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Theoretical Background

Alzheimer's disease (AD) is a neurodegenerative disease that impairs cognition and memory. **Intranasal route of administration for brain drug delivery** offers minimizing the systemic side effects, avoidance of first pass metabolism, ease of self-administration, and enhanced patient compliance. **Chitosan nanoparticles (CS-NPs)** are biodegradable and biocompatible NPs that can be prepared by low cost preparation methods such as ionic gelation. However, Due to the cationic nature of chitosan polymer, entrapping cationic drugs into CS-NPs with reasonable efficiency is challenging. **Galantamine hydrobromide (GH)** is a cationic neurotherapeutic agent whose administration is accompanied by severe side effects; hindering the patient compliance. **The presented study aimed to investigate complexation as an approach to enhance GH entrapment into CS-NPs. Fluorescent GH/chitosan complex NPs were used to detect their uptake into rat brain 1 h after intranasal administration to rats.**

Methods

1. Preparation of GH/chitosan complex nanoparticles

GH was added to chitosan solution in 1% v/v acetic acid, adjusted at pH 4.5, and stirred for 24 h at room temperature. After 24 h, Tween 80 and sodium tripolyphosphate solution were added. The NPs dispersion was stirred for 30 min at 1200 rpm, centrifuged, and finally resuspended in filtered distilled water.

2. Characterization of GH/chitosan complex nanoparticles

To detect GH/chitosan complexation, **FT-IR spectra** for pure chitosan, pure GH and freeze-dried GH/chitosan complex were obtained. **NPs mean particle diameter, zeta potential, and GH entrapment efficiency (EE)** were determined. The shape and size of GH/chitosan complex NPs were examined using **transmission electron microscopy (TEM)**.

3. Detection of GH/chitosan complex nanoparticles in different rat brain regions qualitatively

The control group received 0.9% w/v saline, while **the test group** received 50 μ l of the RBITC-labeled GH/chitosan complex NPs; both by intranasal administration (25 μ l per nostril). Rats were kept in a supine position for 1 h; after which they were euthanized by cervical dislocation. The olfactory bulb, hippocampus, and orbitofrontal and parietal cortices were dissected, frozen, and sectioned using a cryostat. **The sections were examined to localize clusters of fluorescence using a fluorescence microscope with a rhodamine filter set at 100X magnification power.**

Results & Discussion

1. Preparation and characterization of GH/chitosan complex nanoparticles

The FT-IR spectrum of GH/chitosan complex was characterized by a broad band at 3428 cm^{-1} ; which is attributed to the hydrogen bonding, and the intense N-H bending vibration band at 1569 cm^{-1} (**Figure 1**). There is a possible hydrogen bonding between the -OH group of GH and -NH group of chitosan.

