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Original Article

Mucuna Pruriens Seeds Extract Loaded Phytosomal Intranasal Gel for the Effective Treatment of Parkinson's Disease

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ABSTRACT

One phytoconstituent derived from Mucuna pruriens (legumes) is levodopa. This medication's oral usage is limited by its high first-pass metabolism and low absorption. The goal of the present research was to develop a phytosomal gel filled with levodopa extract for better delivery and brain targeting. Various techniques, including solvent evaporation, salting out anti-solvent precipitation, direct egg yolk, and egg lipids methods, were used to create phytosomal formulations. Scanning electron microscopy, particle size, x-ray diffraction, and other techniques were used to characterize phytosomes. And added into gel formation, the more successful batch was examined for several parameters. The final batch underwent a variety of animal tests, including pharmacokinetic analysis, irritation to the nasal cavity testing.

The most effective phytosomes were those made via the antisolvent precipitation approach. In this investigation, a 3^2-randomized complete factorial design was employed. Batch F4 had an entrapment efficiency of 70%, a particle size of 15 (µg) and 60% CDR. The gel-formulated batch F4G3 demonstrated improved results in terms of extrudability (90.82), amount of drug (89.32%), viscosity (5421 cps at 100 rpm), and spreadability (25.18). Batch F4G3 of the Mucuna pruriens phytosome gel exhibited Higuchi's kinetics. According to the findings of the animal study, dopamine levels were significantly elevated. The pharmacokinetic and nasal irritation studies showed notable in vitro penetration of the nasal mucosa without resulting in skin irritation. For improving Parkinson's disease treatment, the phytosomal gel formulation delivered via the nasal route would be the ideal option.

Keywords: L-Dopa extract, Phytosome, gel, *Mucuna pruriens,* Nasal gel, Optimization.

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BACKGROUND

Drugs of origin from nature are significant and vital components of contemporary medicine. as they are occurring in nature, herbal remedies have fewer adverse consequences, which is why many traditional medical practitioners and scientists utilize them to treat conditions including diabetes, cancer, rheumatoid arthritis, inflammation, and several more [1-2]. These products are made to provide specific medical advantages using different ways to deliver drugs (DDS). According to reports, these unique drugs delivery methods provide remarkable benefits over traditional formulations of plant extracts or active ingredients. Improvements in solubility, bioavailability, toxicity, pharmacological activity, stability, tissue distribution, prolonged administration, and protection against physical/chemical deterioration are some of the factors that may be considered. [3]

Phytosome for herbal drug delivery

The reaction between phosphatidylcholine and phospholipids results in lipid-compatible molecular complexes known as phytosome, which are complexes of phospholipids and naturally occurring potent phytochemicals that are bound in their structures.[4-5] It mostly consists of the lipid-bound, bioactive phytoconstituents of the herb. Polar or water-soluble substances constitute most organisms' physiologically active components. However, water-soluble phytoconstituents (such as flavonoids, tannins, glycosidic aglycones, etc.) have poor absorption because of their large molecular size, which hinders passive diffusion, or because they are poorly soluble in lipids, which greatly limits their ability to cross lipid-rich biological membranes, resulting poor bioavailability [5,6,7,8]

Mechanism of Nose-to-Brain Drug Delivery

The nasal mucosa has emerged as a useful site targeting tissue for drug delivery resulting from its accessibility, high blood flow, large surface area, porous endothelial membrane, and ability to avoid hepatic first-pass metabolism.[9] The transport has been reported to involve the combination of the bio-fluids from the vasculature and lymphatic system to be responsible for the transport of bioactive agents from the nasal cavity to the brain. Iintranasal delivery is an alternative administration route for levodopa (L-Dopa) to the brain. It offers high drug permeability across the nasal epithelium and rapid absorption into the central nervous system (CNS) while bypassing first-pass metabolism.[10]

Parkinson's disease and its treatment

Parkinson's disease is a brain disorder that causes unintended or uncontrollable movements, such as shaking, stiffness, and difficulty with balance and coordination. Symptoms usually begin gradually and worsen over time. As the disease progresses, people may have difficulty walking and talking. They may also have mental and behavioral changes, sleep problems, depression, memory difficulties, and fatigue.

Mucuna pruriens is a species of bean that grows in the tropics. It is very rich in natural L-Dopa. Mucuna seed extract has been an effective treatment of

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Parkinson's disease (PD) in many patients.[11] Mucuna seeds are known to produce the unusual nonprotein amino acid 3-(3,4 dihydroxyl phenyl)-l-alanine (l-Dopa), a potent neurotransmitter precursor that is, at least in part, believed to be responsible for the toxicity of Mucuna seeds.[13] One of the causes of neurodegeneration is a disturbed balance between reactive species of oxygen and cellular activity showing an antioxidation effect.[14] It includes Alzheimer's disease (AD) and Parkinson's disease (PD). In Parkinson's disease, there is a loss of dopaminergic neurons in the part of the brain known as substantia nigra with the formation of Lewy bodies.

Need of present investigation and plan of the work

Macuna pruriens seeds have been reported to contain about 8-9% L-Dopa which is known to be active for the treatment of neurodegenerative disease particularly by loss of dopaminergic neurons. Therefore, a systematic probe is necessary to evaluate the synergistic effect of the active moiety present in the herbs.

In the present investigation, we have attempted to prepare the whole extract at a suitable pH ranging from 3-4 in the aqueous solvent system while the organic extracts are attempted in a series of solvents called n-Hexane in various proportions. Thus, one fraction being polar and another fraction nonpolar was a serious challenge for providing a suitable interface for its release and absorption. On the other side for neurodegenerative diseases, the site of action is located somewhere in the brain perhaps beyond Blood Brain Barrier (BBB).[14] Similarly, nasal sites of administration for curcumin and L-Dopa were considered.

To resolve the above complexity suitable nanocarrier system was designed for L-Dopa as the active moiety has been derived from plant material. Phytosome seems to be the appropriate option as a carrier for drug moieties. After having optimized and designed phytosome for the said active moieties the site of administration connected to the brain/ nearest to the brain was opted to be nasal mucosa.

The nasal drug delivery system has its limitations and constraints which could be resolved by formulating suitable aqueous nasal gel.

Finally, the efficacy of the nanodrug delivery system was evaluated *in vivo/in vitro* model which included pharmacokinetic studies, and animal model studies. It was preceded by a preliminary investigation of nasal gel which included the study of nasal irritation, histopathology, dopamine estimation, etc.

EXPERIMENTAL

Authentication of *Plant*

The plant specimen used for the research work was collected from the forest of Kusgaon Bk. Lonavala, Pune, Maharashtra, India. The plant specimen was authenticated by the Botany Department at Agharkar Research Institute, Pune, India.

Pharmacognostic Characterization

Pharmacognostic studies of the plant were conducted by the standard prescribed method, which includes macroscopic, microscopic, and other characteristic properties.

Preparation of the Extract

L-Dopa extract

Different methods and solvent systems were used for the extraction of L-Dopa from Mucuna Pruriens seed powder. The pH of the solvent blend played a significant role.

a) Seed powder was defatted with acetone and then suspended in water: ethanol (1:1) for 3 days at room temperature. Then maceration technique was used for extraction. The extract was passed through a suitable filter medium.[11]

b) A significant amount of seed powder was mixed with a stipulated volume of acidified water and followed by ultrasonication at 250 rpm at room temperature. The extract was passed through a suitable filter medium.[15] The yield obtained was around 5-6 % w/v.

Preliminary Phytochemical analysis of the extract of Levodopa

The extracts were concentrated and subjected to phytochemical screening using standard procedures. Alkaloids, glycosides, flavonoids, tannins, proteins, carbohydrates, amino acids, and steroids are the preliminary phytoconstituents that were analyzed.

Standardization of Extract: L-Dopa

Thin Layer Chromatography TLC[16]

The presence of L-DOPA in the test samples was confirmed by comparing the TLC pattern of testing seeds and the standard L-DOPA using an optimized solvent system; butanol: acetic acid: water (4:1:1). After elution with appropriate solvent, the TLC plates were air dried for 10 min, sprayed with a 0.1% solution of ninhydrin in methanol, and dried at 105 °C for 5 min. The *Rf* values and brownish-pink spots revealed the presence of L-Dopa

High-Performance Liquid Chromatography (HPLC)

HPLC conditions

Column: Lichro CART Purosphere STAR, 4.6 x 250 mm, Rp 18e, 5µm

Mobile Phase

Solvent A: consisted of a 30 mM phosphate buffer pH 2.50 (20 mM sodium dihydrogen phosphate dihydrate and 10 mM disodium hydrogen phosphate dihydrate). The pH of the solvent was adjusted with orthophosphoric acid.

