RESEARCH PAPER

Anti- Stress Potential of Floating Microspheres of Ginkgo Biloba against Neurodegenerative disorder.

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ABSTRACT

Neurodegenerative disorder such as Alzheimer's disease and Parkinson's disease mainly associated with stress, oxidative damage, and gradual loss of brain cells, *Ginkgo biloba* is a well-known herbal plant with antioxidant and neuroprotective properties, but its effectiveness is limited because of poor absorption in the body. This study aimed to improved its therapeutic effect by formulating microspheres for prolonged gastric retention and sustained drug release. Floating microspheres of *Ginkgo biloba* were prepared using solvent evaporation technique with polymers such as Eudragit L-100 and HPMC 3000 cPs+. Phytochemical screening and FTIR studies were performed to confirmed the presence of active constituents and capability between the drug and polymers. The formulated microspheres were evaluated for drug content, incorporation efficiency, percentage yield, and surface morphology using microscopy and SEM analysis. The study successfully developed spherical floating microspheres with porous surfaces, indicating good floating ability and controlled drug release, FTIR studies confirmed capability between Ginkgo biloba and the polymers. The formulation showed 31.5% incorporation efficiency and sustained released characteristics. Overall, the developed microspheres demonstrated potential for improving the bio-availability and anti-stress neuroprotective effects of *Ginkgo biloba* in neurodegenerative disorder.

KEYWORDS: *Ginkgo biloba*, Neurodegenerative disorder, FTIR, SEM, Floating microsphere.

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INTRODUCTION

Neurodegenerative disorders are a category of neurological conditions that impair the structure and function of nerve cells in the brain or spinal cord. The primary feature of these diseases is the loss of neurons. Alzheimer's and Parkinson's disease are common examples of neurodegenerative conditions. For many elderly individuals, neurodegenerative diseases such as dementia and Alzheimer's continue to be a significant clinical issue. This has become more prominent recently due to an aging population and age-related illnesses, increasing in importance because they are irreversible, lack effective treatments, and have considerable social and economic impacts. Neurodegenerative diseases encompass various conditions, including Parkinson's disease (PD), which is marked by the degeneration of dopaminergic neurons in the nigrostriatal pathway; Huntington's disease (HD), which leads to the loss of medium-sized spiny neurons in the striatum; and Alzheimer's disease (AD), attributed to widespread brain atrophy. Certain disorders, such as primary dystonia or tremor, are often classified as neurodegenerative diseases, which are defined by the progressive deterioration of the structure and function of the nervous system.

Patients with neurodegenerative diseases have a lot of different symptoms, many of which are the same. These include problems with memory and thinking, as well as trouble breathing, walking, and talking. These patients often have distinct clinical characteristics, including a gradual deterioration spanning years or even decades [7]. Neurodegeneration is linked to mitochondrial dysfunction, oxidative stress, protein misfolding, neuroinflammation and aggregation, and other biological processes [8,9]. Neurons are important for communication hence they are necessary for the brain to work properly [10]. Although neurons are found throughout the body, the majority of

them start in the brain. Neural stem cells produce most neurons during childhood, drastically decreasing their number in maturity [11,12]. Although neurons are not eternal, neurodegeneration the progressive loss of neurons, their structure, and their functions is a significant health concern and a key factor in the pathogenesis of a number of brain disorders [13]. Neurodegeneration has been identified as the main pathophysiological change in most brain-related diseases [14].

Ginkgo Biloba leaves are rich in several naturally occurring compounds that contribute to their neuroprotective activity. The major constituents include flavonoids such as Kaempferol, Quercetin, and Isorhamnetin, along with terpene including Bilobabide and Ginkgolides A, B, C. These compounds are known for their ability to cross the blood-brain-barrier and their strong antioxidant properties. Researcher suggested that they can help protect brain cells by reducing the accumulation of β-amyloid plaques, which are commonly associated with neurodegenerative disorders like Alzheimer's disease [15]. In addition, ginkgo biloba extract has been reported to reduce neuronal damage, improve communication between nerve cell by enhancing synaptic plasticity, and maintain mitochondrial membrane stability, which is essential for proper cellular energy production [16]. The extract also shows protective effects on dopaminergic neuro, thereby supporting its potential use in conditions such as Parkinsion's disease and cognitive decline [17].

