I Research: Journal of Pharmacy
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Encapsulation of *Phyllanthus Emblica* Fruit Extract Biodegarable Gelatin Nanosperes: Design Characterization and *In Vitro* Antioxidant Assessment

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ARTICLE INFO

Keywords:
Emblica officinalis,
Gelatin nanospheres,
Antioxidant activity,
Solvent displacement method,
Neuroprotection,
Controlled drug delivery.

Article History:
Received : 06th November 2025
Accepted : 24th November 2025
Available online : 24th November 2025

ABSTRACT

Neurodegenerative disorders represent a class of progressive diseases that lead to irreversible loss of neuronal function within the brain and central nervous system. Among them, Huntington's disease (HD) is a fatal neuropsychiatric condition, with its prevalence in India comparable to that observed in Western populations. Despite significant advances in research, no effective curative therapy exists to arrest or reverse disease progression. A major challenge in HD management lies in the restricted permeability of therapeutic agents across the blood–brain barrier (BBB), which limits drug availability within the central nervous system. Moreover, conventional pharmacological treatments are often associated with adverse systemic effects.

In the present investigation, an attempt was made to develop *Phyllanthus emblica* extract–loaded gelatin nanospheres as a novel nanoformulation for potential use in the treatment of Huntington's disease. Pre-formulation studies were carried out to determine the solubility and physicochemical characteristics of the extract. FTIR and UV analyses confirmed the absence of drug–polymer interactions, showing a distinct absorption peak at 273 nm. The nanospheres (formulations F1–F4) were prepared by the solvent displacement method and evaluated for particle size, surface morphology, zeta potential, entrapment efficiency, and drug loading. Entrapment efficiency values ranged from 40.61% to 80.21%, while drug loading varied between 18.79% and 50.33%. Among all, formulation F2 exhibited optimal characteristics with a sustained drug release profile, showing 10% release at 1 hour and 37% release at 6 hours.

The antioxidant potential of the nanospheres was further assessed by DPPH assay, revealing strong free radical scavenging activity. Overall, the results indicate that *Emblica officinalis*–loaded gelatin nanospheres may serve as an effective, biocompatible nanocarrier for targeted brain delivery in the management of Huntington's disease. Further in vivo investigations and optimization are warranted to establish their therapeutic efficacy and clinical relevance.

Introduction

Delivering active compound to the target site is one of the major problems in treatment of disease. A conventional application of drugs is characterized by limited effectiveness, poor biodistribution and lack of selectivity. And these type of limitations and disadvantages can be overcome by controlling drug delivery. In controlled drug delivery systems [DDS] the drug is transported to the targeted place and its influence on vital tissues and unwanted side effects can beminimized . And DDS protects the drug from rapid degradation or clearance and enhances drug concentrations in target tissues, therefore, lower dose of drug are required.[1]

From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, nonspecific toxicity, immunogenicity, biorecognition and efficacy of drugs were generated. These new developments are often called drug delivery system .[2]

Targeting drug delivery system is the ability to direct the drug-loaded particles to the site of interest. Two major mechanism can be described for the desired sites for drug release:

1 Passive Targeting
2 Active Targeting

These system can be generally characterised as controlled drug release system and targeted drug delivery systems. The therapeutic activity of these new system includes;

1 Increased efficacy of the drug
2 Site specific delivery
3 Decreased toxicity/side effects
4 Increased convenience
5 Viable treatments for previously incurable diseases
6 Potential for prophylactic applications
7 Better patient compliance[2]

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Blood brain Barrier

The blood-brain barrier [BBB] is a highly selective permeability barrier that separates the circulating blood from the brain from the brain extracellular fluid [BECF] in the central nervous system [CNS]. BBB is a unique membranous barrier that tightly segregates the brain from the circulating blood. The BBB acts very effectively to protect the brain from many common bacterial infections.[3]

Transport across the BBB

Freely permeable: Diffusion : water, CO_2, O_2 , free forms of steroid hormones , alcohol and lipid soluble substances.

Carrier-mediated transport: glucose [byGLUT-1], amino acids, thyroid hormones, several organic acids, choline, nucleic acid precursors.