Solvent B: was composed of a mixture of water that before mixing had been adjusted to pH 2.50 with orthophosphoric acid and acetonitrile (50:50, v/v). Gradient Elution: HPLC analyses were carried out by applying the gradient conditions [17-18]

High-performance thin layer chromatography (HPTLC)

The HPTLC plates were developed in a saturated twin trough chamber using a mobile solvent phase of n-butanol: glacial acetic acid: water (4:1:1, v/v) for 30 min up to 8 cm and then air dried. After chromatographic development, the peak areas of the individual bands were measured at 280 nm UV using a Camag TLC Scanner 3 integrated with WinCATS software at a slit width of 5 × 0.45 mm. The analysis was done in duplicate and the average was taken for L-dopa estimation. A stock solution of L-dopa standard (0.1 µg/µL) was used in different concentrations (100 ng/spot - 1000 ng/spot) for preparing a calibration graph of peak area versus concentration.[19-20]

Identification of Levodopa in the extracted sample by Fourier Transformed Infrared Spectroscopy (FTIR)

To adapt the conventional KBr disc sampling technique for quantitative determination, the thickness of the KBr disc must be kept constant (to obtain the same path length) for all the analyzed samples. The desired drug quantity was obtained from the 2.0 g% (w/w) drug/KBr mixtures and ground in agate mortar for 2 min, completed to 0.15 g with KBr, then ground again and pressed under 15,000 lbs by the hydraulic pressure system in the die-press for 3 min. The FTIR of the sample was compared with the FTIR of the standard compound.

Preparation of Phytosome [21]

The phytosome were prepared for L-dopa extract by following methods

a) Solvent evaporation method [22-23]

Different molar concentrations of extract and Phosphatidylcholine (1:0.5, 1:1, and 1:2) were taken in the beaker. 50 ml methanol was added. Then the mixture was taken in round bottom flask and evaporated in rotary vacuum at 60^0 C. prepared phospholipids complex washed with N- Hexane with continuous stirring. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant phospholipids complex was placed in an amber-colored bottle stored at room temperature.

b) Salting out anti-solvent precipitation method [22]

Different molar concentrations of extract and Phosphatidylcholine (1:0.5, 1:1, and 1:2) were taken in the beaker with 20 ml methanol refluxed at 60^0 C temperatures for 02 Hrs on a magnetic stirrer at 100 rpm. The solution was later concentrated and anti-solvent like n-hexane was added. The resultant phospholipids complex was placed in an amber-coloured bottle stored at room temperature.

c) Direct egg yolk method

The egg yolk was separated from the egg. The aqueous extract was heated and 50 ml of which was added to 20 ml of egg yolk with homogenization at 250 rpm for not less than 20 min. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried.

d) Egg Lipid method:

20 ml of egg yolk was added into 50 ml of ethanol and 30 ml of ethyl ether. The mixture was stirred continuously. Then 10 ml of extract was added into it with homogenization for 20 min. The Extract–phospholipids complex was precipitated and the precipitate was filtered and dried.

Optimization of the method used for the preparation of phytosome

Phytosomes thus prepared for L-dopa were characterized as follows:

a) Scanning Electron Microscopy (SEM)

The surface morphology of the Phytosome was investigated by scanning electron microscopy. The study provided a better understanding of the morphological characteristics of the Phytosome[24]. The morphology of the phytosome was determined using scanning electron microscopy (Hitachi S-3700N). SEM gives a three-dimensional image of the globules. One drop of phytosome suspension was mounted on a clear glass stub. It was then air-dried and gold-coated using sodium aurothiomalate to visualize under a scanning electron microscope. The studies were carried out at Agarkar Research Institute, Pune.

b) Entrapment efficiency [25]

The entrapment efficiency of phytosome was determined by centrifuging 2 mL of the phytosome formulation at 1500 rpm for 30 min at room temperature. The supernatant was taken carefully using a pipette. Pure supernatant was then dissolved in ethanol to disrupt the vesicles and appropriate dilution was made and measured using a UV spectrophotometer.

c) Selection of method and optimization of Design of Phytosome

Based on the results obtained from Scanning Electron Microscopy and Entrapment Efficiency, the salting out anti-solvent precipitation method was found to be a better method than other tried techniques for the preparation and design of Phytosome. The process parameters like temperature and rotating speed were optimized for better results.

d) Optimization of parameters of the method selected (salting out anti-solvent precipitation method)

The optimum temperature required to form Phytosome was found to be 60^0C

The optimum Rotating Speed required to form a Phytosome was found to be 500 rpm.

Characterization of Phytosome (prepared by Salting out anti-solvent precipitation method): the various batches of phytosome prepared for L-Dopa extract and were subjected to characterization as follows:

a) Surface morphology

The morphology of the phytosome was determined using scanning electron microscopy (Hitachi S-3700N). SEM gives a three-dimensional image of the globules. One drop of phytosome suspension was mounted on a clear glass stub. It was then air-dried and gold-coated using sodium aurothiomalate to visualize under a scanning electron microscope.

b) Zeta potential [26]

Zeta potential was determined using a zeta sizer (HORIBA SZ-100). Measurements were performed for the samples prepared. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion system.

For the measurement, 1ml of the sample was diluted to 10ml with water, 5ml of this diluted sample was transferred to a cuvette, and the zeta potential was measured

c) Particle Size Determination

The mean size of the Phytosomal suspension was determined by photon correlation spectroscopy. The experiments were carried out using a 4.5mW laser diode operating at 670nm as a light source. Size measurements were carried out at a scattering angle of 90^0C.To obtain the mean diameter and polydispersity index of colloidal suspensions, a third-order cumulative fitting correlation function was performed by a Malvern PCS sub-micron particle analyzer. The samples were suitably diluted with filtered water-methanol mixtures at the same ratio used for phytosome preparations and those were placed in a quartz cuvette to avoid the multi-scattering phenomenon.

d) XRD (X ray Diffraction Techniques)

Samples of the prepared phytosomes were examined using Shimadzu XRD -6000 (X-Ray Diffract meter SERIAL NO. Q30344700643CZ, Tokyo, JAPAN). The measurements were carried out using Cu as an anode material and operated at a voltage of 25 KV with a current of 40 mA. The samples were analyzed in the 2 Theta angle ranges of 4 to 50 degrees and a scanning speed of 1.2degree/min.2

e) Drug Entrapment Efficiency and % Drug Loading

The vesicles were separated in a high-speed cooling centrifuge at 10000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using a UV visible spectrophotometer at 624 nm for L-Dopa From this, the entrapment efficiency was determined by the following equation, EE% = (Total drug) - (free drug) X 100 /Total drug.

Drug loading was determined by % Drug Loading = weight of Entrapped drug X 100 /weight of formulation. Based on the results obtained for L-dopa extract phytosomal formulation batch LEF3 (extract: Phosphatidylcholine: cholesterol in the ratio of 1:2:0.25) was further explored for optimization studies.

Optimization of formulation: After having characterized the phytosome of Macuna pruriens extract was further optimized for finalizing the formulation

A 3^2-randomized full factorial design was used in the study. In the design 2 factors identified were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations shown in the table. Percentage of Phosphatidylcholine (X1) and Percentage of Cholesterol (X2) were selected as independent variables. Entrapment efficiency (Y1), and in-vitro drug release (Y2), were selected as dependent variables. The dependent variables were selected based on the fact that the release rate would be directly affected due to an increase in the bond strength (Extract and Lipid) with an increase in the concentration of both excipients and with an increase in the concentration of Phosphatidylcholine and Cholesterol there would be more availability of sites for bonding with lipid and increase in the stability due to cholesterol that will directly affect Entrapment efficiency. The more the entrapment efficiency more will be the economical method. And if the release rate is slowed down that will reduce the dosing frequency. Formulation (Batch F5) containing 2 %w/v of Phosphatidylcholine and 0.5% w/v of Cholesterol was selected as the optimized formulation. The optimized formulations were further ensured by in vitro release and entrapment efficiency.

a) In-vitro drug release studies of optimized batches:

The release tests were performed according to the USP II paddle method at a speed of 50 rpm using 500 ml of SNEF as a dissolution medium at 35°C ± 0.5°C. Aliquots of 1 ml were withdrawn at time intervals of 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min and each aliquot were replaced by 1 ml of fresh SNEF. The samples were measured spectrophotometrically as mentioned earlier.

b) Entrapment Efficiency of optimized batches:

The entrapment efficiency of the Phytosome was determined by centrifugation. The vesicles were separated in a high-speed cooling centrifuge at 10000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using a UV visible spectrophotometer. From this, the entrapment efficiency was determined by the following equation, EE% = (Total drug) - (free drug) X 100 /Total drug

Based on the results obtained for in vitro drug release and entrapment efficiency during the optimization study batch PLEF5 (Phytosomal levodopa extract batch code F5 with 2% Phosphotidylcholine and 0.50% cholesterol) was further transferred into gel as dosage form.

Preparation of Gels: The optimized batches were further transferred into nasal gel as a dosage form

The gels were prepared by dispersion method using Carbopol 940 in different ratios. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was carefully drop wise addition of triethanolamine, followed by adding glycerol was added to gel to balance its viscosity. Finally, the optimized phytosomal dispersion was incorporated in the gel by continuous mixing to form gel. Methylparaben was added as a preservative. The prepared gels were filled in amber colored glass vials and stored at 4-8°C.