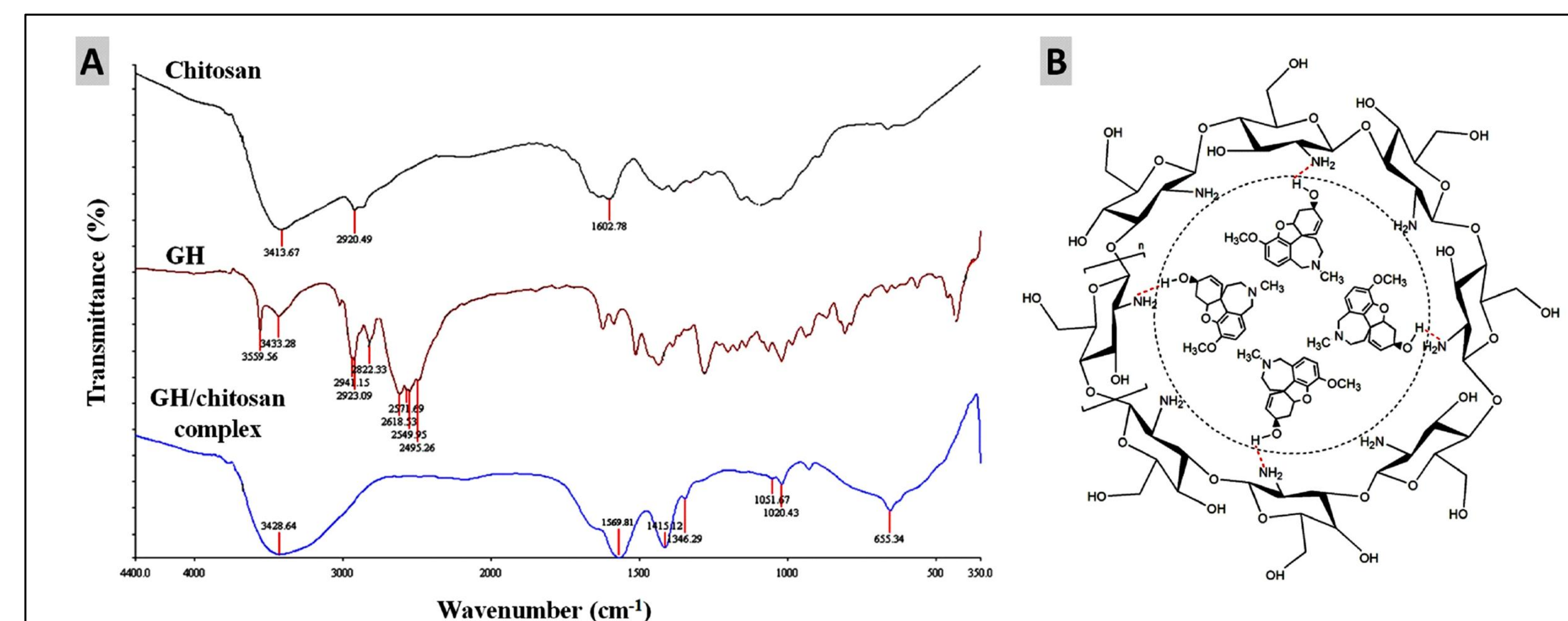


Figure 1: (A) FT-IR spectra of pure chitosan, pure galantamine hydrobromide (GH) and chitosan-GH complex; (B) A hypothetical structure of a nanoparticle showing the possible complex interaction between galantamine hydrobromide and chitosan molecules.

The prepared NPs had an opalescent colloidal appearance (**Figure 2: A**). They had a diameter of 190 nm and a zeta potential of +31.6 mV. The %EE of GH was 23.34%. When visualized by TEM, the NPs were uniform and spherical with a diameter ranging from 48.3 to 68.3 nm. No aggregation was observed. GH encapsulation was manifested as a heavier density white color within NPs (**Figure 2: B & C**).

