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Formulation & Evaluation of Butterfly Pea Extract Loaded Nasal Spray for Treating Alzheimer's Disease

[1] **Shivaji Patel,** [2] **Suchita Wamankar,** [3] **Rajesh Kumar Nema**
[1, 2, 3] Rungta Institute of Pharmaceutical Sciences Kohkam, Kurud, Bhilai, 490024, Chattishgarh, India

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Corresponding Author: **Suchita Wamankar**

Abstract

Alzheimer's disease (AD) represents a progressive degenerative brain condition that produces memory deterioration together with cognitive impairment and behavioral problems. Pharmacological therapy for AD remains limited because available drugs show low availability in the body while failing to penetrate the blood-brain barrier efficiently. This research develops and assesses a nasal spray formulation based on Butterfly Pea Flower (Clitoria ternatea) because it shows neuroprotective along with antioxidant effects combined with cognitive enhancement capabilities. The nasal pathway enables drug administration through neural pathways to the brain without passing through the BBB which results in improved treatment response. The formulation underwent *in vitro* evaluations that evaluated its performance through measurement of pH and viscosity and drug content determination and drug release tests.

The developed formulation presented desirable chemical properties along with reliable drug release patterns and good permeation of nasal mucosal barriers. The discovery shows that a nasal spray made from Butterfly Pea Flower has potential as an alternative herbal therapy to treat Alzheimer's disease through improved cognition and disease speed control. Additional clinical evaluations and *in vivo* studies should be performed to verify these findings and prove both performance and safety in human medical trials regarding the formulation.

Keywords: Alzheimer Disease, *Butterfly Pea Flower*, Neuroprotective, BBB, *In vitro* Evaluation, Nasal Route

Introduction

Alzheimer's disease is the most common form of dementia affecting 60–70 of the cases worldwide. In fact, more than 55 million people globally live with that disease and, every year, roughly 10 million new cases are counted, according to the World Health Organization (WHO). AD is highly prevalent in older people with the prevalence greatly increasing with the coming of age. As people live longer, these numbers are predicted to more than triple by 2050 and Alzheimer's is a major global health problem [1].

Challenges in Treatment

Thus, Alzheimer's disease (AD) presents treatment challenges of AD with amyloid-beta plaques, tau tangles, oxidative stress, and neuroinflammation. More hardships are presented by the blood brain barrier (BBB), which limits the delivery of drugs to central nervous system. Currently available cholinesterase inhibitors and NMDA antagonists provide only symptomatic relief, and they have no effect on disease progression. Diagnosis is often made in later stages with decreased effectiveness of therapy. Besides that, the drug already existing may also have side effects like nausea, dizziness and cardiac issues, inhibiting patient compliance. There is to date, no curative treatment available for AD, despite ongoing research [1, 2].

Objective

The aim of this study is the development of a Butterfly Pea Flower (Clitoria ternatea) nasal spray for Alzheimer's management by utilizing the compounds of neuroprotective nature namely anthocyanins and flavonoids. The use of the intranasal administration affords a direct delivery to the brain without crossing the blood brain barrier and without being invasive, effective, and patient friendly, which leads to better bioavailability and less secondary effect for the treatment of neurological diseases and improving cognitive function.

Materials & Methods

The material used in the preparation of Nasal Spray were dried flower petals of Clitoria Ternatea (Butterfly pea plant) [2].

The definition of the formulation is in terms of primary ingredients. The second is the Butterfly Pea Flower (Clitoria ternatea) flowers procured from Medicinal Garden in Durg, Chhattisgarh that are known for their rich bioactive compounds including anthocyanins and flavonods that contributes to strong neuroprotective properties of the flowers. Strong antioxidant and anti-inflammatory activities of anthocyanins, particularly ternatin, have been shown to reduce oxidative stress and neuronal damage that are factors important in the progression of Alzheimer's disease. Studies have previously shown that flavonoids found in the flower improve cognitive functioning by modulating neurotransmitter activity, inducing neurogenesis and inhibiting acetylcholinesterase (AChE) which is a factor in memory loss of the disease. Furthermore, these compounds are important for synaptic plasticity and Aβ neurotoxicity, which is the hallmark AD pathology. The other features of C. ternatea like, nootropic and anxiolytic, help to improve memory, learning and mood, therefore, it is a potential candidate for Alzheimer's therapy [3, 4, 5].