Characterization of Phytosomal Gels

a) Physical Evaluation

Physical parameters such as color and appearance of the herbal gel were observed manually.

b) Measurement of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done.

c) Spreadability

Spreadability was determined by the modified apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped of from the edges. The top plate was then subjected to pull of 80 gms weight with the help of string attached to the hook and the time (in seconds) required by the top slide to cover 7.5 cm was noted. A shorter interval indicates better spreadability.

d) Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle No.64 at 50, 60 &100 rpm at room temperature. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer manual.

e) Drug content:

For Quantitative determination of drug, we have opted two methods UV/VIS spectrophotometer and HPLC technique. But for providing total concentration in the extract AUC (area under curve) is most reliable method, however for finding single unknown concentration in the various studies UV/VIS spectrophotometer method was found to be more useful. The purpose of using spectroscopic method is not to replace the available methods but to use as an alternative method where advanced instruments like HPLC and GC are not available for routine analysis. Hence to determine drug content UV/VIS spectrophotometer method was used with following treatment done to formulation.

Drug concentration in Gellified Phtytosome was measured by spectrophotometer. Levodopa content in Gellified Phtytosome was measured by dissolving known quantity of Gellified Phtytosome in solvent (Ethanol) by sonication. Absorbance was measured after suitable dilution at 624 nm by using UV/VIS.

f) Extrudability Study of Topical Gel [27]

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by wood. In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented

g) Homogeneity [28]

All the developed gels were tested for homogeneity by visual inspection after setting the gels in the container. They were observed for their appearance and presence of any aggregates

h) Washability

Small amount of gel was taken and applied on the hand and was washed with tap water and observed visually.

Ex-vivo Drug Diffusion Study [29]

The freshly excised sheep nasal mucosa, except septum part was collected from a local slaughter house. The superior nasal membrane was identified and separated from the nasal cavity and made free from adhered tissues. Maintaining the viability of the excised nasal tissues during the experimental period is crucial. Within 10 min of killing the animal, the mucosa was carefully removed, then immediately immersed in phosphate buffer saline solution pH 6.4 for 15 min and was aerated. The membrane was mounted in between the donor and the receptor compartment of the diffusion cell. The position of the donor compartment was adjusted so that the mucosa just touches the permeation medium. Formulation equivalent to 100 mg gel was taken in the donor compartment which was in contact with the mucosal surface of the membrane, while the receptor compartment was filled with 20 ml of SNEF and its temperature was maintained at 37 °C. The content of the receptor compartment was stirred using a magnetic stirrer. An aliquot of 1 ml was withdrawn at suitable time intervals and replaced with the same volume of fresh medium. Absorbance was measured after suitable dilution at 624 nm for L-Dopa by using UV/VIS spectrophotometer (Shimadzu/UV-2600, Japan).

In vitro Pharmacokinetic study [30]

Drug release was monitored by the USP dissolution test apparatus type II A dialysis tube containing 1 ml gel formulation was immersed in 500 ml of SNEF as a dissolution medium at 35°C ± 0.5°C and rotation at 50 rpm. Aliquots of 1 ml were withdrawn at time intervals at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 min and each aliquot were replaced by 1 ml of fresh SNEF. Absorbance was measured after suitable dilution at 624 nm for L-Dopa by using UV/VIS spectrophotometer (Shimadzu/UV-2600, Japan).

Based on the results obtained from above mentioned evaluation parameters batch LF3G3 (Gel formulation of Phytosomal extract of L-Dopa- 5% phytosomal suspension 2% Carbopol and 0.5% triethanolamine) was selected as final batches for further animal study.

Animal Study:

In the present study, the effects of the optimized nasal gel preparation containing L-Dopa was studied on dopamine levels in the wistar rats (350-400 gm). The animals were administered with at 2mg/kg dose by nasal route. The animals were administered with 25 microliters of gel sample by nasal route.

Estimation of dopamine level: Pharmacodynamic study

Preparation of Homogenate

Homogenates were poured in 15ml centrifuge tubes and rotated at 6000 RPM for 20 minutes at 4ºC. The Supernatant was removed in eppendorf tubes and labelled respectively. The dopamine (DA) was determined in the brain homogenates of subjected animals by ELISA assay as per the manufacturer's instructions.

Pharmacokinetic study of nasal gel containing levodopa in Wistar rats

All the groups of rats were subjected for blood withdrawal after test substance administration at various intervals of time. Under slight isoflurane anesthesia, blood 0.2-0.3 mL blood samples were withdrawn in the Eppendorf tubes. Once test compound was administered, the blood samples were withdrawn as per the following schedule in the heparinized/EDTA tubes. Blood samples were withdrawn by microcentrifuge tube (Tarson) at various time intervals o minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, and 48 hours Plasma separation will be done by the following procedure: All the tubes were allowed to stand for 30 minutes and centrifuged at 10000 RPM for 15 minutes at 4 0C (NEAUATION, India) for all remaining groups of the animals, for blood withdrawal, similar procedure will be followed. The plasma samples was separated and subjected for HPLC analysis**.**

Antiparkinsonian activity of test nasal gel formulation

Experimental Animals [31-32]

Healthy either sex, wistar rats (14-18 weeks old) of 350-400 gm, were divided into various groups (n=6). They were housed 3 animals per cage (polycarbonate cages) with normal animal house conditions as temperature 22-25 0C, 40-60 relative humidity and 12:12 light and dark cycle. Animals were fed with standard animal diet (Krishna Aggrotech Ltd, India) with water ad libitum

Parkinson's was induced in the animals by using rotenone and then the effect of gel formulation containing phytosomal extract of Mucuna pruriens administered through nasal mucosal route.

Behavioral evaluations:

All the behavioral evaluation was performed on weekly basis during the treatment regimen. Catalepsy [33] Number of crossing behavior by open field.[34] Muscle grip and postural instability (inclined test), Biochemical estimation On 31st day, after the behavioral evaluation, all the animals were sacrificed by CO2 euthanasia, brain was immediately removed and homogenized (10 % w/v) in phosphate buffer solution (pH 7.0), Homogenates were poured in 15ml centrifuge tubes and rotated at (Neaution, India) at 3000 RPM at 4 0C for 30. The Supernatant was removed in Eppendorf tubes and labeled respectively. The supernatant was separated and stored at -80 0C until used.

Result

Study of plant and extract

Plant authentication and Pharmacognostic evaluation results confirmed the identity of plant. Biological name of plants was confirmed as ***Mucuna pruriens*** (Fabaceae)

Pharmacognostic Characterization

Organoleptic Evaluation

Physicochemical Evaluation

Phytochemical screening:

Phytochemical screening confirmed the presence of phenolic compound in Macuna pruriens extract, which is an antioxidant considered as active moiety available in the plant material. However, the moieties have varying chemical structure reported as L-dopa.

Standardization of plant extract

Thin Layer Chromatography (TLC)

Rf value of Standard and Extract was found to be identical. Thus, the compound extracted is considered as L-Dopa

High-performance liquid chromatography (HPLC)

The HPLC technique was used to determine the quantity of drug in the extract. The area of peak was use to determine the same by using following formula:

Concentration of levodopa in % = Area of sample/area of standard x concentration of Standard

Table 1. Batch code.

Batch code	Variable levels in coded form	
	X_1	X_2
F1	+1	0
F2	-1	-1
F3	0	-1
F4	0	+1
F5	+1	+1
F6	+1	-1
F7	-1	0
F8	-1	+1
F9	0	0

Transformation of code levels in actual units

Variable levels	Low (-1)	Medium 0	High (+1)
Percentage of Phosphatidylcholine (X_1)	1	1.5	2
Percentage of Cholesterol (**X2**)	0.25	0.375	0.50

Table 2. Organoleptic Evaluation (Seeds of Macuna pruriens).

Sr. No	Macroscopic Characters	Seeds
1.	Shape	ovate, oblong, flattened
2.	Size	Length -0.8–1.29 cm, breadth- 0.6–0.9 cm, thickness -0.3–0.63 cm
3.	Surface	hard, smooth, glossy
4.	Colour	brown to black
5.	Weight	Upto 0.5 gm
Sr. No	**Macroscopic Characters**	**Powder**
1	Colour	Creamish green
2	Texture	Course
3	Odor	Odorless
4	Taste	Starchy- Slightly bitter

Table 3. Physicochemical constants of Macuna pruriens seeds.

Sr.No.	Parameters	Value % w/w
1	Total Ash	4.31
2	Acid insoluble ash	2.25
3	Water soluble ash	1.37
4	Water soluble extractives	18.25
5	Alcohol soluble extractives	11.84
6	Loss on drying	6.38

Table 4. Preliminary Phytochemical analysis of the extract of L-Dopa.

Sr. No.	Test	Presence (+)/ Absence (-)
1	Glycosides	+
2	Saponins	-
3	Oils and fats	-
4	Alkaloids	+
5	Steroids	+
6	Flavonoids	-
7	Proteins and free amino acids	+
8	Tannins and phenols	+

Table 5. Macuna pruriens extracts FTIR Results.