Floating microspheres are hollow spherical particles that lack a central core. It contain freely moving particles measuring between 1 and 1000µm. The formulation is designed to remain buoyant in gastric fluid with a density below one, which is regulated by gastrointestinal movement. As a result, food takes more time to pass through the stomach. The medication is gradually released at the intended rate, allowing it to remain in the stomach longer and preventing significant fluctuations in plasma drug concentration. The even distribution of the floating microspheres throughout the GIT may lead to enhanced consistency in drug absorption and a lower chance of local irritation. It resulted in oral controlled drug delivery and prompted the creation of gastro retentive floating microspheres [18].

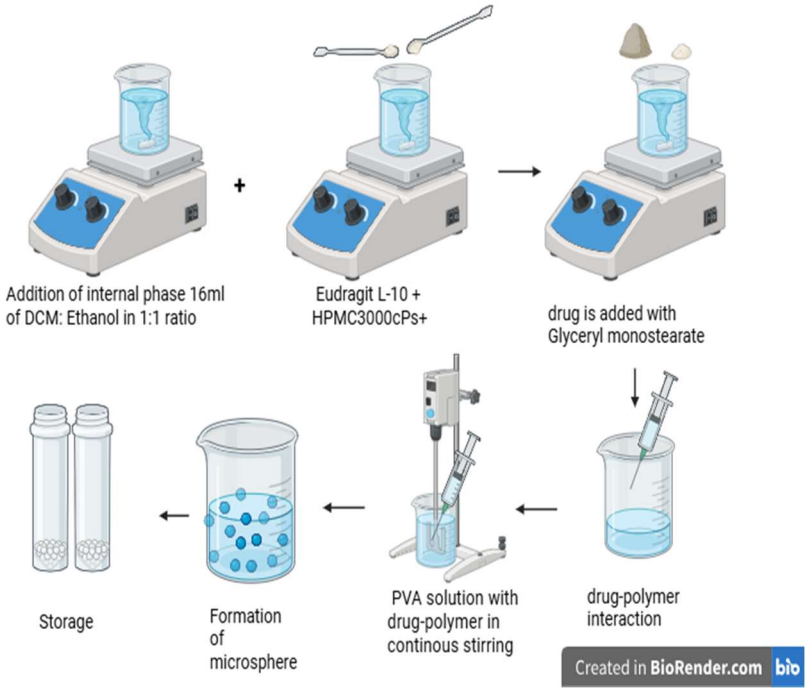

Figure 1: The Process and Formulation of microspheres

MATERIALS AND METHOD

Materials:

Ginkgo biloba leaves extract is collected from Fullmoon Global with its Certificate of Analysis. Excipient like Eudragit L-100, HPMC3000cPs+, Glyceryl Monostearate, Dichloromethane, Ethanol, Polyvinyl Alcohol: Procured from laboratory of SOPR, DBUU.

Ingredients for formulating floating Microsphere

Table 2: formulation ingredients of floating microspheres [19,20].

S.NO.	Chemicals	Purpose	F1	F2	F3
1.	Ginkgo Extract	Active pharmaceutical ingredient	170mg	200mg	230mg
2.	Eudragit L-100	Polymer	90mg	100mg	110mg
3.	HPMC 3000cPs+	Polymer	250mg	300mg	350mg
4.	Glyceryl monostearate	Emulsifying agent	q.s	q.s	q.s
5.	Dichloromethane	solvent	8ml	8ml	8ml
6.	Ethanol	solvent	8ml	8ml	8ml
7.	Polyvinyl Alcohol	Dispersing medium	1.5%	1.5%	1.5%

Methods:

Phytochemical Screening

Flavonoid test:

1. **Alkaline reagent:** To 2ml of the extract were added in 1 ml of 2N sodium hydroxide. When we did this the extract turned yellow. This yellow color is a sign that the extract contains flavonoid [21].

2. **Shinoda's test:** Add a piece of magnesium ribbon to the extract. Add concentrated hydrochloric acid, which results in a red color [22].

Figure 2: Indication of alkaline reagent.