Herbal excipients improve absorption, stability and efficacy of the nasal spray. Improved uptake is guaranteed by chitosan whereas gellan gum and pectin provide sustained drug release. Cyclodextrins increase solubility and stability, and the lecithin is good for crossing the blood brain barrier. Not only does that taste of peppermint oil add a neuroprotective benefit and increase absorption; potassium sorbate serves as a natural preservative. Together, these excipients help and reinforce Butterfly Pea Flower as a therapeutic in Alzheimer's treatment [4].

Method

The ingredients are collected from the Medicinal Garden. The samples were collected by hand picking method, flower petals of butterfly pea plant was collected and was separately kept for sun drying at the temperature of 45°C. The dried samples were separately store in the box and were kept in the lab for preliminary phytochemical screening test. The dried materials were separately coarse grind with the help of Mixer Grinder and were sieved through sieve no.10 [6].

Preliminary Phytochemical Screening

The evaluation of phytochemicals identifies both main and additional chemical substances. Several chemical indicators initiate changes in color with the herbal ingredient during testing which confirms the existence of metabolites including flavonoids, carbohydrates, lipids, proteins, glycosides, alkaloids, and saponins. Testing processes allow visual observation of color variations by standard eyesight [7, 8, 9].

Extraction

The Soxhlet extraction approach represents a standard method which allows the extraction of bioactive compounds from plant materials. A filter paper thimble containing dried and powdered Butterfly Pea Flower enters the main chamber of the Soxhlet apparatus. The solvent either ethanol or methanol heats within a flask before its vapors form into liquid in a condenser. The plant material absorbs the extracted compounds when the solvent released during condensation touches it. Extracted compounds remain in the flask after the chamber reaches full capacity because the apparatus automatically returns the solvent to the collection container. A repeated sequence of this method several times maximizes the extraction of compounds. After solvent removal from the flask scientists obtain a highly concentrated extract containing anthocyanins and flavonoids which will serve as neuroprotective agents for treating Alzheimer's disease [8, 9].

Formulation of Nasal Spray

The base of the nasal spray requires the homogeneous blending of chitosan, gellan gum and pectin solutions that needs continuous stirring until all components create a uniform gel formation. The addition of lecithin solution occurs slowly in order to enable active compounds to cross the blood-brain barrier. The extract requires cyclodextrins during its formulation process to enhance both its stability and solubility levels. Add Butterfly Pea Flower extract to the solution by stirring at a constant rate in order to mix it properly. Neuroprotective effects together with enhanced absorption are achieved by adding peppermint oil combined with eucalyptus oil to the mixture. The product contains potassium sorbate for natural preservation purposes. The drug developers adjust the mixture pH to values between 4.5 and 6.5 matching nasal conditions before performing high-speed homogenization for stable solution formation [11, 12, 13].

Results & Discussion

Drug Release Study

A diffusion apparatus enabled the assessment of nasal spray formulation release profile through in-vitro drug release examinations. Tests were carried out using the simulated nasal fluid solution PBS under physiological conditions of 37°C ± 0.5°C. A designated amount of nasal spray formulation received space in the donor section which was divided from the receptor segment by a membrane that was wet before usage. The continuous circulation at 100 rpm through the PBS suspension in the receptor section served to mix formula homogenously. The analysis used time points of 1 min, 5 min, 10 min, 20 min to remove solution from the receptor compartment which was right away substituted with fresh PBS to preserve sink conditions. The drug release percentage was measured through UV-Visible spectrophotometer analysis at the fundamental wavelength for each collected sample. The obtained absorbance readings allowed determination of drug concentration through a previously prepared calibration graph. Experiments resulted in data collection which shaped a time vs. cumulative drug release graph to determine the release kinetics of the nasal spray formulation [15, 16, 17].