Sr.No.	Vibrational Frequencies	Present Functional group
1	3643	Aromatic OH Group
2	2357	Ammonium Group
3	1714	C=O Stretching, COOH
4	1556	Ring def. coupled OH bend
5	1236	Ring breathing
6	1211	Coupled X-H bending

Table 6. Batch Drug Entrapment Efficiency.

Formulation	Entrapment efficiency (%)
F_1	62.35±0.30
F_2	65.80±0.12
F_3	67.23±.0.41

Table 7. Different batches (F1-F9) with results (Drug Release in 8 Hr and % Entrapment Efficiency).

BATCH	% Phosphotidylcholine	% Cholesterol	% Drug Release in 8hr	% Entrapment Efficiency
F1	2	0.375	59±1.23	75±1.28
F2	1	0.25	80±2.62	50±2.63
F3	1.5	0.25	65±3.67	70±0.87
F4	1.5	0.5	60±0.29	68±0.52
F5	**2**	**0.5**	**58±1.45**	**80±1.25**
F6	2	0.25	58±2.3	78±3.14
F7	1	0.375	70±0.28	55±0.36
F8	1	0.5	60±3.14	68±0.87
F9	1.5	0.375	59±0.69	62±1.027

Table 8. Different Batches of Phytosome Gel (Macuna pruriens extract).

Formulation Code	Phytosome Suspension (ml)	Carbopol 940 (%)	Triethanolamine (%v/v)	Methylparaben (%)	Water (upto 30gms)
LF5G1	5	1	0.5	0.01	q.s
LF3G2	5	1.5	0.5	0.01	q.s
LF3G3	5	2	0.5	0.01	q.s

Table 9. pH readings for all Batches.

Batch	pH
LF5G1	6.3+0.23
LF5G2	6.3+1.01
LF5G3	6.5+0.57

Table 10. % Extrudability.

Formulation	% Extrudability
LF5G1	94.9
LF5G2	90.62
LF5G3	91.81

Table 11. Viscosity Study.

Gel formulation	Viscosity	
	Max100	Min 50
LF5G1	5244	620
LF5G2	3230	570
LF5G3	5690	700

Table 12. Calibration curve of the Drug.

Sr. No	Concentration(µg/ml)	Absorbance
1	0	0
2	2	0.1125
3	4	0.2156
4	6	0.3563
5	8	0.5123
6	10	0.6020

Table 13. Drug content of gel formulation.

Sr. No	Batch	Drug Content in percentage
1	LF5G1	85.20
2	LF5G2	87.85
3	**LF5G3**	**89.32**

Table 14. **Cumulative amount of drug diffused from Phytosomal Gel.**

Time (Hrs)	% CDD of Plain Gel	% CDD of Phytosomal Gel ()
0	0	0
1	16.32±2.010	18.35±1.02
2	21.20±0.23	28.87±0.28
3	34.36±1.002	42.72±2.05
4	38.41±1.52	48.95±0.64
5	41.52±2.36	52.32±3.09
6	45.20±3.07	59.25±1.007
7	48.80±058	68.95±2.30
8	54.37±072	70.85±0.32
10	58.32±1.25	72.52±2.68
12	61.27±0.74	80.23±0.28

Table 15. **Kinetics Release studies for the best fit model.**

Phytosomal Formulation Code	Zero Order Kinetics	First Order Kinetics	Higuchi Kinetics	Peppas Kinetics	Best Fit Model
	r	R	R	r	
F3G2	0.9135	0.9224	0.9844	0.8857	Higuchi

Table 16. **Weights of animals.**

Animal No.	Test Compound	Weight of Animals
	Normal Control	214
	Normal Control	235
	C1	229
	C2	208
	C3	219
	C4	243
	L1	237
	L2	221
	L3	249
	L4	203

Table 17. **Standard values Concentration Vs Optical Density.**

Concentration (ng/ml)	Optical Density at 450 nm
0	0.046
0.3	0.521
0.6	0.81
1.2	1.235
2.4	2.111
4.8	3.319

Table 18. **Effects of levodopa gel on dopamine contents in brain homogenate in rotenone induced Parkinson's disease in rats.**

Particular Treatment and dose	Dopamine Concentration (ng/mL) (Mean±SEM)
Normal Control	0.525±0.06
Disease control	0.129±0.01$^{\#\#}$
Intranasal levodopa gel	0.332±0.02*

$^{\#\#}p<0.0001$ when compared to normal control animals of respective day; ***$p<0.0001$, **$p<0.001$, *$p<0.01$, when compared to Disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test

Table 19. For Standard (Levodopa).

S. No.	RT of Levodopa	Area of Levodopa
1	4.313	801225
2	4.353	802511
3	4.345	804722
4	4.366	802594
5	4.317	805871
Avg.	4.3388	803385
SD	0.023026	1872.36
RSD%	0.530701	0.233059

Table 20. Pharmacokinetics estimation for Levodopa – Test sample.

Levodopa TEST					
Dose	mg/Rat	4 mg			
Time Point	hr	Levodopa N	Levodopa N	Mean	SD
0.00	ug/mL	0.00	0.00	0.00	0.00
0.30	ug/mL	0.32	0.29	0.31	0.02
1.00	ug/mL	3.24	3.10	3.17	0.10
2.00	ug/mL	3.01	2.93	2.97	0.06
4.00	ug/mL	2.34	2.10	2.22	0.17
6.00	ug/mL	1.09	1.22	1.16	0.09
12.00	ug/mL	0.12	0.18	0.15	0.04
24.00	ug/mL	0.02	0.03	0.03	0.01
48.00	ug/mL	0.01	0.01	0.01	0.00
Cmax	ug/mL	3.24	3.24	3.24	0.00
Tmax	hr	1.00	1.00	1.00	0.00
AUC last	ug*hr/mL	16.67	16.67	16.67	0.00
AUC Infinity	h*ug/mL	16.74	16.74	16.74	0.00
CL	L/hr	0.24	0.24	0.24	0.00
Vd	L	1.88	1.88	1.88	0.00
Half Life	h	5.44	5.44	5.44	0.00
AUMC	ug*hr*hr/mL	81.48	81.48	81.48	0.00
MRT	hr	4.89	4.89	4.89	0.00

Table 21. Pharmacokinetics estimation for Levodopa – Reference Standard.

Levodopa reference standard (API)					
Dose	mg/Rat	4 mg			
Time Point	hr	Levodopa RS	Levodopa RS	Mean	SD
0.00	ug/mL	0.00	0.00	0.00	0.00
0.30	ug/mL	0.53	0.45	0.49	0.06
1.00	ug/mL	4.34	4.22	4.28	0.08
2.00	ug/mL	4.10	3.75	3.93	0.25
4.00	ug/mL	3.87	3.67	3.77	0.14
6.00	ug/mL	2.27	2.10	2.19	0.12
12.00	ug/mL	1.02	1.29	1.16	0.19
24.00	ug/mL	0.06	0.19	0.12	0.10
48.00	ug/mL	0.01	0.02	0.02	0.01
Cmax	ug/mL	4.34	4.22	4.28	0.08
Tmax	hr	1.00	1.00	1.00	0.00
AUC last	ug*hr/mL	34.08	37.40	35.74	2.35
AUC Infinity	h*ug/mL	34.15	37.57	35.86	2.42
CL	L/hr	0.12	0.11	0.11	0.01
Vd	L	0.84	0.91	0.88	0.04
Half Life	h	5.00	5.91	5.46	0.65
AUMC	ug*hr*hr/mL	226.66	313.94	270.30	61.72
MRT	hr	6.65	8.39	7.52	1.23

Table 22. Effects of intranasal administration of gel of levodopa or curcumin or their combination on number of crossing behaviour in rotenone-induced Parkinson's disease in rats.

Sr. No.	Treatment and dose	Number of crossings at various days				
		0th day	7th day	14th day	21st day	28th day
1	Normal Control	96.33±9.2	94.01±9.1	95.92±7.9	99.67±9.3	94.12±8.2
2	Disease control	97.2±8.7	96.33±9.2	66.67±8.3$^{\#}$	45.33±5.3$^{\#\#}$	37.12±6.3$^{\#\#}$
3	Intranasal levodopa gel	94.33±8.4	91.67±8.2	94.33±8.9*	72.67±6.3*	81.23±8.4*
4	Intranasal curcumin gel	97.67±9.8	96.67±9.9	72.33±6.7	80.33±8.4*	78.72±7.4**
5	Intranasal combination of levodopa and curcumin gel	93.33±7.9	94.67±9.0	83.67±8.3*	90.33±8.4*	91.43±8.3***

$^{\#\#}p$<0.0001 when compared to normal control animals of respective day; $^{***}p$<0.0001, $^{**}p$<0.001, $^{*}p$<0.01, when compared to Disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test

Table 23. Effects of intranasal administration of gel of levodopa or curcumin or their combination in on catalepsy in rotenone-induced Parkinson's disease in rats.

