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Research article

Benefits of Acetylcholinesterase inhibitor glutamine in Treatment of Alzheimer's disease and instrumental methods for its analysis in medicinal plants

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ABSTRACT

Alzheimer disease is a chronic progressive neurodegenerative disease, leading to memery loss as a result of the formation of amyloid plaques and neurofibrillary tangles.

Galantamine possesses dual mechaunism of action as a reversible blocker of enzyme acetylcholinesterase and an allosterically potentiator of α7 nicotinic acetylcholine receptors, leading to neuroprotection, increased dopaminergic neurotransmission, improved behavioral functions and enhanced concentration.

In Alzheimer's disease with vascular dementia or with cerebrovascular disorders as a result of its antioxidant effect, Galantamine decreases neurodegeneration and cognitive decline by improving the processes of learning, memory and attention.

By inhibition of β-amyloid aggregation and by increasing of Aβ clearance throught an activation of microglia, the drug protects against β-amyloid toxicity and exerts in anti-apoptotic action in cells.

For identification of Galantamine in plant extracts are described thin layer chromatographic methods in stationary phases: Silicagel G60F254 or aluminium and displaying the spots through different approaches: quenching the fluorescence at λ = 254 nm, spraying with Dragendorf reagent or iodine vapour.

For determination of Galantamine in plant extracts are reported the followingo mmethods: 1) thin layer chromatography-densitometry; 2) extraction-thin layer chromatography- spectrophotometry; 3) High Performance Thin Layer Chromatography; 4) High Performance Liquid Chromatography (HPLC) with UV-detection; 5) HPLC-radioimmunoassay; 6) gas chromatogram-phy with mass-spectrometric detection (GC-MS), capillary electrophoresis and polarography.

Keywords: Bioassay, Herbal Drugs, Medicinal Plants.

INTRODUCTION

Alzheimer disease

Alzheimer's disease is a chronic progressive neurodegenerative disorder, wherein the loss of cholinergic neurons in the basal ganglia of the cerebrum cause higher cortical disfunctions and leads to a deficiency in memory, learning, behavioral and functional disorders and total collapse of the intelligence and mental activity. Synaptic dysfunction is mediated from increased accumulation of the peptide ß-amyloid in senile plaques and the formation of neurofibrillary tangles, composed of aggregates of hyperphosphorylated microtubule associated tau-protein.

Risk factors for Alzheimer's disease include genetic predisposition, history of Alzhei-mer's and Down syndrome in a relatives, oxidative stress, depression, epilepsy, Parkinson's disease, migraine, hypothyroidism, brain injury, brain inflammation, high levels of diastolic pressure and cholesterol, atherosclerosis, type 2 diabetes, obesity, tumor

processes, female, pregnancy in old age, increased content of iron in the body, reduced levels of glutathione presence of aluminum in drinking water, alcohol abuse and smoking.

According to the amyloid hypothesis the process of amyloidogenesis – synthesis of neurotoxic Aβ aggregates of extracellular proteins is determined by: 1) E693 δ mutation of the gene for apolipoprotein E on chromosome 19; 2) mutations in the gene for amyloid- precursor protein in chromosome 213) 30 mutations in presenilin 1 gene in chromosome 14 and the presenilin gene in chromosome 2.

It was found that in the body there is a dynamic equilibrium between formation of free radicals and function of the protective antioxidant systems (superoxidedismutase, catalase, glu-tathioneperoxidase, glutathione reductase, glucose-6-phosphate dehydrogenase).

Oxidative stress is the basis of the pathogenesis of aging and a number of diseases neurodegenerative as Alzheimer Parkinson Huntington amyotrophic lateral sclerosis, Lou Gehrig syndrome down autism Schizophrenia atherosclerosis vascular diseases, hypertension, chronic renal failure, diabetes mellitus type 1 and 2, cancer diseases, macular Degeneration and psoriatic arthritis.

The brain is especially sensitive to the effects of free radicals due to high consumption of oxygen, the presence of unsaturated fatty acids and reducing the activity of sensitive oxidation endogenous antioxidant systems [1].

In Alzheimer's disease oxidative stress arises as a result of violation of the balance between endogenous or exogenous overproduction of reactive free radicals and the decrease in antioxidant defense mechanisms. Oxidative disorders are among the early neurologic abnormalities in Alzheimer's disease and selectively occur in areas of the brain responsible for the regulation of memory functions.

Oxidative stress plays an important role in the pathogenesis of neuronal degeneraton because the formation of free radicals determines the inflammatory processes dysfunction of cell membranes and activation of programmed death of nerve cells (apoptosis) by oxidation of proteins, lipids and nucleic acids (DNA, RNA) and disruption of glucose metabolism.

