped (Bactrian) camel (*Camelus bactrianus*), *Acta Neuropathol* 90: 415–8, 1995; Uchida et al, Senile plaques and other senile changes in the brain of an aged American black bear, *Vet. Pathol.* 32: 412–4, 1995).

TAUOPATHIES AND “TANGLES”

[0016] Tau was discovered in the mid-1970s as a microtubule associated protein (Weingarten, 1975). Besides being a stabilizer of microtubules in neurons and other cells, it has since been found to play important roles in cell differentiation, polarization and axonal transport. Normal tau is a soluble protein bound to microtubules, but in a series of neurodegenerative diseases, now known as tauopathies, tau accumulates as a pathogenic insoluble, fibrillar protein. These tau inclusions appear to modulate the severity of dementia and clinical features of these neurodegenerative diseases. Tauopathies include diseases such as Alzheimer’s disease, frontotemporal lobar degeneration with tau inclusions (FTLD-tau) such as Pick’s disease, progressive supranuclear palsy, and corticobasal degeneration, agyrophillic grain disease, some prion diseases, amyotrophic lateral sclerosis/parkinsonism-dementia complex, chronic traumatic encephalopathy, and some genetic forms of Parkinson’s disease (V.M. Lee et al., *Ann. Rev. Neurosci.* 24: 1121–1159, 2001; B. Omalu et al., *Neurosurgery* 69(1):173-83, 2011; A. Rajput et al., *Neurology* 67: 1506–1508, 2006; G. Santpere and I. Ferrer, *Acta Neuropathol.* 117: 227–246, 2009).

[0017] One of the most notable effects of increasing fibrillar tau in the brain is the gradual deterioration of short term memory; that is, the ability to recall immediately those memories only recently stored (P. Giannakopoulos et al., *Neurology* 60: 1495–1500, 2003). As there is no treatment for these disorders, it is important to find a novel invention that could target this pathogenic protein and improve memory deficits.

“TANGLES” ACCUMULATE IN BRAIN IN TRAUMATIC BRAIN INJURY (TBI), CONCUSSIONS, HEAD TRAUMA AND CHRONIC TRAUMATIC ENCEPHALOPATHY (CTE)

[0018] Brain “tangles” consisting of tau protein also accumulate progressively in the brain following blows to the head and include concussions, head injury, post-traumatic stress disorder (PTSD), and blast-induced traumatic brain injury (seen in soldiers and military personnel who have traumatic head injuries induced by a single blast). The movie “concussion” and the NFL Players Association all discuss the dementia-type behavior that has been seen in athletes following repeated concussions and/or blows to the head (known as traumatic brain injury or TBI). Loss of consciousness is a clinical hallmark of concussion but is not required to make the diagnosis. Other symptoms include confusion, disorientation, unsteadiness, dizziness, headache, and visual disturbances.

[0019] The long-term consequences to traumatic brain injury is referred to as Chronic Traumatic Encephalopathy (CTE), which is form of tauopathy (i.e. tau protein containing “tangles” in the brain). CTE is a progressive degenerative disease found in people who have suffered repeated brain trauma including sub-concussive hits to the head that do not cause immediate symptoms. The disease was previously called dementia pugilistica (DP), i.e. “punch-drunk” as it was initially found in those with a history of boxing. CTE has now been found in the brains of professional athletes including NFL athletes who play football, athletes prone to head injury including those that play ice hockey, rugby, skiing, skateboarding, stunt performing, bull riding, rodeo, and all other contact sports where participants experience repeated brain trauma. Individuals with CTE show many signs of dementia such as memory loss, aggression, confusion, and depression, which may appear years or many decades after the trauma. In September 2015, researchers with the Department of Veterans Affairs and Boston University announced they had identified CTE in 96% of NFL football players that they had examined and in 79% of all football players (Jason Breslow, New: *87 deceased NFL players test positive for brain disease*. Frontline January 9, 2016).

[0020] The neuropathology under a microscope is clear – there is primarily an accumulation of “tangles” that consist of tau protein, similar to the “tangles” seen in the brains of Alzheimer’s disease patients. There is also some beta-amyloid protein deposition (i.e. “plaques”), but this is usually uncommon and less of a feature than the “tangle” accumulation in brain. These findings suggest that blows to the head can lead to near immediate brain “tangle” accumulation that then leads to dementia-like symptoms including memory loss and cognitive decline. Identification of a nutraceutical that can help in the reduction and/or clearance of brain “tangles” would be an extraordinary supplement to take every day by all kinds of athletes, NFL players, the military and its soldiers.

DISCLOSURES

[0021] An object of the present invention is to provide the combination of plant extracts from *Uncaria tomentosa* and an oolong tea extract for the development of a cognition and memory supplement, to prevent, reduce and/or clear brain “plaques and tangles” in humans. This invention is predicated on the surprising discovery that various plant extracts, even from the same plant source have differing effectiveness for dissolving plaques and tangles, and that oolong tea extract, in particular LOTE, in combination with *Uncaria tomentosa* extract has a surprising and unexpected activity compared other plant extracts or with each extract alone.

[0022] Another object of the present invention is to provide the combination of plant extracts from *Uncaria tomentosa* and an oolong tea extract to produce a pet food supplement for aged dogs that reduce brain amyloid “plaques” and improve cognition, memory, short-term memory, focus and concentration.

[0023] Another object of the present invention is to provide the combination of plant extracts from *Uncaria tomentosa* and an oolong tea extract to produce a pet food supplement for aged cats that reduce brain amyloid “plaques” and improve cognition, memory, short-term memory, focus and concentration.

[0024] Although some health care providers have suggested that *Uncaria tomentosa* may be used to treat a variety of ailments, nowhere has there been any use, or suggestion of use, of this compound for the treatment of tau fibril formation, deposition, accumulation and/or persistence, such as that which occurs in the tauopathies. Furthermore, nowhere is it suggested that certain other compounds might have synergistic or supplemental efficacy in combination with *Uncaria tomentosa* in treating tauopathies to prevent and/or treat, for example, traumatic

brain injury (TBI), concussions (as observed in most athletes and the military/soldiers), post-traumatic stress disorders, head trauma, blows to the head (either single or repeated), and chronic traumatic encephalopathy (CTE).

[0025] Another object of the present invention is to provide the combination of plant extracts from *Uncaria tomentosa* and an oolong tea extract to produce an effective preventer and/or reducer of brain “tangles,” such as those found in people with traumatic brain injury (TBI), concussions (as observed in most athletes and the military/soldiers), post-traumatic stress disorders, head trauma, blows to the head (either single or repeated), and chronic traumatic encephalopathy (CTE).

[0026] The present invention clearly demonstrates the effectiveness of plant extracts of *Uncaria tomentosa* (cat’s claw) in specific combination with an Oolong tea plant extract for 1) inhibition of tau fibril formation (important for patients in early- to mid-stage tauopathy), 2) inhibition of tau fibril growth (important for patients in early- to mid-stage tauopathy), and 3) causing the dissolution/disruption of preformed tau fibrils (important for late-stage tauopathy).

[0027] An object of the present invention is to use the inner bark and/or roots from *Uncaria tomentosa* (also referred to as Una de Gato or Cat's claw) for the treatment/inhibition of tau deposition, accumulation and/or persistence in tauopathies, in conjunction with an oolong tea extract, disclosed below, to achieve a beneficial therapeutic effect. *Uncaria tomentosa* or Cat's claw is also referred to as, but not limited to, Paraguayo, Garabato, Garbato casha, Tambor huasca, Una de gabilan, Hawk's claw, Nail of Cat, and Nail of Cat Schuler.

[0028] Another object of the present invention is to provide the use of *Uncaria tomentosa* with an oolong tea extract (regardless of commercial source and regardless of final form for consumption by humans, i.e. pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixirs, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, bark bundles and/or bark powder) for inhibition of tau fibril formation, deposition, accumulation, and/or persistence, regardless of its clinical setting.