Sr. No.	Treatment and dose	Catalepsy (second) (Mean±SEM)				
		0th day	7th day	14th day	21st day	28th day
1	Normal Control	1.33±0.2	1.67±0.2	2.33±0.3	2.67±0.4	1.67±0.3
2	Disease control	1.33±0.2	3.67±0.2	5.67±0.5$^{\#}$	15.67±2.3$^{\#\#\#}$	43.67±4.6$^{\#\#\#}$
3	Intranasal levodopa gel	1.33±0.2	2.0±0.2	5.33±0.6	9.67±0.8**	18.67±1.8**
4	Intranasal curcumin gel	1.33±0.2	2.01±0.2	7.02±0.7	14.67±1.7	28.33±2.8**
5	Intranasal combination of levodopa and curcumin gel	1.33±0.2	2.01±0.3	4.33±0.2	10.67±1.0*	6.67±0.7***

$^{\#\#}p$<0.0001 when compared to normal control animals of respective day; $^{***}p$<0.0001, $^{**}p$<0.001, $^{*}p$<0.01, when compared to Disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test

Table 24. Effects of intranasal administration of gel with of levodopa or curcumin or their combinations on fall off latency in rotenone-induced Parkinson's disease in rats.

Sr. No	Treatment and dose	Fall off time (second) (Mean±SEM)				
		0th day	7th day	14th day	21st day	28th day
1	Normal Control	196.2±20.3	184.3±18.2	200.5±19.2	195.5±17.7	205.5±22.3
2	Disease control	202.67±21.8	169.67±12.3	141.2±12.9$^{\#}$	109.67±9.4$^{\#\#\#}$	68.33±4.5$^{\#\#\#}$
3	Intranasal levodopa gel	196.7±17.6	180.33±12.4	171.2±12.4	164.33±15.2*	127.33±11.2*
4	Intranasal curcumin gel	196.2±16.3	171.33±18.2	162.33±17.9	144.2±13.6*	131.67±12.6**
5	Intranasal combination of levodopa and curcumin gel	196.5±18.4	156.33±13.3	162.67±14.2	183.9±12.3**	185.3±12.9***

$^{\#\#}p$<0.0001 when compared to normal control animals of respective day; $^{***}p$<0.0001, $^{**}p$<0.001, $^{*}p$<0.01, when compared to Disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test

Figure 1. Thin Layer Chromatography of Macuna pruriens extracts.

A= Extract 1 B=EXTRACT 2 C=STD Levodopa

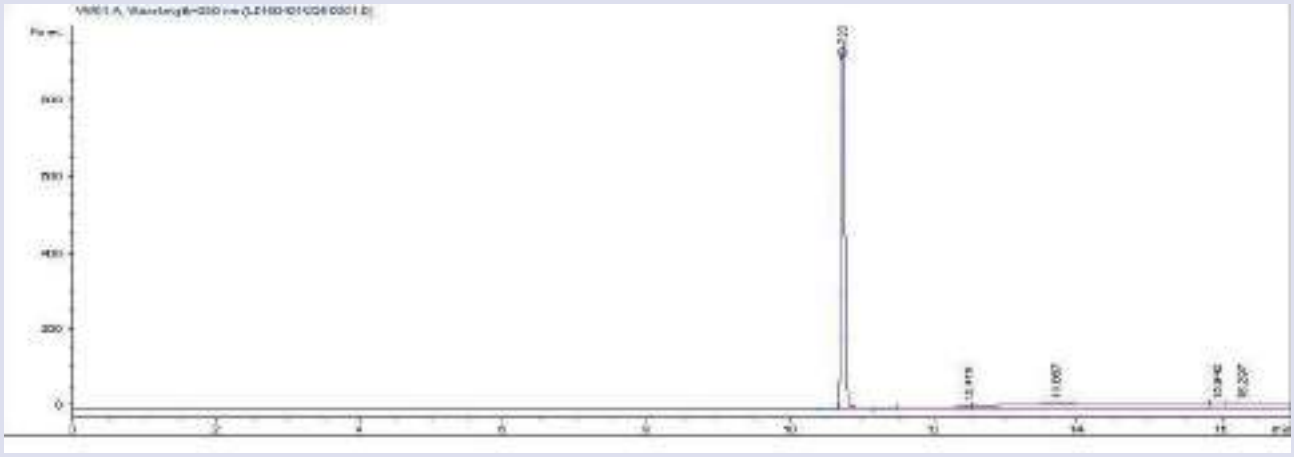

Figure 2. Estimation of Levodopa in the extract samples by HPLC analysis of Macuna pruriens Extract 1.

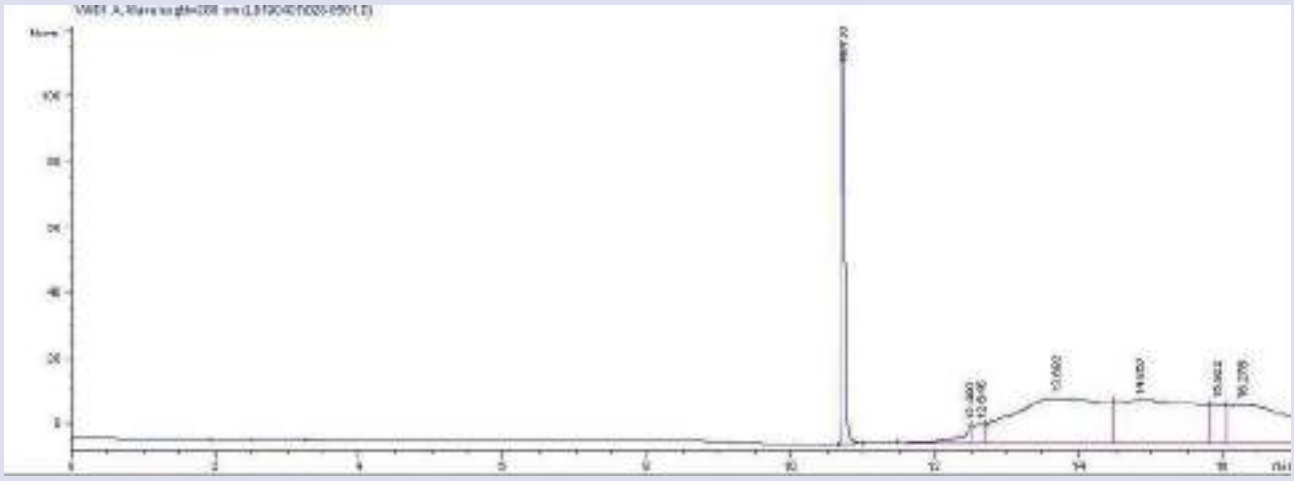

Figure 3. Estimation of Levodopa in the extract samples by HPLC analysis of Macuna pruriens Extract 2.

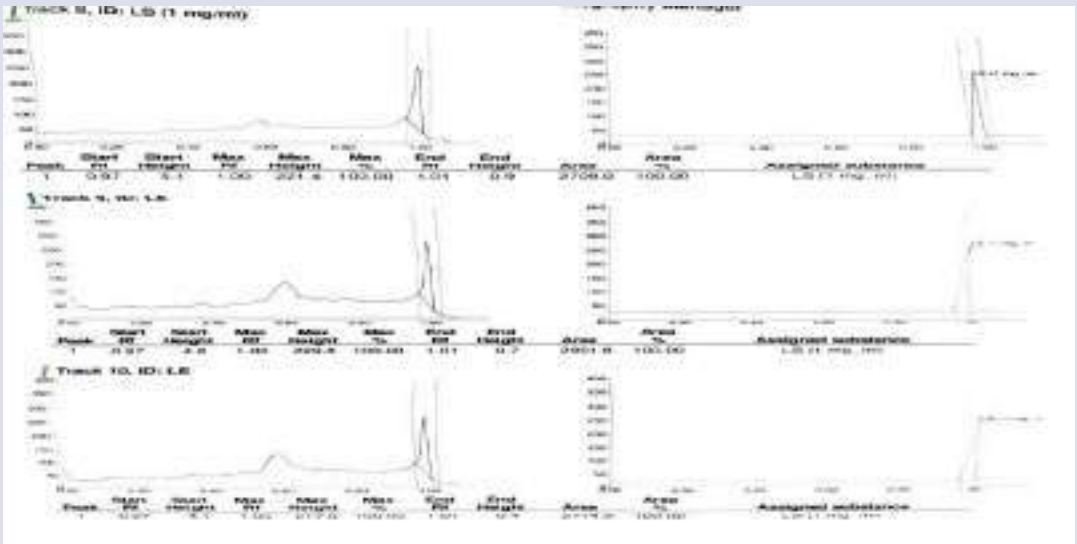

Figure 4. Estimation of Levodopa from the extract samples by HPTLC.