Figure 3: Indication of Shinoda's test

Terpenes test:

3. **Salkowski's test:** The test involved taking half a ml of extract and adding 2ml of chloroform to it. Then concentrated sulfuric acid was added to this mixture. A brown color started to form at the interface. This color change is a sign that terpenoids present, in the extract. The formation of this brown color is a good indicator of the presence of terpenoids. [21].

4. **Copper Acetate test:** Add 2 ml of copper acetate solution to the sample in a test tube, which results in a blue-green color [22].

FTIR Sample preparation

For FT-IR analysis, physical mixtures of the 1 studied polymer are prepared in a ratio of polymer and drug (1:1 ratio). They are weighed and mixed in a mortar to obtain a physical mixture. 2 samples were collected, i.e., ginkgo

biloba extract: Eudragit L-100 and ginkgo biloba extract:
HPMC 3000 cPs+ [23].

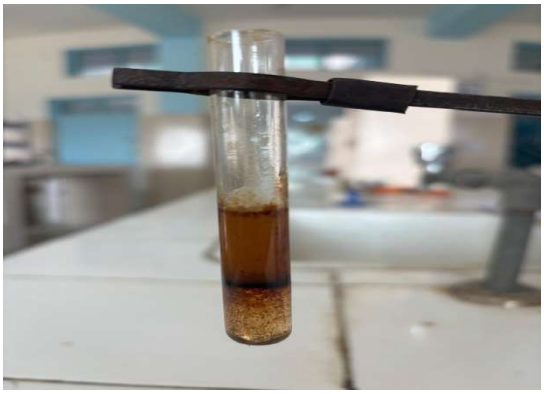

Figure:4 Indication of Salkowski's test

Figure:5 Indication of Copper acetate test

Figure 6: Weighing of polymer

Figure 7: Weighing of Drug

Figure 8: Mixing

Formulation

Preparation of internal phase

1. Mixed 16ml of dichloromethane: ethanol(1:1 ratio)
2. Add Eudragit L-100 + HPMC 3000cPs+
3. Add Ginkgo biloba extract with continous stirring and form drug-polymer solution
4. Add Glyceryl Monostearate with stirring for proper mixing

Preparation of external phase

5. Prepare 1.5% of Polyvinyl Alcohol (PVA)
6. dissolved in 100ml of distilled water

Emulsification process

7. Slowly inject drug-polymer solution into PVA solution (using 22G needle
8. Maintaining constant stirring with its temperature
9. then, Formation of microsphere

Figure 9: Procedure of microspheres by Solvent Evaporation

Figure 10: Process of Formulation, its Trial and formation of Microspheres and storing

EVALUATION

Drug content

1. 10 mg of gingko biloba microsphere was weighed accurately and are crush.
2. Dissolve the polymer in the crushed microspheres by adding 5 mL dichloromethane.
3. Gradually add 0.1 N HCl to the mixture.
4. Filter the mixture into a 50 mL volumetric flask.
5. Dilute to the mark with 0.1 N HCl.
6. Measure the absorbance of the sample solution using a UV spectrophotometer at the maximum absorption wavelength (255.9 nm).
7. Calculate the Ginkgo concentration using a validated calibration curve [24].

Incorporation efficiency

10mg of microspheres were dissolved in 10ml of methanol in an iodine flask. For 4hr, the iodine flask was shaken violently. Shell pieces were removed from

the solution by filtering it [25]. The drug was estimated using a UV double-beam spectrophotometer at an Amax of 311 nm following the proper dilution with methanol. Efficiency of incorporation was assessed using:

Incorporation efficiency = (Calculated drug content/ Theoretical drug content) × 100

Percentage yields of microspheres

After preparation, the microspheres were gathered and weighed. To prepare the microspheres, the measured weight was divided by the total amount of medication and polymer employed [26].

Percentage yield (%) = (weight of microsphere/ total weight of drug and polymer) ×100

Micrographic microscope

A micrograph is a photograph or digital image taken under a microscope that shows a small object at a high magnification (usually more than ten times). The process of creating these images is known as micrography, and it frequently makes use of light microscopes (up to 2,000 times) or electron microscopes for greater resolution [27].

Scanning Electron Microscope

The surface morphology of the produced microsphere was examined using scanning electron microscopy (EHT=15.00kV, Mag=2.00KX) [26].