[0029] These and such other objects of the invention as will become evident from the disclosure below are met by the invention disclosed herein.

APPLICATIONS

[0030] Application of the invention provides for use of plant extracts from *Uncaria tomentosa* and a specific oolong tea extract to benefits aged dogs and cats, who develop brain “plaques” (and to a lesser extent, “tangles”), and have dementia as defined in Canine Cognitive Dysfunction (CCD).

[0031] We have earlier discovered and disclosed a naturally occurring plant product, the inner bark and/or roots from the plant *Uncaria tomentosa*, or Cat's Claw, that we call PTI-00703[®], in WIPO International publication number W098/51302 entitled ‘Composition and Methods for Treating Alzheimer's Disease and other Amyloidoses’ dated November 19, 1998, which is incorporated herein by reference in its entirety. As disclosed therein, this plant compound alone has surprising efficacy in disrupting and/or dissolving amyloid deposits and other accumulations, and is believed to be a potent inhibitor of amyloid formation in Alzheimer's disease, Type II Diabetes, and other amyloidoses.

[0032] However, our data now demonstrates that the formulation of PTI-00703[®] with a specific oolong tea extract (referred to as ‘LOTE’) disclosed herein has surprising and hitherto unsuspected potent efficacy in treating Tauopathies and diseases in which “tangles” are found in the brain (such as in TBI, CTE in humans with concussions, head injury and/or head trauma), and diseases that contain an overabundance of “plaques in brain” such as in aged dogs and cats (in Canine Cognitive Dysfunction).

[0033] The invention relates to the use of mixed compositions including one or more of the following: oolong tea extract and *Uncaria tomentosa* extract PTI-00703[®] referred to as ‘703’ or ‘PTI-703’ for the therapeutic intervention of amyloidosis. Use of a mixed composition of an oolong tea extract, and/or PTI-00703[®], contained within different commercial preparations show unexpected effects on the inhibition of amyloid fibril formation and disaggregation of amyloid fibrils.

[0034] Application of the invention to these needs is especially beneficial in that the invention is the only system that effectively provides for use of extracts from the inner bark and root parts of *Uncaria tomentosa*, together with an oolong tea extract, to benefit human patients with Tauopathies, amyloid diseases, “plaques and/or tangles” in the brain, and those with traumatic brain injury (TBI), concussions (as observed in most athletes and the military/soldiers), post-traumatic stress disorders, head trauma, blows to the head (either single or repeated), and chronic traumatic encephalopathy (CTE).

[0035] Application of the invention to these needs is especially beneficial in that the invention is the only system that effectively provides for use of extracts from the inner bark and root parts of *Uncaria tomentosa*, together with an Oolong tea extract, to benefit human patients with tauopathies, due to the newly discovered ability of *Uncaria tomentosa* in combination with an oolong tea extract, to effectively inhibit tau fibril formation, inhibit tau fibril growth, inhibit fibrillar tau-proteoglycan interactions, fibrillar tau-glycosaminoglycan interactions, and cause dissolution and/or disruption of preformed tau fibrils.

[0036] Over 25 extracts of teas, along with extracts of coffee and yerba mate, were screened for amyloid fibril aggregation inhibition and disaggregation *in vitro* using a variety of assays. One lead oolong tea extract was selected and tested in combination with PTI-00703[®]. It was found that the combination of this specific lead oolong tea extract (LOTE) and PTI-00703[®] inhibited amyloid fibril formation more than the tea extract or PTI-00703[®] alone, and the combination of LOTE and PTI-00703[®] also had the ability to rapidly disaggregate (within 15 minutes) preformed amyloid fibrils.

[0037] The invention also relates to the use of mixed compositions including one or more of the following: oolong tea extract and *Uncaria tomentosa* extract PTI-00703 referred to as '703' or 'PTI-703' for the therapeutic intervention of tauopathies. Use of a mixed composition of oolong tea extract, and/or PTI-00703, contained within different commercial preparations show unexpected effects on the inhibition of tau fibril formation and disaggregation of tau fibrils.

[0038] Over 25 extracts of teas along with extracts of coffee and yerba mate were screened for tau fibril aggregation inhibition and disaggregation *in vitro*. One lead oolong tea extract was selected and tested in combination with PTI-00703[®]. It was found that the combination of this specific lead oolong tea extract (LOTE) and PTI-00703[®] inhibited tau fibril formation more than the tea extract or PTI-00703[®] alone, and also has the ability to rapidly disaggregate preformed tau fibrils.

[0039] It was also found that the combination of this specific lead oolong tea extract (LOTE) and PTI-00703[®] inhibited beta-amyloid protein fibril formation more than the tea extract or PTI-00703[®] alone, and also has the ability to rapidly disaggregate preformed amyloid fibrils.

[0040] A preferred pharmacological agent preferably has a therapeutically effective amount of *Uncaria tomentosa* in a dosage in the range of from about 10 to about 1,000 mg/kg of body weight of the patient, and more preferably in the range of from about 10 to about 100 mg/kg of body weight of the patient.

[0041] The compositions preferably have a therapeutically effective amount of the mixed composition lead oolong tea extract (LOTE) and PTI-00703[®], in a dosage in the range of from about 0.1 to about 500 mg/kg of body weight of the patient, and more preferably in the range from about 1.0 to about 100 mg/kg of body weight of the patient.

[0042] Preferred pharmaceutical agents may also have a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical agent preferably has a fibril inhibitory activity or efficacy greater than 50%.

[0043] The plant matter is preferably comprised of commercially obtained pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixirs, suspensions, emulsions, solutions, syrups, tea bags, aerosols (as a solid or in a liquid medium), suppositories, sterile injectable solutions, sterile packaged powders, bark bundles and/or bark powder, which contain *Uncaria tomentosa*, extracts or derivatives thereof (and may be taken from commercially available gelatin-coated capsules which contain dried powder of *Uncaria tomentosa*, extracts or derivatives thereof), in combination with a specific oolong tea extract.

[0044] An object of the present invention is to use the inner bark and/or roots from *Uncaria tomentosa* (also referred to as *Una de Gato* or Cat's claw) for the treatment/inhibition of amyloid deposition, accumulation and/or persistence in amyloidosis, in conjunction with an oolong tea extract, disclosed below, to achieve a beneficial therapeutic effect. *Uncaria tomentosa* or cat's claw is also referred to as, but not limited to, Paraguaiyo, Garabato, Garbato casha, Tambor huasca, Una de gavilan, Hawk's claw, Nail of Cat, and Nail of Cat Schuler.

[0045] Another object of the present invention is to provide the use of *Uncaria tomentosa* with an oolong tea extract (regardless of commercial source and regardless of final form for consumption by humans, i.e. pills, tablets, caplets, soft and hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixirs, suspensions, emulsions, solutions, syrups, tea bags, beverage preparations, aerosols, as a solid or in a liquid medium, suppositories, sterile injectable solutions, sterile packaged powders, bark bundles and/or bark powder, for inhibition of amyloid fibril formation, deposition, accumulation and/or persistence, regardless of its clinical setting.

[0046] Application of the invention to these needs is especially beneficial in that the invention is the only system that effectively provides the use of extracts from the inner bark and root parts of *Uncaria tomentosa*, together with a specific Oolong tea extract, to benefit human patients with amyloidosis, and aged mammals (such as dogs and cats that develop amyloid

“plaques” in the brain as they age), due to the newly discovered ability of *Uncaria tomentosa* in combination with an Oolong tea extract, to most remarkably and effectively inhibit amyloid fibril formation, inhibit amyloid fibril growth, and cause dissolution and/or disruption of preformed amyloid fibrils.

[0047] A method is also disclosed for treating tauopathy in a patient, comprising the step of administering to the patient a therapeutically effective amount of plant matter from a plant of the genus *Uncaria*, species *tomentosa*, in combination with an Oolong tea extract. The plant matter is preferably administered orally such as in oral capsules, beverage preparations, or any other method, or by aerosol spray or in a parenterally injectable or infusible form.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing will be provided by the Office upon request and payment of the necessary fee.