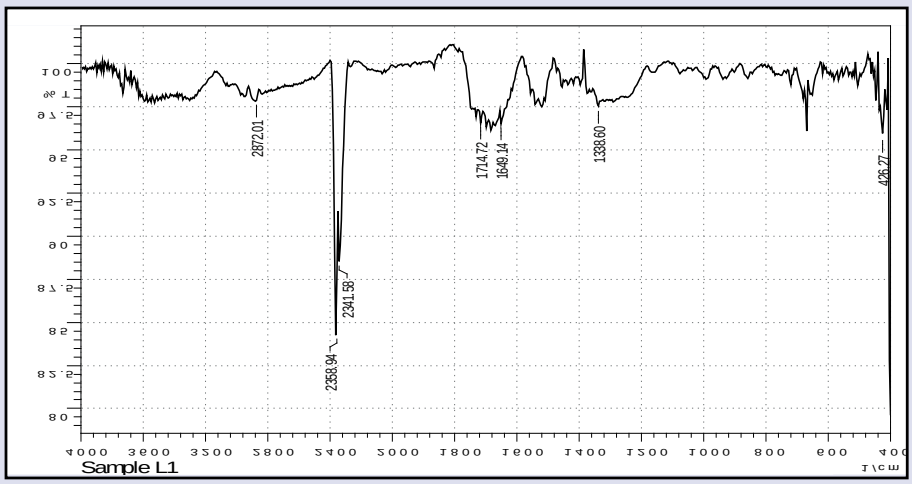

Figure 5. FTIR Spectroscopy of Macuna pruriens extract.

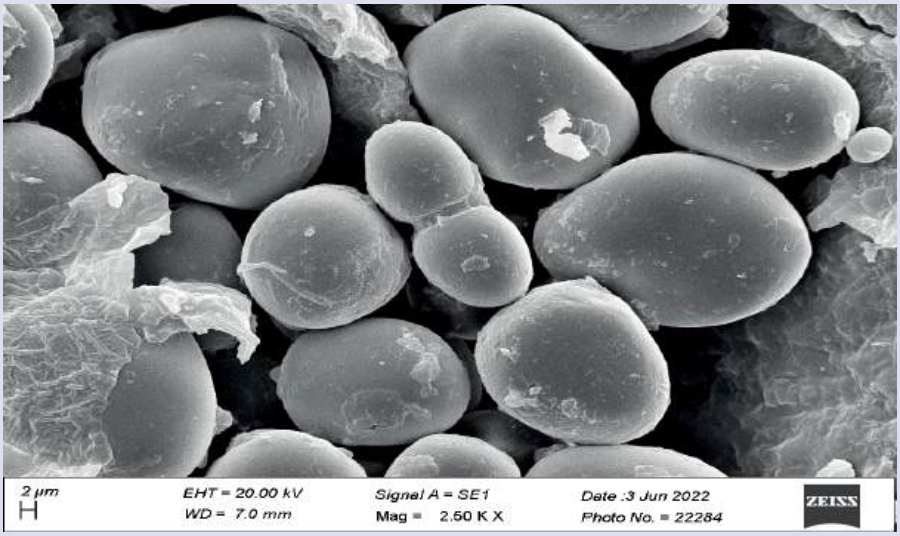

Figure 6. Method 01 Solvent evaporation method (SEM).

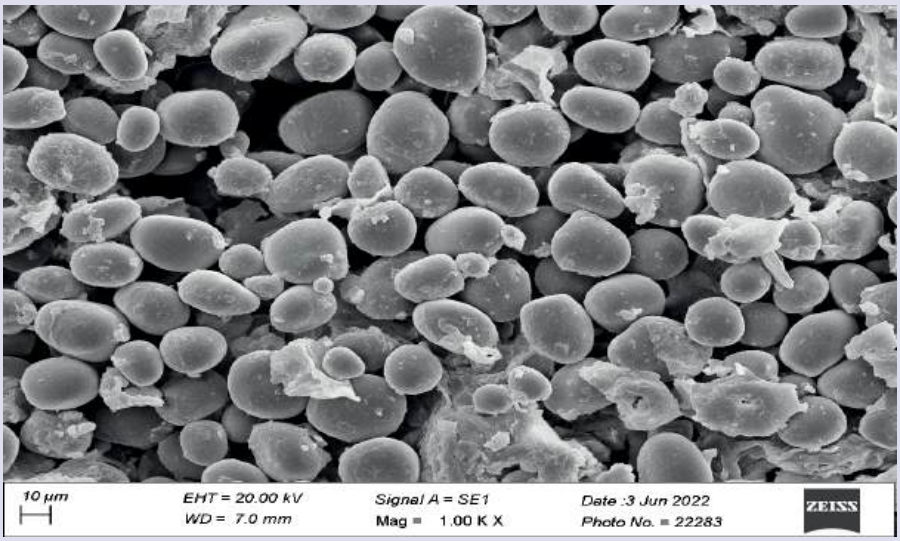

Figure 7. Method 02 Salting out anti solvent precipitation method (SEM).

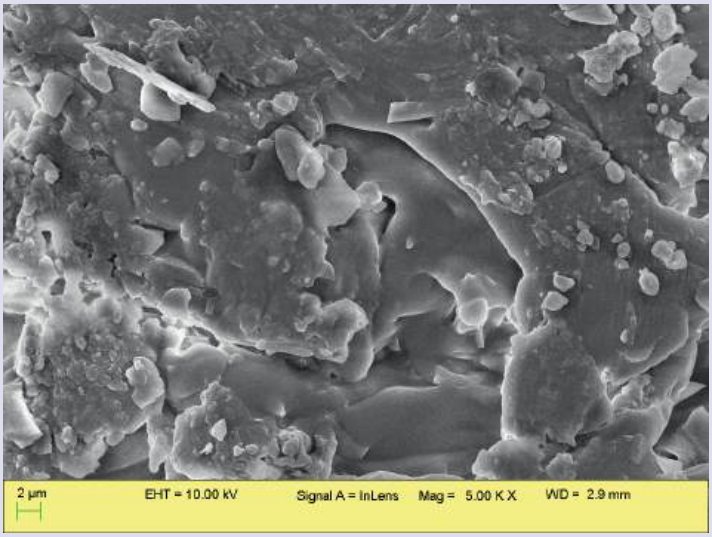

Figure 8. Method 03 Direct egg yolk method (SEM).

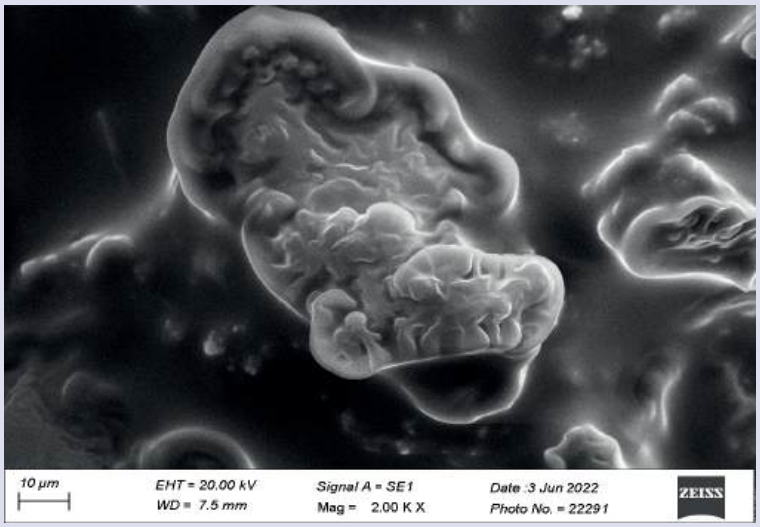

Figure 9. Method 04 Egg Lipid method (SEM).

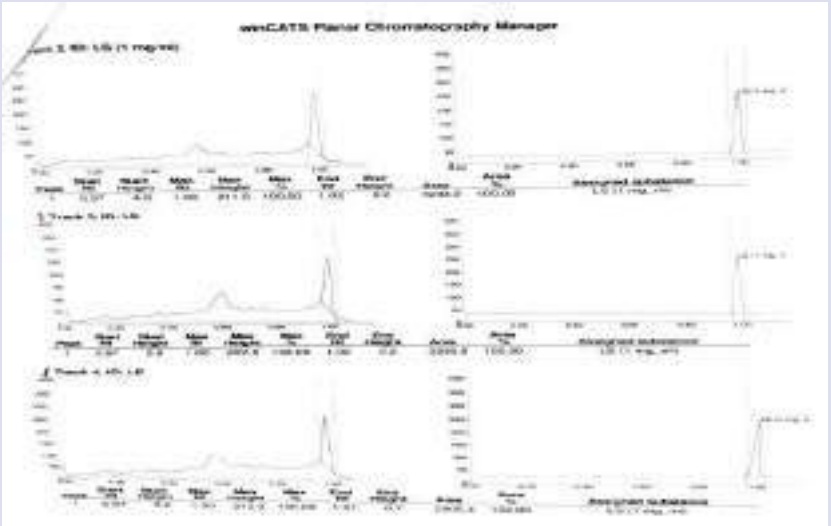

Figure 10. HPTLC study of Standard Drug and Extract (Levodopa and Macuna pruriens).

