# Intranasal Piperine-Loaded Chitosan Nanoparticles as Brain-Targeted Therapy in Alzheimer's Disease: Optimization, Biological Efficacy, and Potential Toxicity

**YOSRA S. R. ELNAGGAR,[1] SAMAR M. ETMAN,[1] DOAA A. ABDELMONSIF,[2] OSSAMA Y. ABDALLAH[1]**

[1]Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt
[2]Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt

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**ABSTRACT:** Piperine (PIP) is a phytopharmaceutical with reported neuroprotective potential in Alzheimer's disease (AD). Oral PIP delivery suffers from its hydrophobicity and pre-systemic metabolism. In this article, mono-disperse intranasal chitosan nanoparticles (CS-NPs) were elaborated for brain targeting of PIP. Formula optimization was based on particle size (PS), zeta potential (ZP), polydispersity index (PDI), % entrapment efficiency (% EE), release studies, and transmission electron microscopy. AD was induced in 48 male Wistar rats on which full behavioral and biochemical testing was conducted. Brain toxicity was assessed based on Caspase-3 assay for apoptosis and tumor necrosis factor for inflammation. Spherical NPs with optimum % EE (81.70), PS (248.50 nm), PDI (0.24), and ZP (+56.30 mV) were elaborated. PIP-NPs could significantly improve cognitive functions as efficient as standard drug (donpezil injection) with additional advantages of dual mechanism (Ach esterase inhibition and antioxidant effect). CS-NPs could significantly alleviate PIP nasal irritation and showed no brain toxicity. This work was the first to report additional mechanism of PIP in AD via anti-apoptosis and anti-inflammatory effects. To conclude, mucoadhesive CS-NPs were successfully tailored for effective, safe, and non-invasive PIP delivery with 20-folds decrease in oral dose, opening a gate for a future with lower AD morbidity. 
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**Keywords:** chitosan; drug targeting; nanotechnology; nasal drug delivery; polymeric drug delivery systems; Alzheimer's disease

## INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia worldwide; it affects more than 35 million people, and this number is expected to triple in the next 40 years because of the increase in life expectancy.[1] AD is a neurodegenerative disorder that is characterized by not only progressive impairment of memory and cognitive functions but also disturbances in behavior.[2,3]

A smaller proportion (5%–10%) of all Alzheimer cases was reported to be caused by genetic abnormalities of chromosomes leading to early AD. On the other hand, the greater proportion of all Alzheimer cases (90%–95%) are found to be of late onset and sporadic in origin.[4] Sporadic dementia of Alzheimer type (SDAT) is shown to be associated with microtubule dysfunction and appearance of cytoskeletal cellular abnormalities together with cognitive impairment.[5] Central administration of microtubule disrupting agents (i.e., colchicine) was shown to result in cell death associated with cognitive impairment that resembles the microtubule dysfunction in AD.[6]

Increased oxidative stress (OS) and decrease in acetylcholine (ACh) concentration are reported in the pathology of AD. To date, the efficacy of drugs for treating AD is very limited. Recently the possible treatment for AD approved by the United States of Food and Drug Administration involves the use of cholinesterase inhibitors such as for example, donepezil, rivastigmine, galantamine, and tacrine. Only donepezil is approved for all stages, whereas others are used in mild to moderate conditions. Side effects of these drugs include nausea, vomiting, loss of appetite, and increased frequency of bowel movements. Tacrine, the first cholinesterase inhibitor approved, is rarely prescribed nowadays because of the possible liver damage associated with its use. Another medication used for mild to moderate AD is memantine that regulates the activity of glutamate by blocking the NMDA receptors but it is also accompanied with headache, constipation, confusion, and dizziness. Another suggested treatment is the use of antioxidants where large number of antioxidants has been used for treatment of various memory related disorders[7,8] Many studies have revealed that increasing the intake of antioxidants available as dietary supplements (such as vitamin E) has been associated with the reversal of cognitive and memory disorders. Nevertheless, the need for newer, better-tolerated, and more efficacious treatments is remaining high.[9]