[0049] **Figure 1** A graph of A β 1-42 aggregation measured by Thioflavin T fluorometry and Congo red binding after incubation with lead Oolong tea extract (LOTE) and PTI-00703[®]

[0050] **Figure 2A-C** Representative images of Congo red, Thioflavin S, and electron micrographs of A β fibrils +/- LOTE and PTI-00703[®]

[0051] **Figure 3** A graph of A β 1-40 aggregation measured by Thioflavin T fluorometry and Congo red binding after incubation with LOTE and PTI-00703[®]

[0052] **Figure 4** Circular dichroism spectra of A β 1-40 after treatment with increasing concentrations of LOTE and PTI-00703[®]

[0053] **Figure 5A-G** Data from various internal tests to characterize TauRD fibrils for screening of tau aggregation inhibitors.

[0054] **Figure 6** A graph of tau aggregation measured by Thioflavin S fluorometry after incubation with tea extracts.

[0055] **Figure 7** A graph of tau aggregation measured by Thioflavin S fluorometry after incubation with LOTE and PTI-00703.

[0056] **Figure 8** A graph of tau secondary structure measured by circular dichroism spectroscopy after treatment with LOTE.

[0057] **Figure 9** Electron micrographs of tau fibril formation with PTI-00703 and LOTE.

[0058] **Figure 10** Electron micrographs of preformed tau fibrils treated with PTI-00703 and LOTE.

DETAILED DESCRIPTION

[0059] A “therapeutically effective amount” in general means the amount that, when administered to a subject or animal for treating a disease, is sufficient to affect the desired degree of treatment for the disease. A “therapeutically effective amount” or a “therapeutically effective dosage” preferably inhibits, reduces, disrupts, disassembles tau fibril formation, deposition, accumulation and/or persistence, or treats a disease associated with these conditions, such as a tauopathy, by at least 20%, more preferably by at least 40%, even more preferably by at least 60%, and still more preferably by at least 80%, relative to an untreated subject. Effective amounts of a compound of this invention or composition thereof for treatment of a mammalian subject are about 0.1 to about 1000 mg/Kg of body weight of the subject/day, such as from about 1 to about 100 mg/Kg/day, especially from about 10 to about 100 mg/Kg/day. A broad range of disclosed composition dosages are believed to be both safe and effective.

[0060] “Amyloid diseases” or “amyloidosis” suitable for treatment with the compositions of this inventions are diseases associated with the formation, deposition, accumulation, and/or persistence of amyloid fibrils, especially the fibrils of an amyloid protein selected from the group consisting of beta-amyloid protein or A β , AA amyloid, AL amyloid, IAPP amyloid, PrP amyloid, α 2-microglobulin amyloid, transthyretin, prealbumin, and procalcitonin, especially A β and IAPP amyloid. Suitable such diseases include Alzheimer’s disease, Down’s syndrome, Mild cognitive impairment (MCI), Cognitive Canine Dysfunction (CDD), traumatic brain injury (TBI), chronic traumatic encephalopathy (CTE), concussions, hear trauma, single- and multiple blows to the head, post-traumatic stress disorders, dementia pugilistica, multiple system atrophy, inclusion body myositis, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, Niewman-Pick disease type C, cerebral β -amyloid angiopathy, dementia associated with cortical basal degeneration, the amyloidosis of type 2 diabetes, the amyloidosis of chronic inflammation, the amyloidosis of malignancy and Familial Mediterranean Fever, the amyloidosis of multiple myeloma and B-cell dyscrasias, the amyloidosis of prion diseases, Creutzfeldt-Jakob disease, Gertsmann-Straussler syndrome, kuru, scrapie, the amyloidosis associated with carpal tunnel

syndrome, senile cardiac amyloidosis, familial amyloidotic polyneuropathy, and the amyloidosis associated with endocrine tumors.

[0061] “Fibrillogenesis” refers to the formation, deposition, accumulation and/or persistence of tau fibrils, filaments, inclusions, deposits, inclusions, or the like.

[0062] “Inhibition of fibrillogenesis” refers to the inhibition of formation, deposition, accumulation and/or persistence of such amyloid “plaque” or tau “tangle” fibril-like deposits.

[0063] “Disruption of fibrils or fibrillogenesis” refers to the disruption of preformed beta-amyloid or tau fibrils that usually exist in a pre-dominant β -sheet secondary structure. Such disruption by compounds of the invention may involve marked reduction or disassembly of beta-amyloid or tau fibrils as assessed by various methods such as circular dichroism spectroscopy, Thioflavin S fluorometry, SDS-PAGE/Western blotting, or negative stain electron microscopy, as demonstrated by the Examples presented in this application.

[0064] “Mammal” includes both humans and non-human mammals, such as companion animals (dogs, cats and the like), laboratory animals (such as mice, rats, guinea pigs, and the like) and farm animals (cattle, horses, sheep, goats, swine, and the like).

[0065] “Plaques” refer to the meatball looking “amyloid deposits” consisting of beta-amyloid protein or A β found in various regions of brain (including hippocampus, cerebral cortex, frontal cortex etc.) that is a pathological hallmark of brain aging, mild-cognitive impairment (MCI), Alzheimer’s disease, and is found in aging mammals such as dogs, cats (referred to as Cognitive Canine Dysfunction or CDD), monkeys, polar bears, horses and the like. Accumulation of amyloid “plaques” in brain is believed to lead to neurodegeneration, loss of synapses and connections between neurons, cognitive decline, memory decline and loss, and loss of focus and concentration.

[0066] “Tangles” refer to the “dried-up” spaghetti looking “tangle-deposits” consisting of tau protein found in various regions of brain (including hippocampus, cerebral cortex, frontal cortex etc.) that is a pathological hallmark of brain aging, mild-cognitive impairment, Alzheimer’s disease, concussions, traumatic brain injury (TBI), single and repeated blows to the head, post-traumatic stress disorders, chronic traumatic encephalopathy (CTE) and the like.

[0067] “Tauopathies” suitable for treatment with the compounds of this invention are also diseases associated with the formation, deposition, accumulation, or persistence of tau fibrils. Suitable diseases include Alzheimer’s disease, frontotemporal lobar degeneration with tau

inclusions (FTLD-tau) such as Pick's disease, progressive supranuclear palsy, and corticobasal degeneration, agyrophilic grain disease, some prion diseases, amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (also called Lytico Bodig disease), dementia pugilistica, chronic traumatic encephalopathy, Parkinson's disease and particularly some genetic forms of Parkinson's disease, tangle-predominant dementia (with neurofibrillary tangles similar to Alzheimer's disease, but without amyloid plaques).

[0068] Tau fibrils is a generic term referring to a group of diverse, but specific intracellular or extracellular protein deposits which all have common morphological properties, staining characteristics, and x-ray diffraction spectra.

[0069] "Treating" or "treatment" of a disease includes preventing the disease from occurring in a mammal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment). Treatment can also mean inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease), such as by disruption of preformed tau fibrils. Treatment does not need be absolute. One such preventive treatment may be use of the disclosed compounds for the treatment of Mild Cognitive impairment (MCI).

[0070] The plant *Uncaria tomentosa*, also known as "Uña de Gato" (in Spanish) or "Cat's claw" (in English) refers to a woody vine which grows within the Peruvian Amazon rain forest. This slow-growing vine takes 20 years to reach maturity, and can grow over 100 feet in length as it attaches and wraps itself around the native trees. It is found abundantly in the foothills, at elevations of two to eight thousand feet. The vine is referred to as "cat's claw" because of its distinctive curved claw-like thorns that project from the base of its leaves. *Uncaria tomentosa* is expected to have immune-supporting, anti-inflammatory, anti-viral, anti-mutagenic and antioxidant properties. The anti-inflammatory properties, for example, are expected to be beneficial for the treatment of arthritis, rheumatism, bursitis and gout. Without being bound by theory, it is believed that is beneficial effects in treating arthritis pain can be due, in part, to its ability to cleanse the digestive tract and aid in removing toxins from the body. Furthermore, *Uncaria tomentosa* or cat's claw is expected to alleviate pain, and is expected to be helpful in reducing pain associated with, for example, chemotherapy, radiation treatment and AZT use.