Ions: transport is regulated and limited

Practically impermeable: proteins and polypeptides

Functions

- Protection-BBB protects the microenvironment or extracellular environment of CNS from extra fluctuation of blood.
- Also protect CNS from toxins.
- Stabilizer-it stabilize the internal environment of CNS including blood and brain
- Holder-it holds the neurotransmitter within CNS[4]

Brain Targeting Drug Delivery System

Rate limiting role of the BBB in brain drug development

In the central nervous system targeted action can be achieved by direct administration of the drugs in to the CNS. Blood brain barrier can considerably impair the effect of the large number of drugs because of its obstinate hindrance affect. From some recent studies it has been represented that the blood brain barrier is usually does not cross by almost 100%of large molecule drugs and 98% small molecule drugs presently numerous approaches with enhanced pharmacodynamics effects, have been developed for the treatment of brain disorders. Drug discovery and drug delivery technologies are the two main fields where advancement is required for drug delivery to the brain[4]

1. BBB is the major confront toward brain targeted drug delivery.
2. BBB have efficient ability to restrict and separate the human brain from circulatory network and only allow the transportation of molecules that play vital role in functional activity of brain.
3. It also limits the transport of water and lipid soluble substances from blood circulation into CNS.
4. Advancement in the perception of the cell biology of blood brain barrier has started the innovative path or opportunities for better drug delivery to the brain.[4]

Nanoparticles

Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. According to the method of preparation, nanoparticles and nanospheres, or nano capsules can be obtained. nano capsules are system in which the drug is confined to a cavity surrounded by unique polymer membrane. And the nanospheres are matrix systems in which the drug is physically and uniformly dispersed in the matrix.[5]

The novel drug delivery systems is used to deliver drugs through oral, nasal, parenteral, etc. through nanoparticles particle size can be easily altered resulting in attaining both active and passive drug targeting after parenteral administration. It become the most advantageous in the treatment of many choric type of diseases. Nanoparticles have the ability to control and sustain the drug before reaching the specific site of action and protects drug from rapid degradation and maintains drug concentration at specific sites or in target tissues hence with lower doses of drug shows high therapeutic efficacy and reduced side effects.[6]

Applications

- Used in targeted drug delivery [therapy] to brain and cancer therapy
- Drug and gene delivery
- Bio detection of pathogens
- Detection of proteins
- Biomarker mapping
- Probing of DNA structure
- Tissue engineering
- Separation and purification of biological molecules and cells
- MRI contrast enhancement

Disadvantages

- In spite of many advantages nanoparticles have some limitations which make researchers to work more on it to attain even more best therapeutic efficacy with lesser side effects.
- Due to its particle size [smaller] and larger surface area nanoparticles are very reactive in the cellular environment.
- Drug loading and burst release is limited because of its smaller particle size.

Polymeric Nanoparticles

The polymeric nanoparticles are prepared from biocompatible and biodegradable polymers in size between 10-1000nm where the drug is dissolved , entrapped ,encapsulated or attached to a nanoparticle matrix.[7]

Types of Nanoparticles

Fullerences

A fullerence is any molecule composed entirely of carbon. These are in the form of hollow sphere, ellipsoid or tube. Fullerences are similar in structure to the graphite which is composed of stacked grapheme sheets of linked hexagonal rings, additionally they may also contain pentagonal rings to give potentially porous molecules.[8]

Solid Lipid Nanoparticles

Solid lipid nanoparticles comprise of lipids that are in solid phase at room temperature and surfactants for emulsification .solid lipid nanoparticles offer uniquepropertiessuchassmallsize,largesurfacearea,highdrugloading,the interaction of phases at the interfaces and are attractive for their potential to improve performance of pharmaceuticals and other materials.

Liposomes

Liposomes are vesicular structure with an aqueous core surrounded by hydrophobic lipid bilayer created by the extrusion of phospholipids. Solute such as drugs in the core cannot pass through the hydrophobic molecules can be absorbed into the bilayer, enabling the liposomes to carry both hydrophilic and hydrophobic molecules.