RESULT AND DISCUSSION

FTIR

The compatibility studies of ginkgo biloba and the polymers (Eudragit L-100) i.e. Formula-1 shows that the FTIR spectrum of the formulation shows characteristic peaks of ginkgo such as O-H stretching (3400cm^{-1}) and aromatic C=C stretching (1600cm^{-1}), confirming the presence of active phytoconstituents. Peaks at 1715cm^{-1}(C=O) and 1200-1000cm^{-1}(C-O-C) indicate the presence of polymer. Indicating the compatibility of the drug and polymer.

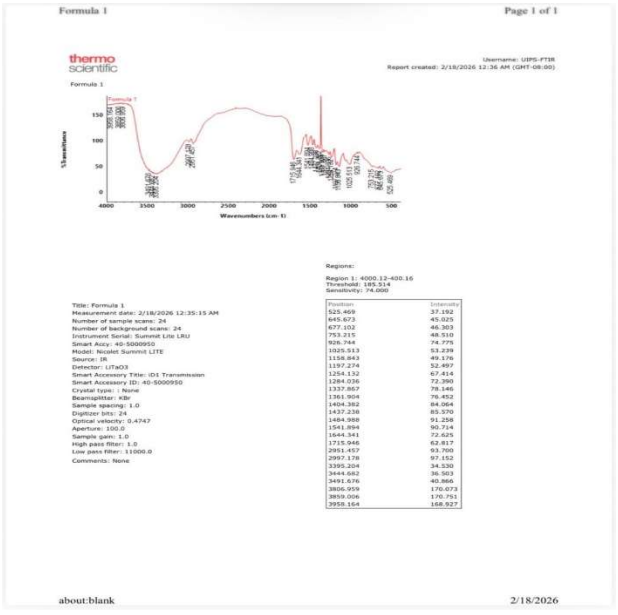

Figure 11: Result of formula 1

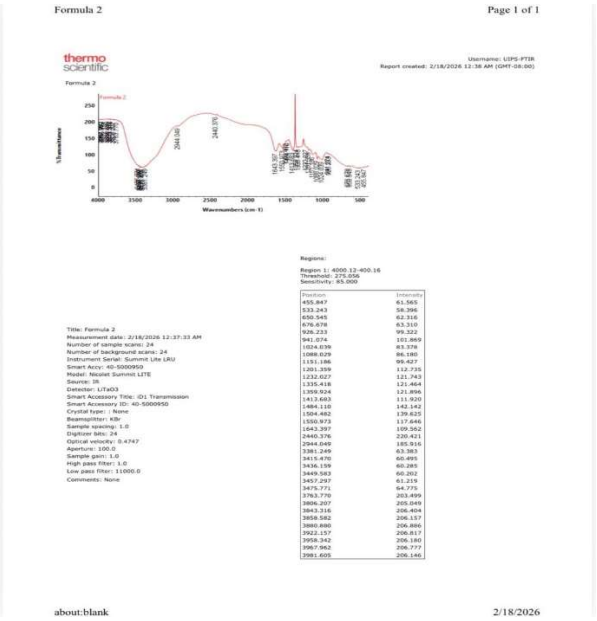

figure 12: Result of Formula 2

The compatibility studies of ginkgo biloba and the polymer (HPMC 3000cPs^{+}) i.e. Formula-2 shows that FTIR spectrum of formulation 2 shows a broad peak around 3400 cm^{-1} corresponding to O-H stretching of Ginkgo biloba. Peaks at 2940cm^{-1} indicate C-H stretching. Aromatic C=C stretching is observed near 1643cm $^{-1}$. peaks in the region 1200-1000cm^{-1} confirm C-CO-C stretching of polymer. Indicating the compatibility of the drug and polymer.

Drug Content

UV spectroscopic analysis was performed using dilute HCL as blank. The sample containing microspheres extract with DCM and HCL shows an absorption peak at 255nm, corresponding to flavonoids of ginkgo biloba. This result confirmed successful in drug ectaction and presence of active constituent.