[0071] *Uncaria tomentosa* or cat's claw is also expected to be useful in stopping viral infections in stopping viral infections in the early stages, fighting opportunistic infections in

AIDs patients and decreasing the viable size of some skin tumors and cysts. *Uncaria tomentosa* can also be used to treat a variety of ailments, including cancer, AIDs, Crohn's disease, respiratory infections, allergies, herpes, prostate problems, lupus, Epstein Barr virus, chronic fatigue syndrome, and a variety of stomach and bowel disorders.

[0072] For additional and further information and background on *Uncaria tomentosa*, the reader is also referred to the inventors' WIPO International publication number W098/51302, which is incorporated herein by reference in its entirety.

Further Aspects and Utilizations

[0073] Another embodiment of the present invention is to formulate, prior to administration in a patient, a pharmaceutical blend comprising *Uncaria tomentosa* and an oolong tea extract in one or more pharmaceutical acceptable carriers, diluents or excipients.

[0074] In another embodiment *Uncaria tomentosa* obtained commercially in any form could be further modulated using suitable carriers, excipients and diluents including lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed response of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 to about 10,000 mg of *Uncaria tomentosa* (or its active ingredients), more usually about 500 to about 2,000 mg of *Uncaria tomentosa* (or its active ingredients).

[0075] However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the clinical condition to be treated, the organ or tissues affected or suspected to be affected with tau fibril accumulation, and the chosen route of administration. Therefore, the above dosage ranges are not intended to limit the scope of the invention in any way.

[0076] The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity

of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

[0077] Use of extracts from the inner bark and root parts of *Uncaria tomentosa*, and its blends benefit human patients with tauopathies due to the newly discovered ability of *Uncaria tomentosa* in combination with an Oolong tea extract to inhibit tau fibril formation, inhibit tau fibril growth, inhibit tau fibril-proteoglycan interactions, inhibit tau fibril-glycosaminoglycan interactions, and cause dissolution and/or disruption of preformed tau fibrils.

Compositions and Administration

[0078] In general, isolated, and/or purified *Uncaria tomentosa* and oolong tea plant extracts are administered in therapeutically effective amounts by any of the usual modes known in the art, either singly or in combination with at least one of the extracts of the disclosure. Administration will be by one of the following routes: oral, topical, and synthetic (e.g. transdermal, intranasal or by suppository), or parenteral (e.g. intramuscular, subcutaneous, or intravenous injection). Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixers, aerosols, or any other appropriate compositions, and comprise at least one pharmaceutically acceptable excipient. Suitable excipients are well known to persons of ordinary skill in the art, and they, and the methods of formulating the compositions, can be found in standard references as Alfonso A R: Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa, 1985. Suitable liquid carriers, especially for injectable solutions, include water, aqueous saline solution, aqueous dextrose solution, and glycols.

[0079] In particular, the compound(s) can be administered orally, for example, as tablets, trouches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixers. In one embodiment, only one such compound is administered in any particular dosage form. Compositions intended for oral use can be prepared to any method known in the art for the manufacture of nutraceutical compositions and such compositions can contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide nutraceutically elegant and palatable preparations.

[0080] Tablets contain the plant extracts in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium

phosphate or sodium phosphate; granulating and disintegrating agents, for example, maize starch or alginic acid; binding agents, for example, maize starch, gelatin or acacia; and lubricating agents, for example magnesium stearate or stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glycerol monostearate or glycerol distearate can be employed. Formulations for oral use can also be prepared as hard gelatin capsules wherein the compounds are mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules, wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

[0081] Aqueous suspensions contain the compound in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include, for example, suspending agents, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl cellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; and dispersing and wetting agents that are naturally occurring phosphatides, for example lecithin, or condensation products of an alkylene oxide with fatty acids; for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids such as hexitol, for example polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters from fatty acids and a hexitol anhydride, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and/or one or more sweetening agents, such as sucrose or saccharin.

[0082] Oily suspensions can be formulated by suspending the extracts in a vegetable oil, for example arachis oil, olive oil, sesame oil, or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth below, and flavoring agents can be added to provide a palatable oral preparation. These compositions can be preserved by the addition of an antioxidant such as ascorbic acid.

[0083] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredients in admixture with a dispersing

or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already described above. Additional excipients, for example sweetening, flavoring and agents, can also be present.

[0084] The plant extracts can also be in the form of oil-in-water emulsions. The oily phase of a vegetable oil, for example, olive oil or arachis oils, or a mineral oil, for example liquid paraffin, or mixtures thereof. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyethylene sorbitan monooleate. The emulsion can also contain sweetening and flavoring agents. Syrups and elixirs can be formulated with sweetening agents, for example, glycerol, sorbitol or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents.

[0085] The following non-limiting Examples are given by way of illustration only and are not considered a limitation of this disclosure, many apparent variations of which are possible without departing from the spirit or scope thereof.

EXAMPLES

Composition Preparation

[0086] For these studies, commercially available tea leaves or extracts were obtained from various vendors. To make tea extracts from tea leaves, 2 g of tea leaves were extracted into 100 °C deionized water for 20 min with occasional mixing. Tea extracts were filtered through a >10 µm cut-off filter to remove large particulates. Extracts were snap frozen in dry ice/ethanol then lyophilized to obtain a dried, concentrated powder. Dried powder was weighed and resuspended in DMSO to make concentrated 100 mg/ml stock solutions. Stock solutions were diluted into aggregation reactions such that DMSO concentration was less than 0.28 % in the final reaction.

[0087] LOTE was prepared from oolong tea (AuNutra Industries Inc., Chino, CA). PTI-00703[®] is a powder extract made from water extraction of the inner bark of Peruvian *Uncaria tomentosa* (RFI Ingredients, Blauvelt, NY). Methods for preparing PTI-00703[®] have been described, for example in WIPO International Pub. No. W098/51302. Ethanol extracts of oolong tea and/or *Uncaria tomentosa* are within the scope of the current invention.

Example 1: Compositions of this Invention are Potent Disrupters/Inhibitors of Alzheimer's A β

Fibrils or Aggregates

[0088] The compositions were found to be potent disrupter/inhibitors of A β protein fibrils or aggregates. In a set of studies, the efficacy of the compositions to cause disassembly/disruption/disaggregation of pre-formed amyloid fibrils of Alzheimer's disease (i.e. consisting of A β 1-42 or A β 1-40) was analyzed.

Part A: Thioflavin T Fluorometry

[0089] In this study, Thioflavin T fluorometry was used to determine the effects of the compositions. Thioflavin T specifically binds to fibrillary amyloid, and this binding produces a fluorescence enhancement at 485 nm that is directly proportional to the amount of amyloid fibrils formed. The higher the fluorescence, the greater the amount of amyloid fibrils formed (Nakai et al., Lab. Invest. 65:104-110, 1991; Levine III, Protein Sci. 2:404-410, 1993; Amyloid: Int. J. Exp. Clin. Invest. 2:1-6, 1995).

[0090] In this study, 40 μ l of a 1 mg/ml solution (in distilled water) or pre-fibrillized human A β 1-42 (rPeptide) was incubated at 37 °C for 3 days either alone (control), or in the presence of LOTE + PTI-00703[®] (at test composition: A β weight ratios of 1:1 and 1:0.1) (referred to as "Cognitive Clarity[™]"). The final concentration of A β in the reaction was 0.4 mg/ml (88 μ M) in phosphate-buffered saline (PBS), pH 7.4 + 0.02% sodium azide in 100 μ l final volume. Following 3-days of co-incubation, 12.5 μ l of each incubation mixture was transferred into a 96-well microtiter plate containing 37.5 μ l of PBS and 200 μ l of a Thioflavin T solution (i.e. 125 μ M Thioflavin T in 62.5 mM phosphate buffer, pH 6.8). The emission fluorescence was read at 485 nm (444 nm excitation wavelength) using an ELISA plate fluorometer after subtraction with buffer alone or composition alone, as blank.