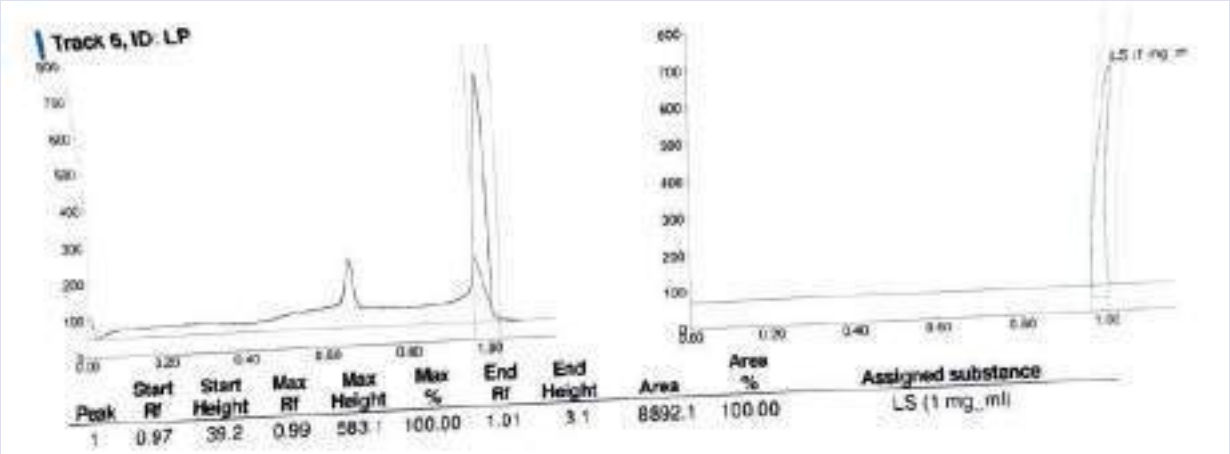

Figure 11. HPTLC study of Phytosomal extracts (Macuna pruriens).

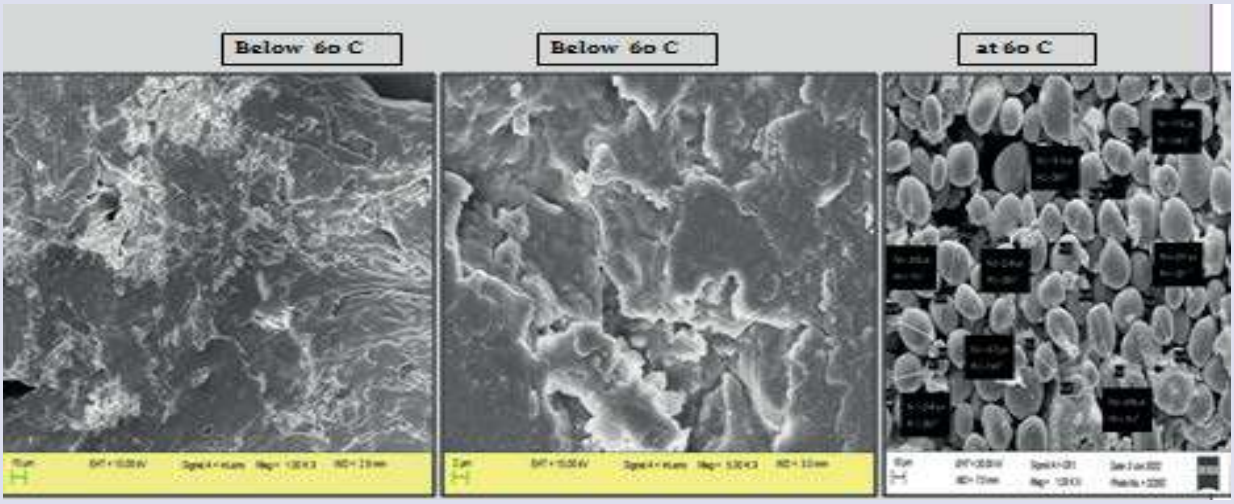

Figure 12. Based on SEM result Selected optimized temperature was 60^0 C.

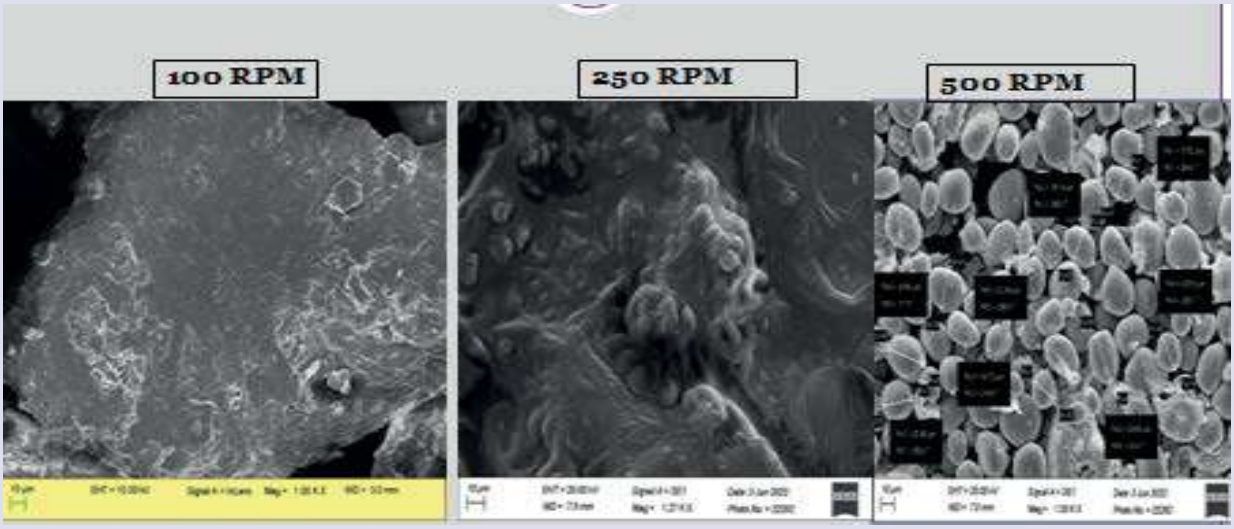

Figure 13. Based on SEM result Selected optimized rotating was 500 RPM.

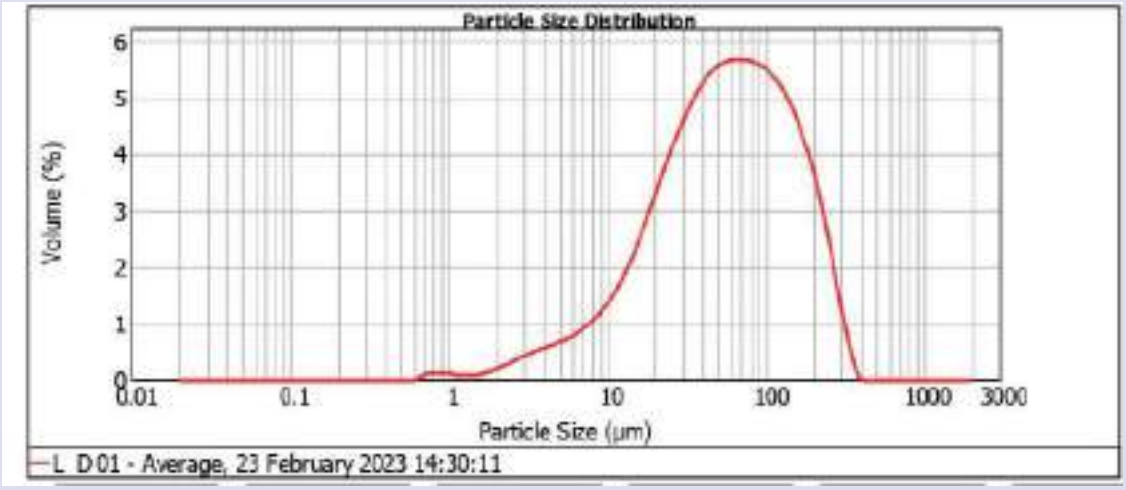

Figure 14. Batch F1 Particle Size Distribution.

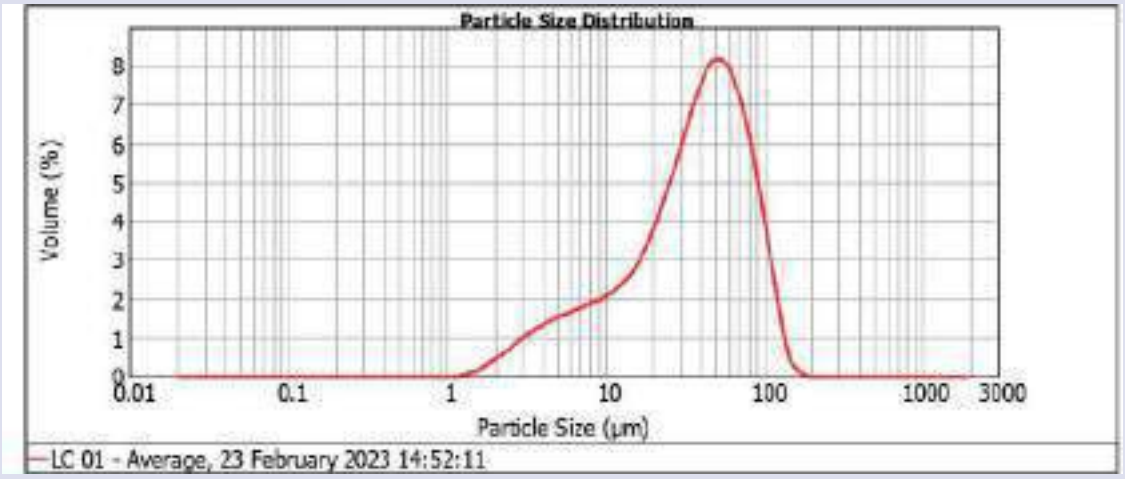

Figure 15. Batch F2 Particle Size Distribution.