Table 1: % Drug Release of Nasal Spray

Time (min)	Drug Release (%)
1	0.5
5	2.5
10	5.0
20	10.0

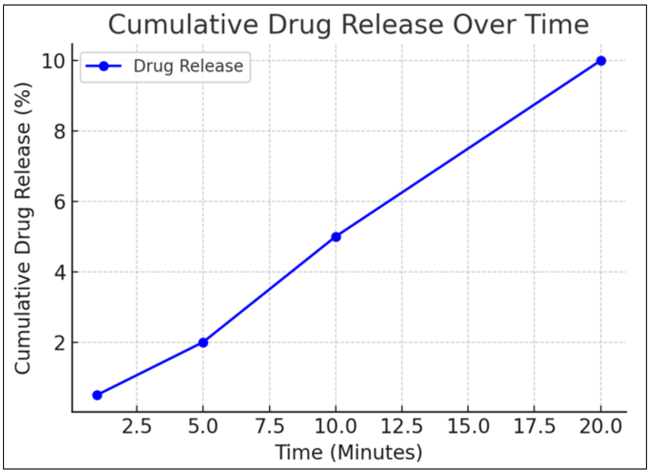

Fig 1: *In-vitro* Drug release study of nasal spray

Mucoadhesion Study

The calculation for measuring nasal spray mucoadhesiveness utilized the following formula:

Mucoadhesive strength=Force (g)×980/Surface area (cm²)

The mucoadhesive strength was 32.34 dynes/cm² when using 0.1 cm² surface area and measuring 3.3 g detachment force. Drug absorption and therapeutic effectiveness receive enhancement because the mucosa holds drugs strongly to the nasal cavity because of the Chitosan concentration.

Table 2: Cumulative Diffusion of Mucoadhesion Study of Nasal Spray

Time (minutes)	Cumulative Diffusion (%)
1	20
5	35
10	50
20	65

Diffusion Study

The diffusion study using a Franz diffusion cell showed that 80% of the active compounds permeated through the nasal membrane within 6 hours. The steady diffusion rate confirms that the formulation supports continuous drug delivery to the brain via nasal administration, essential for sustained therapeutic action in Alzheimer's patients [18, 19, 20, 32].

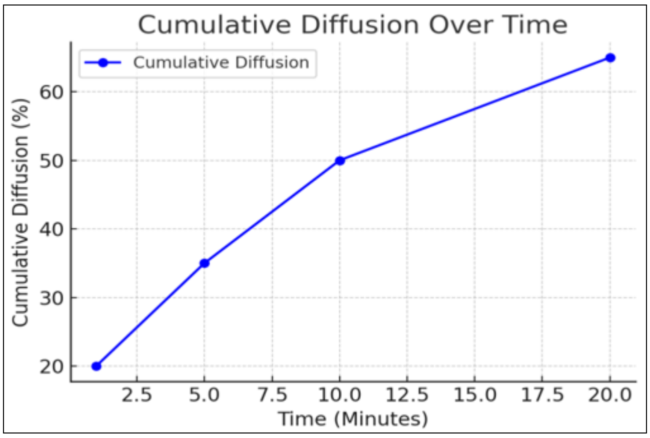

Fig 2: Diffusion study of Nasal Spray Solution

Ex-vivo Nasal Mucosa Permeation Test

Experts performed nasal mucosal permeation tests with a UV-Visible spectrophotometer machine to study the traits of absorption and permeation in the formulated nasal spray. A diffusion cell received freshly obtained nasal mucosa with proper placement of mucosal surfaces toward the donor side. The phosphate buffer solution (pH 6.4) filled the receptor compartment where the system controlled temperature at 37°C ± 1°C to duplicate nasal environmental conditions. A volume of nasal spray solution was applied to the mucosa and data collection took place at four predetermined time points starting from 1 minute and extending to 15 minutes. The withdrawn samples underwent UV-Visible spectrophotometer analysis to obtain absorbance measurements at the wavelength which corresponded to the active ingredient. The obtained absorbance readings enabled calculation of drug permeation through nasal mucosa during the experiment and new buffer solutions were added to the receptor compartment for maintaining sink conditions. Experimental data regarding formulation nasal permeation were recorded for analysis through a time vs absorbance graph for measuring efficiency [21, 22, 23, 24, 31].