Nano shells

Nano shells are also notorious as core shells, these are spherical cores of a compound surrounded by a shell or outer coating of a thin layer of another material which is a few1-20nm thick.

Quantum dots

The quantum dots are semiconductor nanocrystals and core shell nanocrystals contain interface between different semiconductor materials. Thesize can be turned from 2-10nm . which after polymer encapsulation , generally increases to 5-20 nm in diameter. Particles smaller than 5 nm are quickly cleared by renal filtration.[7]

Gelatin Nanoparticles

Gelatin is a natural versatile biopolymer. It has several important applications due to low cast, easy availability, biodegradable and biocompatible nature as well as the presence of abundant active groups. Gelatin is poly-ampholyte in nature because it contains both cationic and anionic groups. Gelatin polypeptide is composed of repeating triplets of alanine, glycine and proline residues, responsible for typical triple helical structure of gelatin. Gelatin is a protein obtained from the hydrolysis of collagen. The type of gelatin obtained [type A or type B] is governed by the process of collagen hydrolysis[i.e. acidic and basic hydrolysis respectively]. It is shown that type B gelatin nanoparticles show better potential in terms of drug delivery than type A. Moreover, gelatintype B physically captures DNA molecule, thus increasing the transfection efficiency of carriers.[9]

e.g: gelatine, sodium alginate, albumin, chitosan

Applications of GNPs in nano-biotechnology and biomedical sciences

- Targeting human malignancies with nanoparticles
- Crossing the blood-brain barrier
- Macrophages targeting; chasing HIV by GNPs
- GNPs as DNA carrier
- Management of infectious tuberculosis via GNPs
- GNPs targeting Leishmaniasis
- GNPs as bacterial bodies
- GNPs monitoring restenosis (vascular malady)
- The role and potential of gelatinin tissue engineering
- Gelatin lowers quantum dots related toxicity[9]

Polymerization Method

In this method, monomers are polymerized to from nanospheres in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption on to the nanospheres after polymerization completed. The nanospheres suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles is an isotonic surfactant- free medium.

Huntington's Disease

HD is a neurodegenerative disease, characterized by chorea, involuntary movements and anxiety.[10] It is caused by an autosomal dominantly inherited CAG trinucleotide repeat expansion in the huntingtin[HTT] gene on chromosome 4. This results in the production of mutant huntingtin [mHTT] protein with an abnormally long polyglutamine repeat. Those with greater than 39 CAG repeats are certain to develop the disease, whilst reduced penetrance is seen between 36and 39 repeats.[10] It is a neurodegenerative disorder passing within families from generation to generation with onset in middle age and characterized by unwanted choreatic movements, behavioural and psychiatric disturbances and dementia.[11] Forthe first time, actualpremanifest diagnosescould be made and as more disease involving trinucleotide repeats of CAG were found, HD served as a model for manystudies in medicine. CAG [cytosine(C), adenine (A), and guanine (G)], is a trinucleotide, the building stone of DNA.[11]

Signs and Symptoms

- Involuntary, unwanted movements.
- Walking becomes unstable and the person can look as if he/ she is slightly drunk.
- Talking and swallowing gradually become more problematic leading to choking at any time in some patients.
- Dysarthria and dysphagia become more prominent during the course of the disease.
- Develop hypokinesia, akinesia, and rigidity leading to slower pace of all activities.

- Theylose flexibility of mind, and can no longer make mental adjustments.[12]

Secondary symptoms and signs

- Slower functioning
- Decreased appetite
- Difficulty in handling of food
- Swallowing.

Drug Profile

Emblica Officinalis

It is also known as Phyllanthus emblica, emblic, Emblic myrobalan, myrobalan, Indian gooseberry, Malacca tree. Amla is widely used as a mainly medicine. It hasarole intraditionalayurvedic medicine. It containhighamountof ascorbic acid [vitamin C].[22]

Figure1 Emblica Officinalis

Molecular weight

- Plant name : *Phyllanthus emblica*
- Family : Phyllanthaceae
- Part used : Fruit
- Synonym : Amla
- English : Amla
- Distribution : Many parts of India

Phyto constituents

Emblica Officinalis (EO), especially fruit, contain numerous phyto constituents viz. higher amount ofpolyphenols like galic acid , ellagic acid ,different tannins , minerals, vitamins aminoacids, fixed oils and flavonoids like rutin and quercetin.[23]

Mechanism of action

Alteration in basic homeostatic balance of the body is the origin of disease. Imbalance between pre-oxidant and anti-oxidant homeostasis place a major role in majority of ailments. Amla inhibits the growth and spread of various cancers like breast, uterus, pancreas, stomach and liver cancers.