Numerous medicinal plants possessing profound central nervous system effects and antioxidant activity have recently received attention to improve cognitive function in AD. Therefore, the effect of piperine (PIP), a main active alkaloid in Piper nigrum, on memory performance and neurodegeneration in animal model of AD has been investigated.[10] Imperative pharmacological effects discovered for this household

**Abbreviations used:** ACh, acetylcholine; AD, Alzheimer's disease; CS-NPs, chitosan nanoparticles; ICV, intracerebroventricular; i.n., intranasal; PIP, piperine; PIP-NP, piperine-loaded chitosan nanoparticles; MDA, malondialdehyde; MWM, Morris water maze; SOD, superoxide dismutase; TAC, total antioxidant capacity; TPP, sodium tripolyphosphate.

*Correspondence to*: Yosra Elnaggar (Telephone: +20-1147591065; Fax: +20-3-4873273; E-mail: yosra_pharm@yahoo.com)

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phytopharmaceutical encompassed anti-inflammatory and analgesic potential,[11] anticonvulsant,[12] anticancer,[13] antidepressant,[14] and cognitive enhancing effects.[15] PIP was reported to possess beneficial effect on cognitive function in AD.[10] Structure–activity relationship for PIP infers that polyene bond system makes it a potent antioxidant, whereas the presence of tertiary nitrogen like in ACh can inhibit acetylcholenesterase enzyme or enhance cholinergic system in the brain.[16] Nevertheless, oral PIP delivery is hampered by first pass effect by CYP 450 (cytochrome P 450). To circumvent pre-systemic clearance of PIP by oral delivery, intranasal (i.n.) route was proposed.[17]

Intranasal delivery is non-invasive, painless, does not require sterile preparation, and can be easily administered by the patients themselves.[18,19] It has been shown that direct nose to brain delivery is a potential method for bypassing the blood–brain barrier and consequently brain targeting.

This route involves the olfactory or trigeminal nerve systems, which initiate in the brain and terminate in the nose at the olfactory neuroepithelium or respiratory epithelium, respectively. Being the only exposed parts of the CNS, they represent an interesting route for direct non-invasive brain delivery.[20] However, the bioavailability of the drugs administered through the nasal cavity was only 0.1%.[21] Taking the proposed advantages into consideration, such a therapy would be desirable for patients especially for diseases that require chronic treatment such as those related to dementia.[17] Nevertheless, efficient i.n. delivery of PIP would necessitate its encapsulation inside nanocarrier to mask its pungency. Furthermore, brain targeting via i.n. route could be achieved with nanocarriers possessing particular particle size (PS) and mucoadhesive potential.[22] In this context, ionically cross-linked chitosan nanoparticles (CS-NPs) have proved efficacy.[23,24] CS possesses some ideal properties of polymeric NPs as it is biocompatible, biodegradable, nontoxic, and inexpensive. Moreover, it possesses positive charge that offers better interaction with cellular membranes,[25] exhibits absorption enhancing effect by its ability to open the tight junctions, and has mucoadhesive properties.[26] CS was also reported to have anti-inflammatory effect by blocking the secretions of TNF-alpha.[27] Furthermore, PIP encapsulation into CS-NPs would protect it from degradation and decrease irritability. The toxicity of CS-NP on nasal mucosa has been previously reported by measuring the ciliary beat frequency and was found to be cilio-friendly.[28,29] CS was previously involved in a micro-system (121 μm) for oral delivery of PIP as hepatoprotective.[30] Nevertheless, a crucial requirement for efficient brain targeting via i.n. route is a nanocarrier of CS with a nanometric PS cutoff (250 nm) and low polydispersity index (PDI),[22] which was not so far tailored for PIP.