[0091] The results of the 3-day incubations are presented in Figure 1. Incubation of A β 1-42 with "Cognitive Clarity" (i.e. combination of LOTE + PTI-00703[®]) caused a dose-dependent disruption

[0092] disassembly/disaggregation of preformed A β 1-42 fibrils. At a test composition: A β weight ratio of 0.1:1 LOTE+PTI-00703 inhibited fibrils 62.8%. At equal weight equivalents (test composition: A β weight ratio of 1:1) there was a 93.6% inhibition

of Thioflavin T fluorescence. This study indicated that this unique combination is a potent disruptor/inhibitor of Alzheimer's disease type A β fibrils, and exerts its effect in a dose-dependent manner.

Part B: Congo red

[0093] In the Congo red binding assay the ability of a test composition to alter β -amyloid binding to Congo red is quantified. In this assay, A β 1-42 (as prepared for the Thioflavin T assay) was incubated for 3 days either alone (control), or with increasing amounts of test compositions and then vacuum filtered through a 0.2 μ m filter. The amount of A β 1-42 retained in the filter was then quantitated following staining of the filter with Congo red (125 μ M Congo red, 100 mM Tris, 50 mM NaCl, pH 7). After appropriate washing of the filter, any lowering of the Congo red color on the filter in the presence of the test composition (compared to the Congo red staining of the amyloid protein in the absence of the test composition) was indicative of the test composition's ability to diminish/alter the amount of aggregated and congophilic A β .

[0094] In one study, the ability of A β fibrils to bind Congo red in the absence or presence of increasing amounts of LOTE+PTI-00703[®] (at test composition:A β weight ratios of 1:1, and 0.1:1) was determined. The results of 3-day incubations are presented in Figure 1. LOTE+PTI-00703[®] caused a dose-dependent inhibition of A β binding to Congo red. At a test composition:A β weight ratio of 0.1:1, LOTE+PTI-00703[®] inhibited Congo red binding 20.9% (p<0.001). At equal weight equivalents (test composition:A β weight ratio of 1:1) there was a 58.6% (p<0.001) inhibition of Congo red binding.

[0095] Similar to the results for the Congo red binding assay, this study also indicated that this combination of a specific lead oolong tea extract (LOTE) and PTI-00703[®] are potent disruptors /inhibitors of A β fibrils as assessed by a Thioflavin T fluorometry assay, and exerted its effects in a dose-dependent manner (Figure 1). The combination of LOTE + PTI-00703[®] caused a dose dependent reduction of Thioflavin T binding (indicating disruption/reduction of A β fibrils) by 70% (p<0.001) at a test composition: A β weight ratio of 0.1:1 (Figure 1), and by 95% at a test composition:A β weight ratio of 1:1 (Figure 1).

Part C: Slide-based Congo red Binding, Thioflavin S and Electron Microscopy

[0096] In the slide-based Congo red assay, Congo red dye is incubated with A β 1-42, dotted on a slide and imaged under polarized light. Amyloid fibrils bound by Congo red emits a characteristic "apple-green birefringence" under polarized light. In this study, pre-fibrillarized 0.4mg/ml A β 1-42 (as prepared for the Thioflavin T assay) with or without test compositions was incubated for 3 days. 10 μ l Congo red solution (250 mg Congo red dye (54% pure; Sigma) dissolved in 1 L dH₂O) was added to 10 μ l of A β 1-42 +/- test compositions and mixed by vortexing for 30 seconds. Samples were incubated 10 minutes at room temperature with Congo red solution. Samples were then centrifuged at 2000g for 3 minutes and 10 μ l of supernatant was removed. 2 μ l glycerol was added to the pellet and mixed by pipetting up and down 15 times. Sample was vortexed then 10 μ l of stained protein was dotted onto 18-well 5MM HTC(R) autoclavable blue slides. Samples were covered with small circular coverslips and then immediately imaged under polarized light. Images were captured with a Zeiss Axioscope 2 Plus microscope with HBO 100 illuminator equipped with a Q-Imaging Retiga 1300 digital camera.

[0097] In Figure 2A (left panel), a representative image shows that untreated A β 1-42 has characteristic apple-green birefringence and abundant fibrillar protein uniformly distributed across the viewing field. In Figure 2A (right panel) treatment with LOTE+PTI-00703 (0.1:1 weight ratio with A β 42) resulted in substantially less Congo red stained fibrils, demonstrating that the plant extract combination can reduce and disaggregate/dissolve pre-formed A β 1-42 pre-formed fibrils..

[0098] Similar to Thioflavin T, Thioflavin S is a related anionic dye that binds to fibrillar amyloid proteins and can be used to detect fibrillar proteins bound to a glass microscope slide. In this study, pre-fibrillarized A β 1-42 (as prepared for the Thioflavin T assay) with or without test compositions was incubated for 3 days. 4 μ l of 0.4 mg/ml A β 1-42 +/- test compositions were dotted onto 18-well 5MM HTC(R) autoclavable blue slides. Samples were allowed to air dry for 2h. 10 μ l of Thioflavin S solution (31 mg of Thioflavin S dissolved in 50 mL dH₂O) was gently applied to dried protein on slides. Protein was stained for 1 minute, then Thioflavin solution was removed by pipette. 40 μ l of a 70% ethanol solution was gently pipetted onto stained protein for 1 minute to rinse. This solution was gently removed by pipette. 2 μ l of Vectashield (Vector) mounting media was applied to the stained protein and then covered with circular coverslips. Images were viewed under fluorescent light and images captured with a Zeiss Axioscope 2 Plus

microscope with HBO 100 illuminator equipped with a Q-Imaging Retiga 1300 digital camera.

[0099] In Figure 2B (left panel), a representative image of untreated A β 1-42 shows abundant Thioflavin S fluorescent fibrils uniformly distributed across the viewing field. Preformed A β 1-42 fibrils incubated with LOTE+PTI-00703[®] (at 0.1:1 weight ratio with A β 1-42) for 72 hr showed a substantial reduction in fluorescent fibrils (Figure 2B, right panel).

[0100] In Figure 2B (right panel), representative images show that with LOTE+PTI- 00703 treatment, the Thioflavin S-fluorescent fibrils disappeared.

[0101] Negative stain electron microscopy (EM) analysis was used to independently monitor the effectiveness of different compositions to disrupt pre-formed A β fibrils. In these experiments, pre-formed A β 1-42 fibrils (as prepared for the Thioflavin T assay) were incubated in the absence (control) or presence of increasing concentrations of test compositions. After the 3-day incubation, 10 μ l samples were spotted onto grids, stained with 2% uranyl acetate, and visualized at 8,000x to 30,000x magnifications with a JEOL 1010 transmission electron microscope.

[0102] In Figure 2C (bottom panel), EM analysis confirmed formation of A β fibrils in the absence of treatment (i.e. Fig 2C, Control). Without treatment (2C, left panel), A β formed large clumped fibrils that uniformly covered the field. These samples were also tested by the Thioflavin T assay and confirmed to be Thioflavin T-fluorescence positive fibrils. In the presence of LOTE+PTI-00703[®] (Figure 2C, right panel), the number of clumped A β fibrils was significantly reduced and dissolved. These results correlate well with the Thioflavin T fluorometry data that showed a reduction in Thioflavin T fluorescence with treatment and Congo red binding data that showed a reduction in Congo red binding with treatment. Using these independent methodologies, we have identified and validated the lead oolong tea extract (LOTE) in combination with PTI-00703[®] (i.e. LOTE + PTI-00703[®]) as potent A β disruptors/inhibitors.