Other applications includes

- Antiviral and cardio protective
- Antioxidant and anti-proliferative
- Best regulation of glucose level
- Inhibits oxidation of DNA.
- Suitable for using skin anti-aging.
- Enhancing natural killer and cell activity

Medicinal uses

Radio protective activity, antibacterial activity, antifungal activity, antioxidants and free radical scavenging activity, insectidal activity, hypolipidemic activity, antidepressant activity, anti-inflammatory activity, anti diabetic and hypoglycaemic activity.[23]

It can be used for the management of dementia, Alzheimer's disease, Parkinson's disease due to its anticholine esterase activity. Amla also has antioxidant and anti-inflammatory property. It fight against free radicals and inhibits the inflammatory mediators to reduce brain damage and improve cognition.[24]

Dose

Diabetes/hyperlipidemia:1to3gofpowdered,driedfruitwas consumed daily in 30ml of water for 21days in 1 clinical trial. Another trial used amla 300 mg tablets (each containing 50% amla extract and 50% dextrin) 3 times per day. Hypercholesterolmia/hyperlipidemia: 500 mg standardized extract of *Phyllanthus emblica* twice daily.

Contraindication

- Contraindications have not been identified.
- Pregnancy/Lactation: Information regarding safety and efficacy in pregnancy and lactation is lacking.

Experimental Methods

Collection of plant material

The fruits of EO was collected and identified and authenticated by Tamil Nadu Agriculture University, Coimbatore India.

Extraction by Hot Decoction

The fruits of EO was first cut into small pieces, seed removed and dried completely without any moisture content present in it. The dried fruit was then pulverized and made into fine powder. About 75 g of the powder was taken in a beaker with 100 ml of distilled water. This was boiled to 1000 c for 15 minutes and stirred continuously. Then the solution was filtered and filtrate was taken for solvent evaporation process. This process was done for 24 hrs at 700 c till a dry powder extract of the fruit was obtained.[19]

Physical characteristics

Solubility studies

2mg of drug was dissolved in 5 mlof distilled water, ethanol and methanol.

Calibration of the EO extract:

The aqueous extract of EO was dissolved in water in a volumetric flask to get a concentration of 100µ g/ml. This was treated as stock solution. Various aliquots of stock solution were diluted further to get different concentrations. The resultant solutions were scanned for wavelength in the range of 273.7 nm using UV-spectrometer.[19]

Fabrication of EO loaded Gelatin nanospheres

The nanospheres of EO extract with gelatin polymer were formulated by solvent displacement method. In this process, 200mg of gelatin was dissolved in 10 ml of hot water at 500c. To this 50mg of dry extract of EO was added and dispersed. 100ml of non- solvent ethanol with water in the ratio of 65:35v/v was taken and stabilized with 7%w/v of Tween 80. To this drug loaded gelatin polymer solution was added in drops under continuous stirring in a magnetic stirrer at 500rpm for 2hrs. This was followed by addition of 1ml of formaldehyde solution as a cross linking agent of gelatin nanoparticles. The drug loaded gelatin nanoparticles were centrifuged at 15000 rpm for 30 minutes, washed twice with deionized distilled water for 90 minutes and lyophilized.[19]

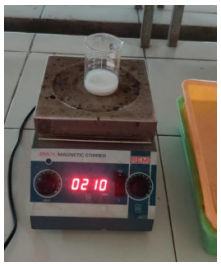

Figure2: Fabrication of EO loaded Gelatin nanospheres

Table1: Formulating of EO loaded gelatin nanospheres

S.No.	Formulation code	Drug (mg) EO extract	Gelatin (mg)
1	F1	50	50
2	F2	50	100
3	F3	50	150
4	F4	50	200

This process was performed to formulate the nanospheres in different comparison. A blank nanosphere containing the polymer and surfactant (without drug) was also formulated for comparison.