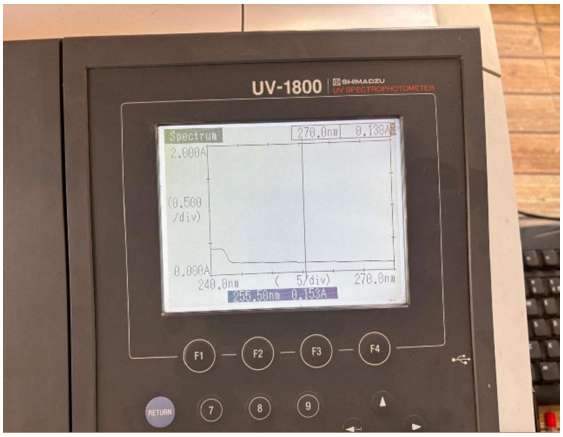

Figure 13: Curve of Blank sample

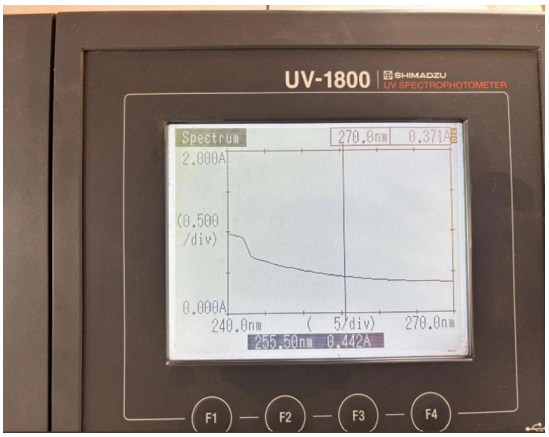

Figure 14: Curve of drug sample

Incorporation efficiency

The blank methanol showed an absorption of about 0.300, which represent baseline interference. The microspheres sample shows a higher absorbance of 0.615 at 311nm, which confirms the presence of drug.

Microsphere taken = 10mg

Methanol = 10ml

λ max = 311nm

Incorporation Efficiency= (calculated drug content/ theoretical drug content) ×100

Drug content = C×V

= 31.5×10 =0.315mg

Theoretical drug in 10mg microsphere = 1mg

% Incorporation Efficiency = (0.315mg/ 1mg) ×100

= 31.5%

Percentage yield of floating microsphere

% yield= (actual wt. of the product/total weight of excipient & drug) ×100

Weight of product= 0.07g

Total weight of the drug & polymer = 0.200g+ 0.100g+ 0.300g = 0.6g

$$\% \text{ yield} = \frac{0.07}{0.6} \times 100$$

=0.116×100

= 11.6%

Micrographic microscope

The particles are spherical with smooth and well-defined boundary. Their uniform shape indicates successful formation of microsphere in wet and drying state.

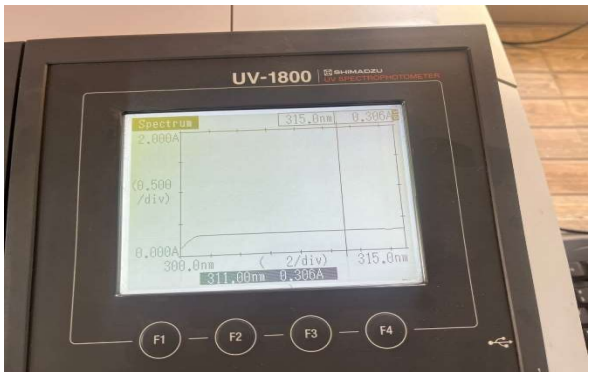

Figure 15: Curve of blank sample

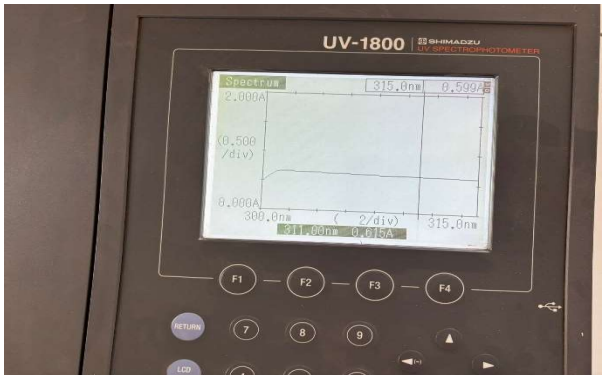

Figure 16: Curve of Drug sample

Figure17: Shape of a dry microspheres

Figure 18: Shape and structure of dry microsphere

Figure 19: Shape and Structure of microsphere in a solvent

Scanning Electron Microscope

The SEM image shows a porous and rough surface morphology of microspheres. The presence of

interconnected pores and hollow cavities indicates successful solvent evaporation and formation of low-density floating microspheres. This porous structure may enhance the buoyancy and contribute to sustained drug release behavior.