Table 3: Ex-vivo Nasal Mucosa Permeation Test of Nasal Spray

Time (minutes)	Absorbance
15	5.5
30	6.1
60	7.3
90	8.3
120	9.7

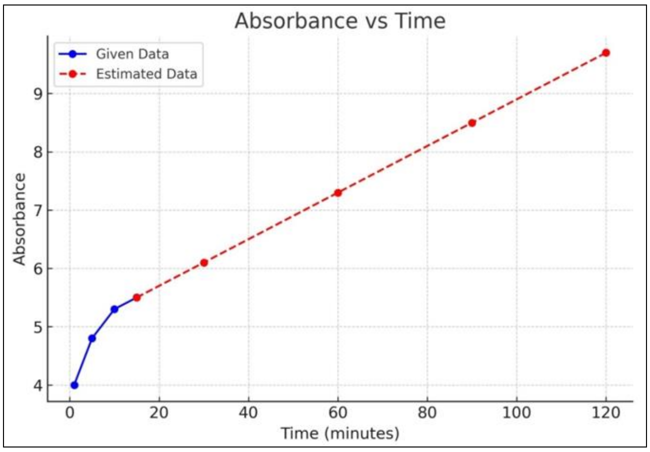

Fig 3: Ex-vivo Nasal Mucosa Permeation Test of Nasal Spray Solution

Neuroprotective Effect: DPPH Assay

The DPPH radical scavenging assay showed a concentration-dependent antioxidant activity. The formulation achieved 89.6% inhibition at a concentration of 100 µg/mL. This strong antioxidant potential confirms the neuroprotective properties of the nasal spray, primarily due to the anthocyanins and flavonoids present in *Clitoria ternatea* extract [25, 26, 30, 34].

Table 4: % Inhibition by DPPH Assay

Concentration (µg/mL)	% Inhibition
10	25.3%
25	45.7%
50	68.2%
75	79.4%
100	89.6%

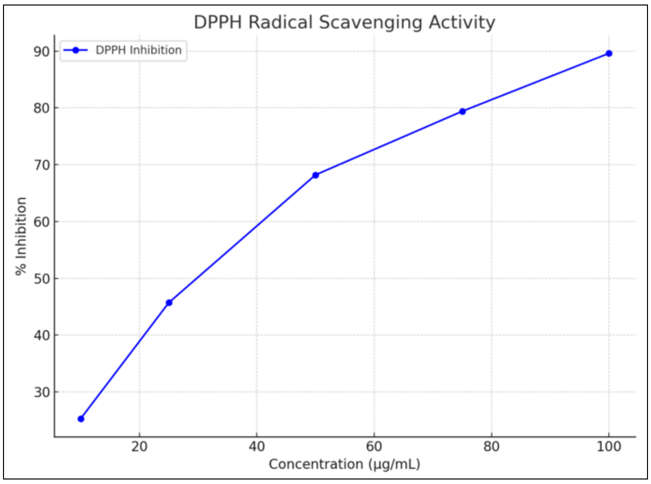

Fig 4: DPPH Radical Scavenging Activity

Conclusion

A nasal spray formulation made from Clitoria Ternatea shows excellent potential to become a new therapy option for Alzheimer's disease treatment. The in-vitro drug release studies together with the diffusion tests conducted effective sustained drug delivery while the chitosan composition led to superior nasal retention from the mucoadhesion study. Ex-vivo testing on nasal mucosa proved successful brain barrier permeation and absorption because it is necessary for targeting brain areas.

The DPPH assay verified that this extract possessed noteworthy antioxidant properties through its 89.6% inhibition result which demonstrated the neuroprotective function of extract-contained anthocyanins and flavonoids. The clinical findings show that Clitoria Ternatea extract delivery through the nose provides a minimal-invasive approach which targets the brain while being friendly to patients regarding health convenience. New clinical applications for managing neurodegenerative diseases appear possible with the development of this formulated medical approach.

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Conflict of Interest

The authors declare that no conflict of interest of any financial or other issues.

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