Characterization of Nanospheres

Drug polymer interaction studies

A few mg of dried extract of EO and gelatin were mixed with 100mg of KBr [dried at 400-500°C]. The mixture was taken and compressed under 10- ton pressures in hydraulic press to form a pellet and was scanned from 4000-400cm^{-1} in IR spectrometer.

Determination of percentage yield

EO loaded gelatin nanospheres were weighed after freeze dried.

$$\text{Percentage yield} = \frac{\text{Practical weight of nanospheres obtained}}{\text{Theoretical weight [drug + polymers]}} \times 100$$

Scanning electron microscopy

Particle morphology and particle shape were examined by scanning electron microscopy [SEM]. SEM analysis was performed to determine their microscopic characters [shape and morphology] of prepared EO nanospheres. Nanospheres were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy [Hitachi X650, Tokyo, Japan] in different magnifications. Samples were placed on glass slide kept under vacuum and then by using sputter coater unit, samples were coated with a thin gold layer, operated at 15 Kv acceleration voltage.[19]

Particle size and surface charge analysis of the nanospheres

The nanospheres of EO formulated were analysed by a zeta analysis [Malvern nanosizer ZS ,UK] to verify for the particle size based on dynamic light scatteringtechniques and the zeta potential based on charge conductivity principle to ensure the uniformity of size distribution and the stability of the formulation respectively.[19]

Entrapment efficiency of the polymeric herbal nanospheres and percentage drug loading.

The nanospheres suspensions formulated with extract and gelatin was ultra centrifuged at 18000 rpm for 30 mins and then the supernatant solution was diluted suitably to measure the absorbance from which the concentration of drug in supernatant was calculated using the standard calibration graph. The entrapment efficiency of EO in gelatin nanospheres was calculated using the formula.[19]

$$\% \text{ entrapment efficiency} = \frac{\text{Total drug content-drug content in supernatant}}{\text{Total drug content}} \times 100$$

Entrapment efficiency and drugloading were calculated as follows:

$$\text{Drug loading} = \frac{\text{Total drug content-drug content in supernatant}}{\text{Total weight of nanospheres}} \times 100$$

In vitro release studies

A volume of 2.0 ml of nanosphere suspension was enclosed in dialysis bag (cellulose membrane, molecular weight cut off 12400) and incubated in 90ml of distilled water was used as the release medium slightly agitated with magnetic stirrer. Predetermined time intervals, 500µl samples were withdrawn from the incubation medium and analysed for drug by UV-spectroscopy at 273nm. After sampling, 500µl of fresh medium was added in the incubation medium.[25]

In vitro antioxidant activity

100 mg of EO gelatin nanospheres [100µg] was blinded with 0.5 ml of DPPH solution [250µg in methanol] and 1ml of 0.1 ml of acetate buffer and final volume was made up to 3ml with methanol. The reaction mixture was shaken thoroughly and kept in dark for 30 mins at 27±20c. Then the absorbance was measured at 515nm using UV visible spectrophotometer. The reaction mixture without nanospheres was used as control and ascorbic acid was used as standard. *In vitro* antioxidant activity can be formulated using this formula,[26]

$$AA\% = \frac{1-A_{ts}}{A_c} \times 100$$

where, Ats- Absorbance of test samples,

Ac—without nanospheres respectively

Results and Discussions

Physical characteristics

EO dried extract was checked for its colour, odour and texture. The pulverised EO extract was light brown coloured powder, with pungent odour and smooth texture.

Solubility studies

Table2: Solubility test of EO in varioussolvents

S.No.	Solvent	Solubility
1	Water	Soluble
2	Ethanol	Soluble
3	Methanol	Soluble

Construction of calibration curve of Emblica officinalis

In the calibration curve, linearity was obtained between 2-12µg/ml concentration of EO and the regression value was found to be r^2=0.9996. Hence we can conclude that EO obeys Beer Lamberts Law at the concentration 2-12 µg/ml. The results are shown in table and figure.