Example 2: Compositions of this Invention Directly Inhibit/Disrupt the In Vitro Conversion of A β to β -sheet Containing Fibril Structures

Part A: Thioflavin T Fluorometry

[0103] To test whether LOTE+PTI-00703[®] can inhibit the β -sheet formation of A β , the same Thioflavin T assay as described in Example 1 was utilized, but with A β 1-40 as a substrate instead. Similar to A β 1-42, A β 1-40 forms Thioflavin T positive aggregates but requires >24h incubation at 37 °C shaking to become fully fibrillarized. Since A β 1-40 is in a non-fibrillar state at the start of the assay, this protein can be aggregated in the presence of compositions to measure aggregation inhibition. Lyophilized human A β 1-40 (rPeptide) was dissolved to 1 mg/mL (220 μ M) in dH₂O. In separate test tubes, test composition stocks were prepared in PBS at various concentrations such that final reactions containing equal volumes of the test composition stocks and the A β solution would result in a final A β concentration of 0.5 mg/mL (110 μ M) with test composition:A β weight ratios of 1:1, 0.5:1, 1:1, and 0.2:1. The reactions containing A β + test compositions (or A β + PBS as a control for A β aggregation) were then incubated for 2 days. The incubation mixtures were diluted 1:10 to 0.05 mg/ml. A β and 50 μ L of each diluted incubation mixture was transferred into a 96-well microtiter plate containing 200 μ L of Thioflavin T solution (i.e. 125 μ M Thioflavin T in 62.5 mM phosphate buffer, pH 6.8). The fluorescence was read at 485nm (444 nm excitation wavelength) using an ELISA plate fluorometer after subtraction with PBS buffer alone or composition alone, as blank.

[0104] The results of this study presented in Figure 3 indicated that LOTE+PTI-00703[®] of this invention interfered with A β aggregation as indicated by its ability to prevent the formation of fibrils as assessed by Thioflavin T fluorometry. At a test composition:A β weight ratio of 0.2:1 LOTE+PTI- 00703[®] inhibited fibrils by 14.1% and at 0.5:1 LOTE+PTI-00703[®] inhibited fibrils at 73% ($p < 0.001$). At equal weight equivalents (test composition:A β weight ratio of 1:1) there was an 89.3% inhibition ($p < 0.001$) of Thioflavin T fluorescence. This study indicated that LOTE+PTI-00703[®] is a potent inhibitor of β -sheet rich-A β fibril formation as assessed by Thioflavin T fluorometry, and this combination exerts its effect in a dose-dependent manner.

Part B: Congo red

[0105] To test whether LOTE+PTI-00703[®] can inhibit β -sheet formation of A β , the same Congo red assay as described in Example 1 was utilized, but with A β 1-40 as a substrate instead. In this assay, A β 1-40 (as prepared for the Thio T assay) and test compositions were incubated for 2 days and then vacuum filtered through a 0.2 μ m filter.

The amount of A β 1-40 retained in the filter was then quantitated following staining of the filter with Congo red (125 μ M Congo red, 100mM Tris, 50mM NaCl, pH 7). After appropriate washing of the filter, any lowering of the Congo red color on the filter in the presence of the test composition (compared to the Congo red staining of the amyloid protein in the absence of the test composition) was indicative of the test composition's ability to diminish/alter the amount of aggregated and congophilic A β .

[0106] In one study, the ability of A β fibrils to bind Congo red in the absence or presence of increasing amounts of LOTE+ PTI-00703[®] (at test composition: A β weight ratios of 1:1, 0.5:1, and 0.2:1) was determined. The results of 2-day incubations are presented in Figure 3. LOTE+PTI-00703[®] caused a dose-dependent inhibition of A β binding to Congo red. At a test composition: A β weight ratio of 0.2:1 LOTE+PTI-00703 inhibited Congo red binding 41.7% and 0.5:1 LOTE+PTI-00703[®] inhibited Congo Red binding 62.5% ($p < 0.001$). At equal weight equivalents (test composition: A β weight ratio of 1:1) there was an 83.3% inhibition ($p < 0.001$) of Congo red binding. Similar to the results for the Thioflavin T fluorometry assay, this study also indicated that LOTE+ PTI-00703[®] is a potent inhibitor of A β fibrils as assessed by A β fibril binding to Congo red, and exerts its effect in a dose-dependent manner.

Part C: CD Spectroscopy

[0107] CD spectroscopy was performed to determine LOTE+PTI-00703[®]'s potency to inhibit formation of A β 1-40 β -sheet secondary structure under aggregation-prone conditions. Since β -sheet structure is characteristic of A β fibrils, monitoring secondary structure of protein can provide additional proof of a compositions' effectiveness at inhibiting aggregation. CD spectra of A β 1-40 samples with increasing +/- concentrations of compositions were analyzed at 25°C on a JASCO Model J-810 Spectropolarimeter. CD spectroscopy and ThioT assays were analyzed in parallel from the same sample preparation in order to correlate the results from two independent assays.

[0108] In Figure 4, the CD spectra of A β 1-40 treated with LOTE+PTI-00703[®] indicated a dose-dependent inhibition of β -sheet containing fibrils. A minima at 218 nm indicates the presence of β -sheet structure. A positive shift of ellipticity at 218 nm indicates less β -sheet structure. At a test composition: A β weight ratio of 0.2:1 LOTE+PTI-00703[®] demonstrated 30.9% less β -sheet structure from untreated. 0.5:1 LOTE+PTI-00703[®] showed 53.9% less β -sheet structure than control. At equal weight equivalents (test

composition: A β weight ratio of 1:1) there was 64.8% less β -sheet structure compared to control.

[0109] These data verified LOTE+PTI-00703[®] has a significant ability to inhibit abnormal assembly of A β into fibrillar, β -sheet assemblies and maintains A β 1-40 in a less pathogenic form.

Use of Recombinant Tau Repeat Domain for *In Vitro* Screening of Tau Aggregation Inhibitors

[0110] During *in vitro* screening for identification of tau aggregation inhibitors, we found that under the same experimental conditions, formation of paired helical filaments (PHFs) from commercially-purchased full-length tau protein (Tau441; from rPeptide) was much slower (>11 days; data not shown) than that from the tau repeat domain (TauRD; containing Q244-E372 of Tau441) ($\geq$ 24 hr) (S. Barghorn et al, Methods Mol Biol, 299: 35-51, 2005).

[0111] Because of the remarkably short turn-around time and common aggregation properties, we used TauRD for *in vitro* screening to identify tau aggregation inhibitors in our current invention. Since the TauRD protein is not commercially available, we produced our own protein for this project. A cDNA fragment coding for the human TauRD was cloned into a bacterial expression vector and the construct was then expressed in *E. coli*. Bacterial clones demonstrating high levels of expression of TauRD were then selected for protein purification. The recombinant TauRD protein was then purified by heat-stability treatment and cation exchange chromatography as described with minor modifications (S. Barghorn et al, Methods Mol Biol, 299: 35-51, 2005). Using this method, we achieved a protein yield of 20 mg per liter of bacterial culture). Aggregation and PHF formation of purified TauRD were evaluated and validated by independent assays including Thioflavin S (ThioS) fluorometry, a dye that fluoresces after binding to fibrils (Figure 5D), circular dichroism (CD) spectroscopy, a method that detects changes in secondary structure of proteins (Figures 5B and 5C) and electron microscopy (Figures 5E-G). The results consistently demonstrate that TauRD (10 μ M) is able to form ThioS-positive, β -sheet-containing PHFs when incubated with equimolar heparin (Sigma-Aldrich, St. Louis, MO) at 37°C, with shaking at 800-1000 rpm for $\geq$ 1 day.