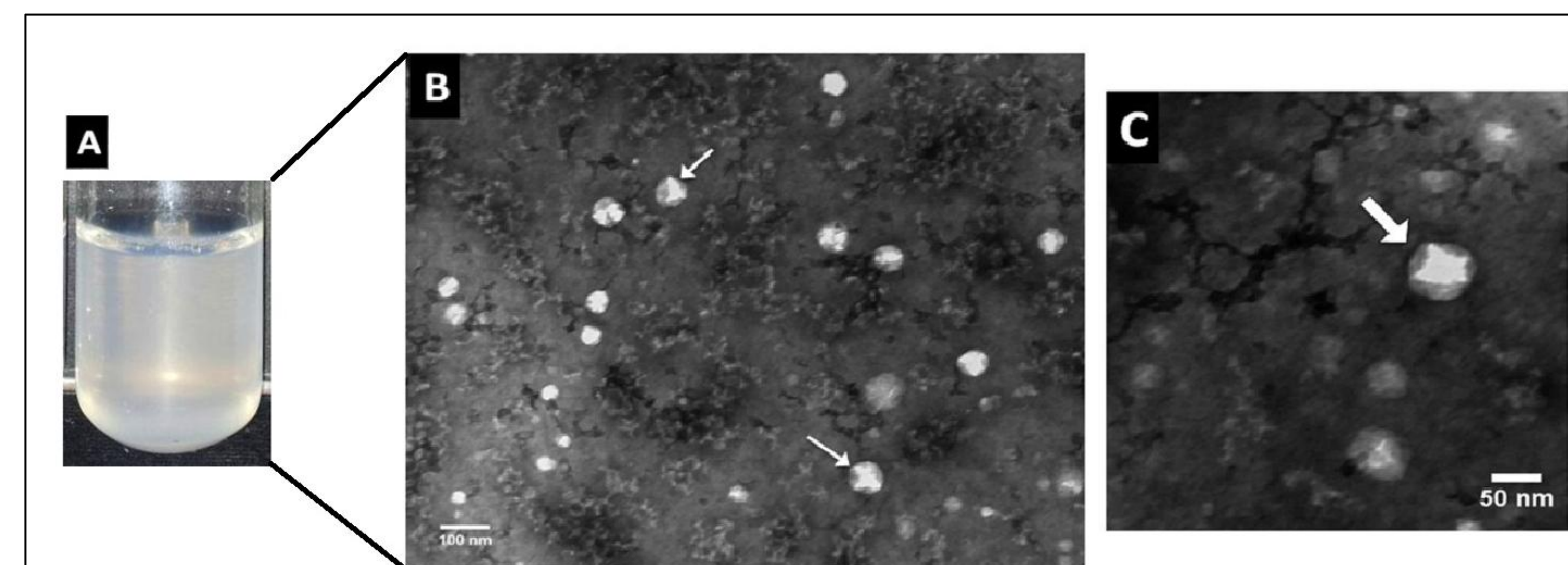


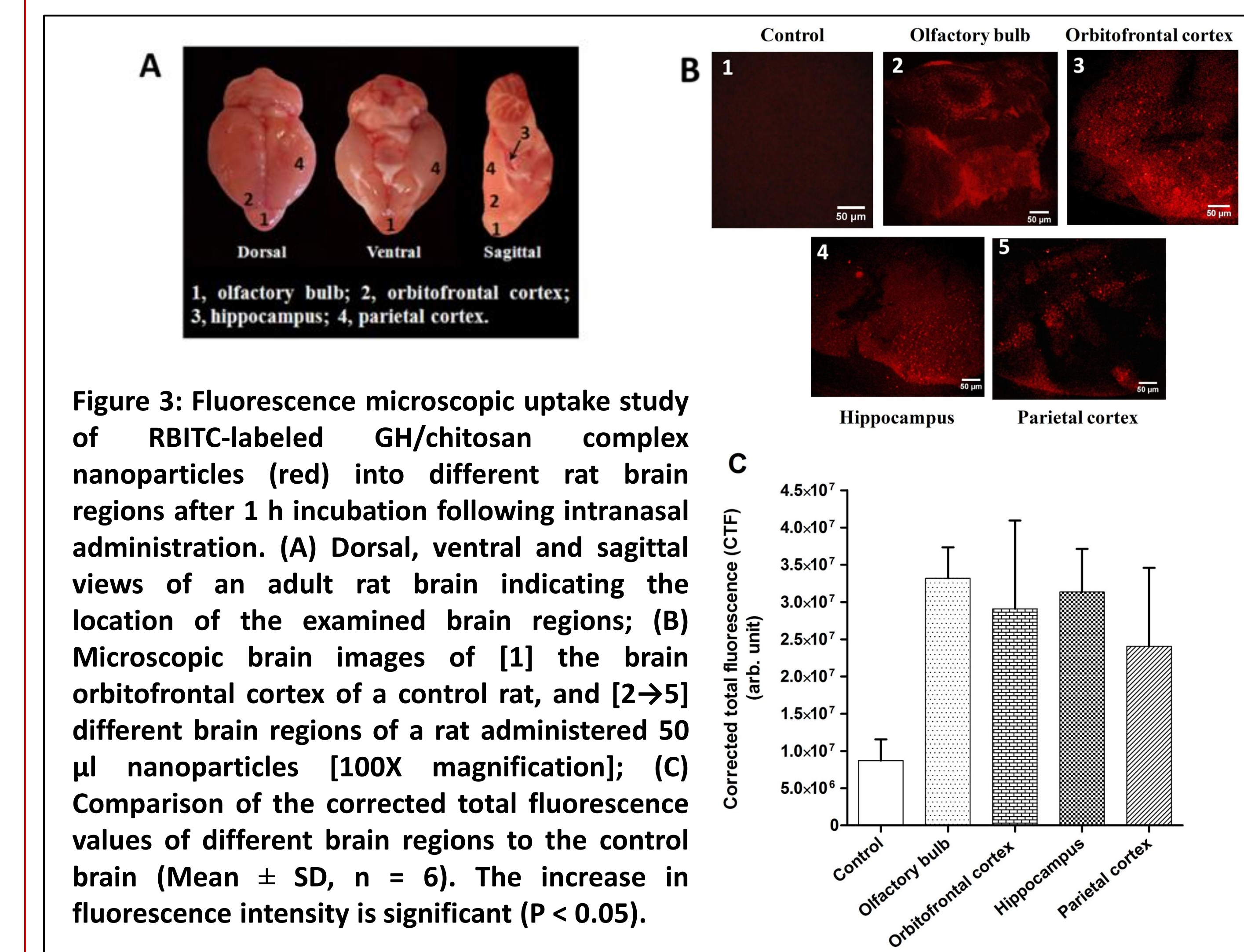
Figure 2: (A) Photograph of GH/chitosan complex nanoparticles colloidal dispersion; (B) TEM photographs of GH/chitosan complex nanoparticles, and (C) a magnified single nanoparticle [the arrows point at the drug encapsulated within the nanoparticles].

2. Detection of GH/chitosan complex nanoparticles in different rat brain regions qualitatively

Distinguishable patterns of red fluorescence were observed in all brain regions 1 h after administering the NPs intranasally (**Figure 3: B**). The computed mean corrected total fluorescence (CTF) values computed indicate the fluorescence intensity in various brain regions compared to the control brain (**Figure 3: C**).

Results & Discussion

It is inferred that the prepared NPs were delivered to the brain after intranasal administration. **They could have been transported across the olfactory and respiratory epithelia reaching the olfactory bulb and trigeminal nerve fibers**, respectively, and then spreading intracellularly (via synapses) or extracellularly (by diffusion) to different brain regions.



Conclusion

Complexation is a promising approach to overcome the repulsive forces between GH, a cationic drug, and CS-NPs, cationic NPs. GH/chitosan complexation enhanced the %EE. Additionally, GH/chitosan complex NPs were detected in the olfactory bulb, hippocampus, orbitofrontal and parietal cortices 1 h after intranasal administration; suggesting their potential as intranasal brain delivery system for AD management.

Declaration & Acknowledgement

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