Table3 Calibration data

S.No	Concentration(µg/ml)	Absorbance at 273nm
1	0.0200	0.0714
2	0.0400	0.1429
3	0.0600	0.2199
4	0.0800	0.2807
5	0.1000	0.3552
6	0.1200	0.4272

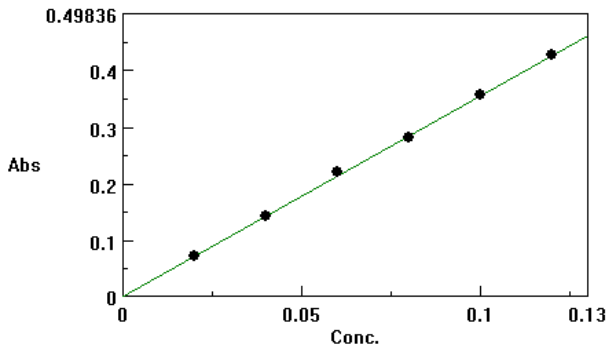

Figure 3: Calibration graph

Drug excipient interaction studies

Fourier Transform Infrared [FT-IR] spectra of the samples were obtained using a FTIR Jasco 4100 spectrometer by KBr disc method.

Fabrication of EO loaded gelatin nanospheres by solvent displacement method:

The F1,F2,F3,F4 were formulated using solvent displacement technique and formaldehyde was used as a cross linking agent.

Percentage yield analysis

The percentage yield of the EO loaded gelatin nanospheres was found to be 80% given for analysis.

Scanning Electron Microscopy

SEM analysis was used to evaluate the surface morphology of nanospheres.

Particle Size Measurement

The particle size is one of the most important parameters for the characterisation of nanoparticles. The average particle sizes of the prepared EO nanospheres were measured using Malvern zeta sizer.

Determination of zetapotential

Zeta potential was determined using Malvern zeta sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability.

Entrapment efficiency

Table 4: % of Entrapment efficiency and % of Drug loading

S.No	Formulation code	% entrapment efficiency	% Drug loading
1	F1	40.61±0.08	50.33±2.58
2	F2	50.42±0.59	43.12±1.21
3	F3	68.5±0.23	32.61±1.8
4	F4	80.21±0.47	18.79±2.3

n=3

The entrapment efficiency of drug depends on polymer concentration. As the polymer concentration increases % Entrapment efficiency increases % Drug loading decreases. So F4 had high %Entrapment efficiency and low% drug loading & F1 had low % Entrapment efficiency whereas high % drug loading.

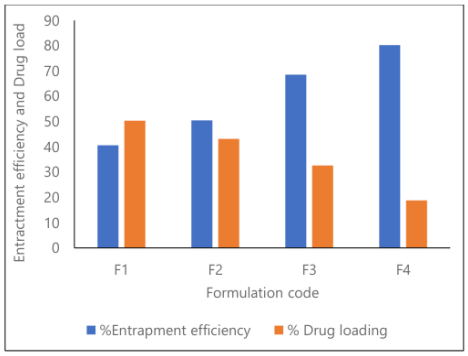

Figure4: Percentage Entrapment efficiency and % Drug loading

In vitro release studies

The release of the drug from gelatin nanospheres prepared from different concentration of polymer was compared. F1,F2,F3,F4 showed 62%, 37%, 23%, 12% release respectively at the end of 6th hour. All the formulations showed that was some initial burst release and this depends upon the ability of the polymer matrix to encapsulate the drug making it unavailable for immediate release and thus could be useful in controlling the release. The burst release does not depends upon the encapsulation efficiency alone but also depends on polymer hydrophilicity. As gelatin is hydrophilic it facilitates water uptake and cause initial burst release.

Figure 5: In vitro release Cumulative percentage release studies

Table 5 Cumulative % release

Time (mins)	Cumulative Percentage Release			
	F1	F2	F3	F4
0	0	0	0	0
15	10	5	4.1	2
30	13	6	4.9	4
45	15	8	6	5
60	19	10	7.3	6
120	32	12	8.5	6.8
180	41	14	10	7.2
240	49	18	12	8.5
300	56	22	14	10
360	62	37	23	12

F2 was found to have a satisfactory release at the end of 6th hour. So it was selected for further pharmacological studies.