[0112] In Figure 5A, TauRD (15kDa) protein purified from *E. coli* was evaluated by SDS-PAGE/silver stain with typical purity of >95%. In Figure 5B-C are examples of CD spectroscopy of non-aggregated and aggregated TauRD proteins. TauRD aggregates were

prepared in the presence of equimolar (10 μ M) ratios of TauRD and heparin in 20mM sodium phosphate buffer (pH7.4), incubated at 37°C with shaking for 0-120h. In the absence of heparin, CD spectra of non-aggregated TauRD was random coil with ellipticity minima at 195nm (Figure 5B). In the presence of heparin, CD spectra showed time-dependent conformational changes of TauRD protein from random coil (minima at 195nm) at time 0 to β -sheet (minima at 218nm) at 20-120h (Figure 5C). Figure 5D is an example of TauRD aggregation monitored by ThioS fluorometry over time. The results in Figure 5D, show formation of ThioS-positive TauRD fibrils after 20h incubation. In the absence of heparin without fibril formation, tauRD ThioS signals were <200 arbitrary units (A.U.) of fluorescence at all time points. Tau fibril formation was confirmed by negative stain EM in Figure 5E-G. Representative images show formation of Tau fibrils after 48h incubation with heparin. TauRD monomers at time 0 are shown in Figure 5E (Bar = 200nm). Formation of TauRD fibrils at 48 hr are shown in Figures 5F-G (Bar = 50nm). Both straight and paired helical filaments were found.

Example 3: Identification of Novel Tau Aggregation Inhibitors by Thioflavin S Fluorometry

Screening

[0113] A well-known method for measuring fibril formation is Thioflavin T (ThioT) fluorometry (H. Naiki et al, Lab. Invest. 65:104-110, 1991; H. Levine III, Protein Sci. 2:404-410, 1993; H. Levine III, Amyloid 2:1-6, 1995; H. Naiki and K. Nakakuki, Lab. Invest. 74:374-383, 1996). ThioT is known to bind to fibrillar proteins, and an increase in fluorescence correlates with an increase in fibril formation, whereas a decrease in fluorescence correlates with a decrease in fibrils due to disassembly and/or disruption. We modified the assay by replacing ThioT with Thioflavin S (ThioS), a related anionic dye with similar properties, because the latter has been shown to be more sensitive, and reproducible for quantifying Tau PHFs (data not shown; P. Friedhoff et al, Biochemistry, 37(28): 10223-30, 1998). ThioS fluorometry was employed to assess whether the above described mixed compositions were capable of causing a disassembly/disruption of fibrils.

[0114] Aggregated tau fibrils were prepared in the presence of equimolar ratios of TauRD and heparin (10 μ M each) in 20mM sodium phosphate buffer, pH7.4. The reaction mixture was incubated at 37°C with shaking (800-1000 rpm) for 24 h to 72 h. Test compositions were tested at varying weight to weight concentrations with 0.14 mg/ml TauRD

with heparin. The same reaction mixtures (+increasing concentrations of test compositions) but without TauRD were also set up in parallel to serve as background controls. For all test compounds background ThioS fluorescence readings were very low, usually <5% of those of the TauRD-containing wells.

[0115] Following 24-72h of co-incubation, 50 μ l of each incubation mixture was transferred into a black 96-well microtiter plate (Santa Cruz Biotechnology, Inc., Dallas, TX) with 50 μ l of phosphate buffered saline (PBS; Sigma-Aldrich, St. Louis, MO) and 25 μ l of Thioflavin S solution (500mM Thioflavin S; Sigma-Aldrich, St. Louis, MO in PBS, pH 7.4). Using an ELISA plate fluorometer, fluorescence was read at 485 nm (444 nm excitation wavelength) after subtraction with buffer alone or composition alone, as blank.

[0116] Inhibition of tau aggregation by 50%, IC_{50} , was calculated using Prism version 5 software (GraphPad Software) by nonlinear regression [(Log [inhibitor] vs. normalized response; variable slope)]. In initial screenings, the test compounds demonstrated a broad range of activities for inhibiting tau protein fibril formation: IC_{50} values ranged from <.14mg/ml (less or equal to the concentration of tauRD in the assay) to infinity (i.e. no activity at all). The results suggested that the inhibitory activities were test subject specific. A few samples were also tested for their ability to disrupt preformed tau fibrils (disruption assay).

[0117] Over 25 tea extracts were tested for inhibition of ThioS-positive tau aggregates. A majority of teas were oolong tea extracts. Figure 6, is an example of ThioS fluorescence of tau aggregated in the presence of a few oolong tea extracts, chamomile tea and green tea. Increasing concentrations of extracts were incubated with 0.14mg/ml (10 μ M) tauRD and equimolar heparin for 24-72h, shaking at 1000rpm, 37°C. The lead oolong tea extract (LOTE) inhibited tau aggregates more than other oolong teas, chamomile tea or green tea extract. LOTE inhibited tau aggregation 50% (IC_{50}) with a ratio of 0.23:1, weight:weight, of composition to tau. This experiment demonstrates LOTE has a superior ability over other compositions to inhibit abnormal assembly of tau into fibrillar, pathogenic assemblies.

[0118] After identifying a tea extract that has excellent tau aggregation inhibition, lead oolong tea extract (LOTE) was incubated alone or in combination with PTI-00703[®] in the tau aggregation inhibition assay. A combination of specific oolong tea and PTI-00703[®] inhibited ThioS-positive tau aggregates more than LOTE or PTI-00703[®] alone (*significant by paired t-test, $p < 0.05$) (Figure 7). This combination of lead oolong tea extract and PTI-00703[®] is a

potent inhibitor of tau aggregation/fibrillogenesis that could be used as a novel therapeutic for tauopathies.

Example 4. Tau aggregation inhibition by lead oolong tea extract confirmed by analysis of protein secondary structure by circular dichroism (CD) spectroscopy

[0119] CD is a powerful method that provides dye-independent confirmation that the structure of a protein has changed. CD measures the differential absorption between left and right handed circularly polarized light. Proteins contain elements of asymmetry that exhibit distinct CD signals measured in units of ellipticity. Thioflavin S fluorescent, tau aggregates have an ellipticity minima of 218nm, which is characteristic of β -sheet containing proteins. Non-aggregated tau has an ellipticity with a characteristic minima at 195nm indicating random coil structure. CD spectroscopy was performed to determine each composition's potency to inhibit formation of β -sheet secondary structure in TauRD under aggregation-prone conditions. CD spectra were taken from samples containing +/- TauRD with increasing concentrations of compounds, and analyzed at 25°C on a JASCO Model J-810 Spectropolarimeter. CD spectroscopy and ThioS assays were analyzed in parallel from the same sample preparation in order to correlate the results from two independent assays.

[0120] In Figure 8, tau was treated with lead oolong tea extract (LOTE) or chamomile tea extract for 48h. Tau treated with LOTE is dose-dependently inhibited from converting to β -sheet containing fibrils. At the highest concentration of LOTE treatment, tau remains in soluble, random coil form with a minima ~195nm. Conversely, at the highest concentration of treatment with chamomile tea extract, tau still changes to an aggregated, β -sheet structure similar to the untreated control. These data confirm LOTE has a significant ability over other compositions to inhibit abnormal assembly of tau into fibrillar, β -sheet assemblies and maintains tau in a non-pathogenic soluble, random coil form.

Example 5: Inhibition of Tau Protein Fibrillogenesis and Disaggregation of Preformed Tau Fibrils by PTI-00703 + LOTE Determined by Negative Stain Electron Microscopy (EM)

[0121] EM analysis was used to independently monitor the effectiveness of compositions to inhibit tau fibrillogenesis. In these experiments, tau fibrils were assembled by incubating equimolar ratios of TauRD protein and heparin (10 μ M each) in the absence (control) or presence of increasing concentrations of test compositions. After the 2-day incubation, samples were spotted onto grids, stained with 2% uranyl acetate, and visualized at 8,000X to 30,000X magnifications with a JEOL 1010 transmission electron microscope.