In the neuropathology of Alzheimer's disease important markers of oxidative stress in the brain are:

Extracellular senile plaques and intracellular β-amyloid peptides Aβ1-42. Intracellular neurofibrillary tangles composed of aggregated paired filaments connected hyper phosphorylated microtubule associated tau-protein and surrounded by activated microglia and astrocyte cells and cells of Hirano

Products of glycosylation

Oxidized proteins and nucleic acid nuclear DNA mitochondrial DNA and ribosomal RNA products of lipid peroxidation acrolein 4-hydroxy-2-nonenal Hydroxy oktadekadienic acid and peroxynitrite elevated levels of gamma-glutamyltransferase and the factors of inflammation microglial interleukin 1, α-1- antichymotrypsin synthesis in astrocytes reactive C-protein decreased concentration of enzymes hydrolysed β-amyloid (neprilysin) and sortrilin 1 (intracellular amyloid part suppresses the expression of neprilysin).

The basis of the pathogenesis of Alzheimer's disease are two main mechanisms progressive accumulation of intraneuronal Aβ peptides and extraneuronal Aβ oligomers into amyloid plaques and the formation of intracellular neurofibrillary tangles of hyperphosphorylated tau (τ)-protein [2].

Methodology

The LC analyses were performed to assess the presence of the diosgenin in samples of DV. The antinociceptive study of DV was performed using models of acetic acid-induced writhing and formalin-induced pain in mice. The anti- inflammatory study was accomplished by leukocyte migration to the peritoneal cavity. A dry extract of DV was tested at doses of 100, 200 and 400 mg/kg (per os or p.o.). The toxicological properties of the dry extract were evaluated by toxicity assays of acute (5 g/kg, single dose) and subchronic (1 g/kg/day, 30 days) treatment. Haematological, biochemical, and histopathological parameters were studied. The results are expressed as mean ± S.D., and statistical analysis of the data were performed with the Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's test. In all cases differences were considered significant if $p < 0.05$ [3].

RESULTS

HPLC-DAD analysis of the extract from DV revealed the presence of diosgenin as the major compound. Doses of 200 and 400 mg⁄kg significantly reduced the amount of acetic acid- induced writhing in relation to the vehicle ($p < 0.0001$). In the first phase, using the formalin-induced neurogenic pain test, only the 400 mg/kg dose of DV showed significant inhibition of neurogenic pain ($p < 0.001$). In the second phase, 200 and 400 mg/kg of DV showed significant inhibition of inflammatory pain ($p < 0.0001$). Significant inhibition of leukocyte migration was observed with doses of 100 ($p < 0.001$), 200 ($p < 0.01$) and 400 mg/kg ($p < 0.01$). Haematological, biochemical and histopathological data obtained in both acute and subchronic toxicological assays revealed only unremarkable changes, which are unlikely to indicate DV toxicity with oral administration.We found that DV possesses antinociceptive and anti-inflammatory properties in rodent models. In addition, no acute or subchronic toxicity was evident when the herbal extract was administered orally. These results supporting the folkloric usage of the plant to treat various inflammatory diseases [4, 5].

Bioassay Guided Evaluation of Medicinal Plant (Pseudelephantopus Spicatus)

Material and Methods

Collection of Plant Material

For the purpose of botanicalidentification, small branches or twigs with reproductivestructures, healthy leaves, stipules, bark and wood

samples from each plant were collected in duplicate following accuratedocumentation. Mature *P. spicatus* leaves were collected from

Preparation of Plant Extract

For the preparation of the crude extract, about 2-3kg of plant or plant parts were cleaned bywashing with tap water followed by distilled water. The sampl was air-dried for about 2-3 weeks, and the dried samples werepulverized using a sterile electricblender. The half portion ofthe pulverized leaves was soaked in pure absolute ethanol, and the other was soaked in 50:50 ethanol-water mixtures for threedays. Each solution was filtered with Whatman No. 1 filterpaper and concentrated at 40°-50°C under reduced pressure using the rotary evaporator21.For the preparation of the plant decoction, about 1 kg fresh and clean samples of the plant were cut into pieces and boiled insufficient amount of distilled water (1:2 ratio) for 5 minutes.The mixture was filtered, cooled and stored in glass containersand freeze-dried until all the water was removed to giveconcentrated decoction. It was then kept until required.Stock solutions were prepared. Thirty milligrams of driedsamples from decoction, crude ethanol extract and crudeethanol-water extract, were dissolved with 3000 ppm ethanol and distilled water respectively and then sonicated to dissolvethe dried samples. From the stock solution, 10, 100, 500, and1000 ppm concentrations were prepared by the addition of 5 ppm, 50 ppm, 250 ppm and 500 ppm of solution, respectively ina 20 mL test tube. Addition of a minimal amount of dimethylsulfoxide (DMSO) was done to completely dissolve the solution in each test tube.