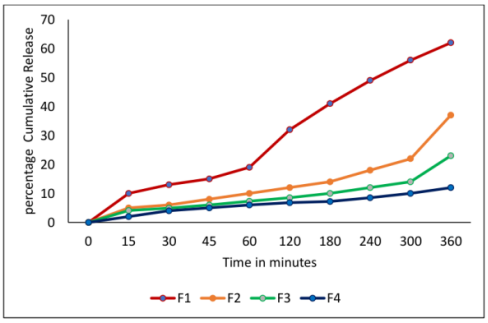

Figure 6: Cumulative % release

In vitro antioxidant activity

The DPPH method [2,2-diphenyl-1-picrylhydrazyl] is based on the capture of the DPPH free radical by antioxidants producing a decrease in absorbance at 515nm wave length when DPPH radical reacts with an antioxidants compound which can donate a hydrogen atom, it is reduced to stable DPPH-H molecules. This reduction is accompanied by the colour change from purple to yellow, leading to decrease in absorbance. This result aids in making EO gelatin nanospheres to treat Huntington's disease as most of the research reports say that oxidative stress is one of the cause of the progression of Huntington's disease.

Table 6 Antioxidant activity

S.No	Concentration of samples (µg/ml)	Antioxidant activity	
		Ascorbic acid (standard)	F2 (test sample)
1	100	0.0336±0.02	0.307±0.004

n=3

Summary and Conclusion

Neurodegenerative disease are debleating disorders that primarily affect the neuron cells and they degenerate the brain and central nervous system. The symptoms of neurodegenerative disease often progress slowly over the years with complete disabilities or death. Huntington's disease is fatal neuropsychiatric degenerative disease with a prevalence in India similar to that of west and more than any other Asian countries. Till date no cure has been found to stop the progression of the disease.

Drug delivery to the CNS has several draw backs in which BBB is the main drawback for treatment of neuro degenerative disease. Current treatments have considerable side effects thus nano formulation of herbal extracts are effective strategics to overcome such problems and to target the drug to brain. In the present research work an attempt has been made to fabricate *Emblica Officinalis* loaded gelatin nanospheres to treat Huntington's disease.

Pre formulation studies were carried out to find the solubility of Eo and its physical characteristics. FTIR and

UV spectral studies authenticate that there was no polymer drug interaction and absorption peak at 273nm respectively. Biodegradable gelatin nanospheres of *Emblica Officinalis* (F1-F4) were prepared with different concentration of polymer by solvent displacement technique.

The prepared EO loaded gelatin nanospheres were characterised for its surface morphology, particle size and zeta potential. Entrapment efficiency ranged from 40.61%to80.21%and drug loading ranged from18.79%to50.33% and this revealed that EO was entrapped well inside gelatin nanospheres.

In vitro release studies of EO loaded gelatin nanospheres showed initial burst effect. The polymer concentration was found to influence the % entrapment efficiency and drug loading. The release of F2formulation was found to be satisfactory as it showed37% release at theendof6th hour and 10% release at end of 1st hour. The development of EO loaded gelatin nanospheres was found to be very successful in providing linear sustained release with initial burst release due to swelling of hydrophilic biodegradable gelatin. Because of substantial evidence of antioxidant activity of the prepared EO loaded gelatin nanospheres were studied for its potential antioxidant effect using DPPH assay.

The result showed EO loaded gelatin nanospheres possess good antioxidant effect and this could makes EO gelatin nanospheres as a suitable candidate for delaying the progression of Huntington's disease.

The validity of the present investigation needs to be confirmed with its reproducibility, optimization of the formulation factors and with *in-vivo* studies.

Acknowledgement:

Authors are thankful to Pannai College of Pharmacy, Tamilnadu, India and The Tamilnadu Dr.M.G.R. Medical University, Chennai, Tamilnadu, India

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