[0122] In Figure 9, left panel, EM analysis confirmed formation of tau fibrils in the absence of treatment. Without treatment, tau formed a mixture of paired straight and helical filaments similar to those found in human tauopathies (V.M. Lee et al., Ann. Rev. Neurosci. 24:1121–159, 2001). These samples were also tested by the ThioS assay and CD and confirmed to be ThioS-fluorescence positive and β -sheet in structure. In the presence of PTI-00703[®] and PTI-00703[®] + LOTE (Figure 9, middle and right panel), tau fibrils become shortened and sparse, indicating an inhibition of tau fibril formation. Even less fibrils are apparent in tau samples treated with both PTI-00703[®] and LOTE. These results correlate well with ThioS fluorometry and CD analysis, which showed a reduction in ThioS fluorescence and decrease in β -sheet structure. Using three independent methodologies, we have identified and validated PTI-00703+lead oolong tea extract as a potent inhibitor of tau aggregation/fibrillogenesis.

[0123] Utilizing EM analysis, preformed tau fibrils were shown to rapidly disaggregate in the presence of both PTI-00703[®] and LOTE. TauRD was incubated with equimolar heparin to form fibrils as described in the previous assay. TauRD was diluted with or without test compositions and incubated, shaking at 37°C for various time points. At each time point tauRD+/-test compounds was assayed for ThioS fluorescence and snap frozen for EM analysis. In Figure 10 (left panel) without treatment, pre-fibrillarized tau remained in long filaments. In the next panels, pre-fibrillarized tau was found to rapidly disaggregate in the presence of PTI-00703[®] + LOTE. As early as 15 minutes incubation with PTI-00703[®] + LOTE, tau fibrils are shorter and sparser than without treatment. Disruption of tau fibrils was also confirmed by ThioS assay (data not shown). These data indicate the composition of PTI-00703[®]+LOTE not only can inhibit tau aggregation but can disaggregate preformed tau fibrils.

Example 6: Further *In Vivo* Testing of the PTI-00703[®] + LOTE for Improvement of Cognition and Memory

[0124] Further *in vivo* studies are used to test the PTI-00703[®] + LOTE plant extract combination for their effectiveness in the reduction of brain “plaque and tangle” load and improvement of cognition and memory. 40-60 men and women are selected for a clinical study. Subjects have age-associated memory impairment (AAMI), and expecting to have worsening symptoms of memory loss within the 6-month study period, but are in good general health otherwise.

[0125] This study includes a placebo group, i.e. the subjects are divided into two groups, one of which receives the PTI-00703[®] + oolong tea extract combination capsules (two 390 mg capsules containing the PTI-00703[®] + oolong tea extract in a 1:1 weight/weight combination with a meal; preferably lunch) of this disclosure, and the other receives a placebo (two capsules containing capsules without the study product active ingredients). The patients are benchmarked as to memory, cognition, focus, concentration, reasoning and other symptoms associated with mild-cognitive impairment (MCI). Subjects in the test groups receive a therapeutic dose of the combination study product extract or placebo for 6 months, with analysis for short-term memory, cognition, focus and concentration to be examined at 0, 1, 3, and 6-months of treatment. Accurate records with regards to memory, focus and concentration are kept as to the benchmarked symptoms in both groups and at the end of the study these results are compared. The results also are compared between members of each group. In addition, the results for each patient are compared to the symptoms reported by each patient before the study began. Activity of the combination PTI-00703[®] + oolong tea extract study product is illustrated by the attenuation of the typical cognitive decline, decline in short-term memory, cognition, focus and concentration, and/or associated behavioral disruptions associated with age-associated memory impairment (AAMI)

[0126] All references and patent publications provided herein are hereby incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. Use of a therapeutically effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for the treatment of amyloidosis in a mammal in need thereof wherein the medicament is to be administered to the mammal.
2. The use of claim 1 wherein the amyloidosis is selected from Alzheimer's disease, Down's syndrome, dementia pugilistica, cognitive dysfunction syndrome, canine cognitive dysfunction, multiple system atrophy, inclusion body myositis, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, Nieman-Pick disease type C, cerebral β -amyloid angiopathy, dementia associated with cortical basal degeneration, the amyloidosis of type 2 diabetes, the amyloidosis of chronic inflammation, the amyloidosis of malignancy and Familial Mediterranean Fever, the amyloidosis of multiple myeloma and B-cell dyscrasias, the amyloidosis of prion diseases, Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru, scrapie, the amyloidosis associated with carpal tunnel syndrome, senile cardiac amyloidosis, familial amyloidotic polyneuropathy, and the amyloidosis associated with endocrine tumors.
3. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for treating the formation, deposition, accumulation, or persistence of amyloid fibrils in a mammal.
4. The use of claim 3 wherein the extracts are formulated for administration by one of the routes selected from pills, tablets, caplets, soft or hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixirs, suspensions, emulsions, solutions, cold and hot beverage preparations, syrups, tea bags, aerosols, suppositories, sterile injectable solutions, or sterile packaged powders.
5. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for treating the formation, deposition, accumulation, or persistence of beta-amyloid containing plaques in a mammal.
6. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for the treatment of a tauopathy in a mammal.

7. The use of claim 6 wherein the tauopathy is selected from Alzheimer's disease, frontotemporal lobar degeneration with tau inclusions (FTLD-tau), Pick's disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, prion disease, amyotrophic lateral sclerosis-parkinsonism-dementia complex of Guam (also called Lytico-Bodig disease), Parkinson's disease, tangle-predominant dementia, ganglioma, ganglioglioma, gangliocytoma, meningiomas, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, traumatic brain injury (TBI), chronic traumatic encephalopathy (CTE), dementia pugilistica, concussion, single or repeated blows to the head, and post-traumatic stress disorder.
8. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for treating the formation, deposition, accumulation, or persistence of tau protein containing tangles in a mammal.
9. The use of claim 8 wherein the extracts are formulated for administration by one of the routes selected from pills, tablets, caplets, soft or hard gelatin capsules, lozenges, sachets, cachets, vegicaps, liquid drops, elixirs, suspensions, emulsions, solutions, beverage preparations, syrups, tea bags, aerosols, suppositories, sterile injectable solutions, or sterile packaged powders.
10. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for improving cognitive performance and/or slowing cognitive decline in a patient suffering from an amyloid disease wherein the medicament is to be administered to reduce formation, deposition, accumulation or persistence of beta-amyloid protein plaques or fibrils in the patient.
11. The use of claim 10 wherein the amount of *Uncaria tomentosa* extract and an oolong tea extract is between about 100-500 mg of *Uncaria tomentosa* extract and about 100-500 mg oolong tea extract.
12. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for preventing, reducing or treating tangles in the brain of a patient who has experienced single or multiple concussions, traumatic brain injury (TBI), chronic traumatic encephalopathy (CTE), post-traumatic stress disorder, brain aging, mild-cognitive impairment or Alzheimer's disease.

13. The use of claim 12 wherein the amount of the combination of *Uncaria tomentosa* extract and the oolong tea extract is between about 100-500 mg of *Uncaria tomentosa* extract and about 100-500 mg oolong tea extract.
14. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for preventing, reducing or treating plaques and tangles in the brain of an aged dog or cat.
15. The use of claim 14 wherein the amount of the *Uncaria tomentosa* extract and the oolong tea extract is between about 100-500 mg of *Uncaria tomentosa* extract and about 100-500 mg oolong tea extract.
16. Use of an effective amount of a composition comprising an *Uncaria tomentosa* extract and an oolong tea extract in the manufacture of a medicament for improving cognitive performance and/or slowing cognitive decline in an aged dog or cat suffering from brain formation, deposition, accumulation and/or persistence of plaques and tangles.
17. The use of claim 16 wherein the amount of *Uncaria tomentosa* extract and oolong tea extract is between about 100-500 mg of *Uncaria tomentosa* extract and about 100-500 mg oolong tea extract.