Brine Shrimp Lethality Bioassay: Hatching of Brine

Shrimp: Brine shrimp (*Artemia salina*) lethality bioassay wascarried out to investigate the cytotoxicity of extracts ofmedicinal plants. Artificial seawater was prepared by dissolving 40 grams of natural table salt in every liter of distilled water.Sea water was kept in a small tank, and *A. salina* eggs wereadded to the divided tank21. Constant temperature (around 37° C) was maintained and constant supply of oxygen was carriedout. Brine shrimps were allowed to mature and hatch as naupliifor two days. The newly hatched shrimp was collected using a dropper.

Assay Proper

Ten brine shrimp larvae were introduced intoeach sample vials containing different concentrations of theextracts. Seawater was added to make a total volume of 5 ml.

The vials were maintained under illumination. Survivors werecounted after 6, and 24 hours and the deaths at control and eachdose level were determined [6].

Lethal concentration Determination

After 6h and 24h, thelethal concentrations of the *P. spicatus* extract resulting to 50%mortality of the brine shrimp (LC50) were determined. Then, by means of a trendline fit linear regression analysis (MS Excelversion 7) the dose-response data were transformed into astraight line. From the best-fit line obtained the LC50 wasderived.

Statistical Analysis: Reed-Muench statistical method was used to determine the relative toxicity of the *P. spicatus* extracts to living organisms. It was done by testing the response of *A. salina* under various concentrations of the extract. LC50 represents the dose lethal to the half members of the A. salina.This was determined by plotting the mortality (y-axis) versus log of concentration (x-axis). The concentration that rendered 50% mortality was the LC50.

RESULTS AND DISCUSSION

spicatus extracts on the brine shrimp after 6 and 24-hour exposure. The extractsobtained from decoction and ethanol-water extract exhibited no lethality on the brine shrimps at any of the concentrations at 6hand 24h. The brine shrimps were still actively moving, and nosigns of behavioral changes were observed. Crude plant extract with LC50 value of less than 1000 ppm is toxic while non-toxic (inactive) if it is higher than 1000 ppm20. Since the LC50 in theboth of this extract taken from decoction and ethanol-water mixture was higher than 1000 ppm, it was considered inactive.It may be because the active components present in the *P.spicatus* were not extracted through the two methods mentioned above. Even though decoction process is economical due to itslow cost in terms of instrumentation and reagents 21 it may be aninefficient process given that ingredients may be damagedduring the prolonged heating of substances, and otheringredients may be oxidized and lose activity 22.

During thedecoction process, many aromatic herbs with high levels ofvolatile oils are easily lost through evaporation 23.

Also, ethanolwatermixture extraction process was stillineffective and it is inaccordance with the previous study in which the alcohol/water mixture (typically 20–40% alcohol) is actually a poor mediumfor extraction. It is because it causes the desired components tocondense out of the liquid therefore none is left in the finished product24. The ethanol extract of *P. spicatus* showed a toxicityeffect at 6h and 24h, with LC50 value at 944.07 and 266.07 ppm,respectively. This suggests that the extract could have compounds that are cytotoxic as the LC50 value was lower than1000 ppm [4].

shrimp mortality rate at differentconcentrations in the ethanol extract was found to increase with increasing concentration of the sample, and it clearly shows thatthe extraction with ethanol was a better way of obtaining *P.spicatus* extract bioactive components. The previous studies show that ethanolic extract of *P. spicatus* demonstrated strongbiological activity toward *Leishmania amazonensis*8. Ursolicacid and the two hirsutinolides (the 8-acetyl-13-O-ethylpiptocarpholand 8, 13-diacetyl- piptocarphol 8-acetyl-13-Oethyl-piptocarphol) isolated through phytochemical screeningmight be responsible for its pharmacological activities thus giving support to its use in Peru [7].

CONCLUSION

Nanosponge are nano sized colloidal carrier so they easily penetrate through skin. Due to their small size and porous nature they can bind poorly- soluble drugs within the matrix and improve their bioavailability of drug and they also increase the solubility of poorly soluble drugs. The nanosponges have the ability to incorporate many drugs and release them in a controlled and predictable manner at the target site. Topical nanosponge can be more patient compliant and provide sufficient patient benefits by reducing repeated doses and side effects. Nanosponge can be effectively incorporated into topical drug delivery system for retention of dosage form on skin. Nanosponges are tiny mesh- like structures that may revolutionise the treatment of many diseases and this technology is five times more effective at delivering drugs for cancer than conventional methods. These are self sterilizing as their average pore size is 0.25µm where bacteria cannot penetrate. .

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