The current article is the first one to elaborate ionically cross-linked CS/tripolyphosphate (TPP) NPs for brain targeting of PIP via nasal pathway. Challenges addressed in this article encompass loading of lipophilic drug in hydrophilic carrier and formulation of monodisperse NPs of a natural polymer. To address these challenges, optimization process of 10 variables based mainly on PS, PDI, zeta potential (ZP), and entrapment efficiency (EE) was conducted. *In vivo* study was carried out on male Wistar rats including behavioral test and biochemical assessment of AD mediators. The two major pathways for AD pathophysiology (OS and acetyl choline) were investigated.

## MATERIALS AND METHODS

### Materials

Chitosan with medium molecular weight ($M_w$ = 100,000–300,000 Da) and degree of deacetylation >90% and PIP ($M_w$ = 285.34 Da, purity 98%) were purchased from Alpha Aeser (Ward Hill, MA 01835, United States, Massachusetts), sodium TPP was purchased from Sigma–Aldrich (St. Louis, Missouri). Poloxamer 188, Tween 80, glacial acetic acid, methanol AR, and all other reagents were of analytical grade. Colchicine, donepezil hydrochloride, thiobarbituric acid (TBA), trichloracetic acid (TCA), nitroblue tetrazolium (NBT), acetylthiocholine iodide (ACTI), dithiobisnitrobenzoic acid (DTNB), Folin phenol reagent, and other buffers and reagents of *in vivo* study were purchased from Sigma–Aldrich.

### Solubility Study

Saturation solubilities (PIP) in different release media and formulation ingredients were adopted using shake flask method.[31] An excess amount of PIP was added into 5 mL of each solubilizing medium and mixed by vortex for 1 min (GEMMY vortex mixer; VM-300, Germany), mixtures were shaken for 24 h at 25 °C in a thermostatically controlled shaking water bath (type 3047; Kottermann, Hanigsen, Germany) followed by equilibrium for 48 h. Mixtures were then centrifuged at 905.58 g for 10 min and the supernatant was filtered through a Millipore membrane filter (0.22 μm). Samples were diluted with the suitable solubilizing medium and measured against its corresponding blank at 342 nm by UV-VIS spectrophotometer (UV-160A; Shimadzu, Kyoto, Japan).[32] The experiment was repeated in triplicates. Results were represented as mean value (μg/mL) ± SEM.

### Preparation of CS-NPs

Chitosan-nanoparticles were prepared by ionic gelation method.[33–35] Briefly, CS was dissolved in 1% acetic acid to reach a final concentration of 1, 1.25, 1.5, 1.75, and 2 mg/mL by magnetic stirring overnight followed by filtration (0.45 μm Millipore filter). Poloxamer 188 (1%, w/v) was added as stabilizer to CS solution and sonicated for 20 min. pH of CS solution was adjusted to 4.8 using NaOH solution (2N). Sodium TPP solution (1 mg/mL) was prepared by dissolving TPP in ultrapure deionized water by sonication for 2 min followed by filtration (0.45 μm Millipore filter). To CS solution, TPP solution was added drop wise with a syringe under stirring to yield different ratios of CS:TPP (3:1, 4:1, 5:1, and 6:1).

Piperine-loaded CS-NPs were prepared using the aforementioned methodology and ratio. PIP in acetic acid in different amounts (300, 600, 900, and 3000 μg) was added to CS solution and sonicated for 5 min before the addition of TPP solution. The prepared dispersions were allowed to stabilize by magnetic stirring for 30 min. CS-NPs were collected by centrifugation at 22639.5 g for 30 min at 4°C (cooling centrifuge, Sigma 3–30K, Germany). The supernatant was removed by aspiration with syringe needle and used to determine EE. Precipitate was redispersed in 5 mL deionized water by sonication (bath sonicator, Julabo sonicator USR-3, Ceelbach, Germany) for 10 min.

### Physicochemical Characterization of CS-NPs

#### *PS, ZP, and PDI*

The PS, PDI, and ZP were determined by dynamic light scattering technique using Zetasizer Nano ZS. (Malvern, Instruments