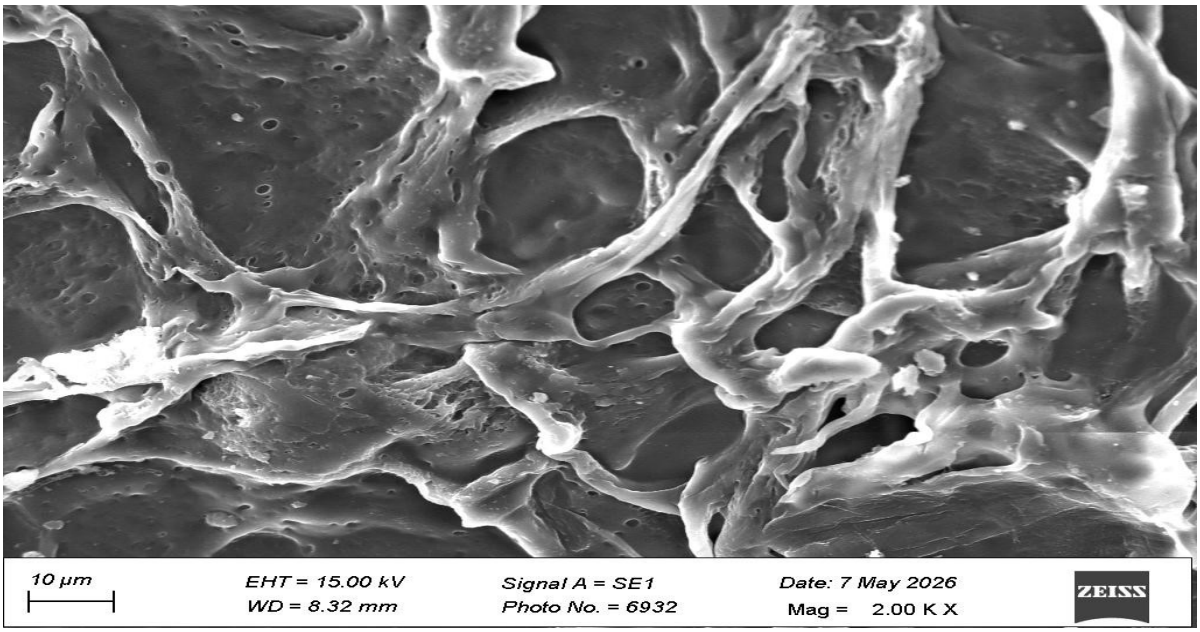

Figure 20: SEM micrograph of Ginkgo biloba floating microspheres showing porous and rough surface morphology.

CONCLUSION AND FUTURE SCOPE

Conclusion

The present investigation successfully formulated and optimized floating microspheres of Ginkgo biloba with desired physicochemical and functional properties. The developed microspheres displayed longer gastric retention and sustained drug release that are vital for enhancing the bioavailability of active phytoconstituents. The formulation was assessed for anti-stress activity using established behavioral and biochemical models, and for potential neuroprotective effects under experimental conditions.

The results suggest an improved therapeutic efficacy of floating microsphere system with controlled release and better systemic absorption. In addition, the formulation showed potential in alleviating stress-induced neuronal injury, possibly through its antioxidant, anti-inflammatory and neurotrophic effects. The present study proved good scientific evidence to support the application of gastro-retentive microsphere technology in enhancement of herbal drugs.

Future scope

The further studies can be directed towards detailed pharmacokinetic and pharmacodynamic assessment to understand the in vivo behavior of the developed formulation. More advanced molecular studies can be performed to elucidate the exact mechanisms of its neuroprotective and anti-stress effects. Long-term toxicity and safety studies will be required to ensure clinical applicability.

In addition, the translational translation of such findings into useful therapeutic applications can be facilitated by the scale-up of the formulation and clinical trials. The proposed method can be further extended to other herbal drugs with poor bioavailability which will widen the scope of gastro-retentive drug delivery systems for treatment of stress related neurodegenerative disorders.

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