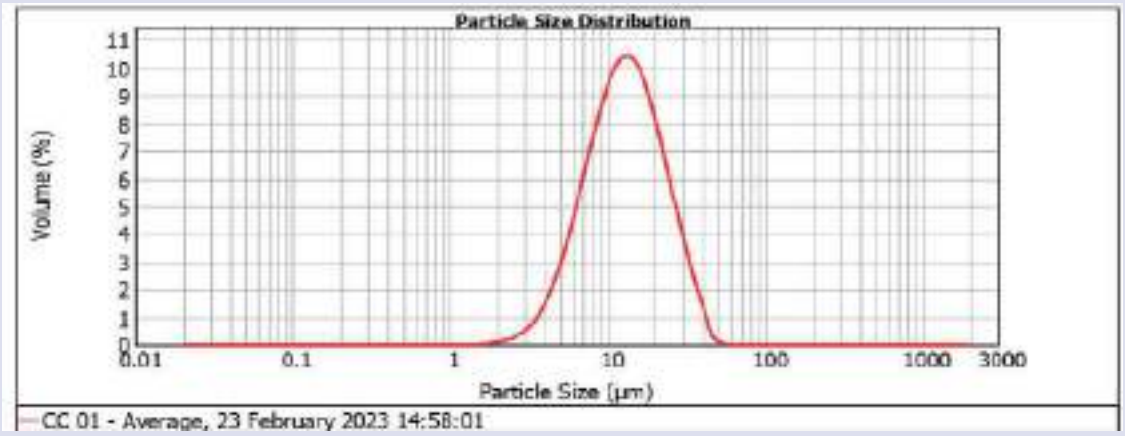

Figure 16. Batch F3 Particle Size Distribution.

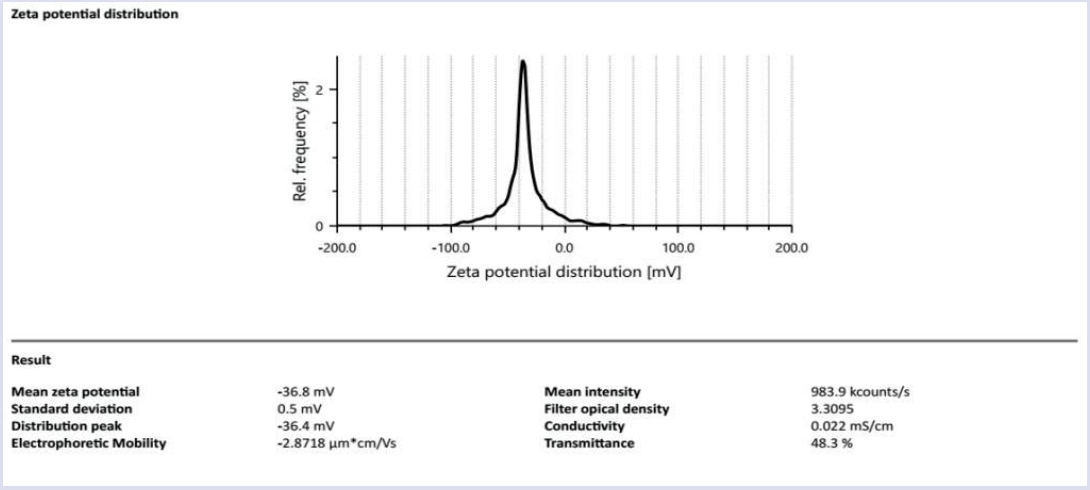

Figure 17. Zeta Potential Batch F1.

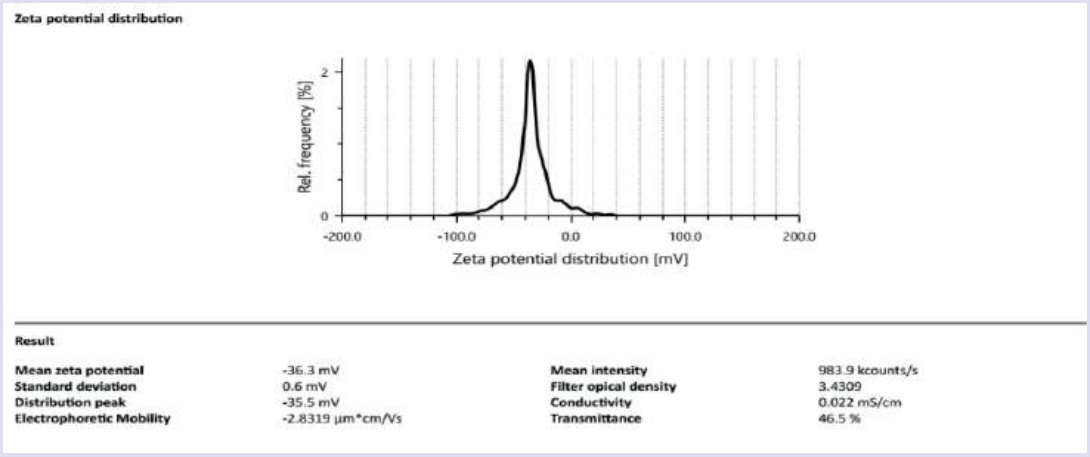

Figure 18. Zeta Potential Batch F2.

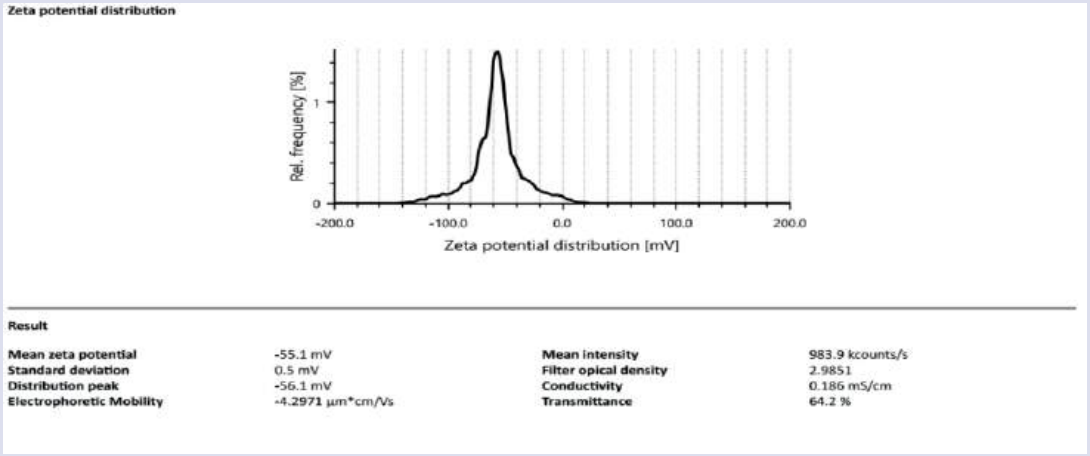

Figure 19. Zeta Potential Batch F3.

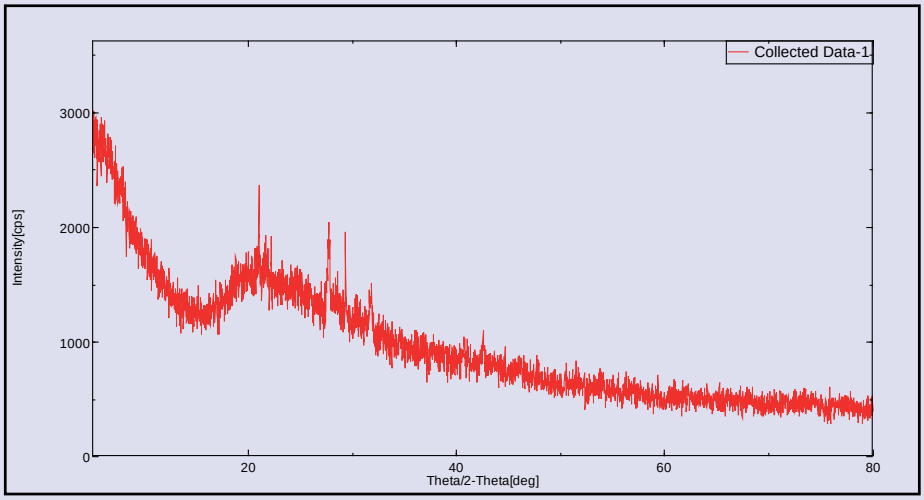

Figure 20. Batch F1 XRD.

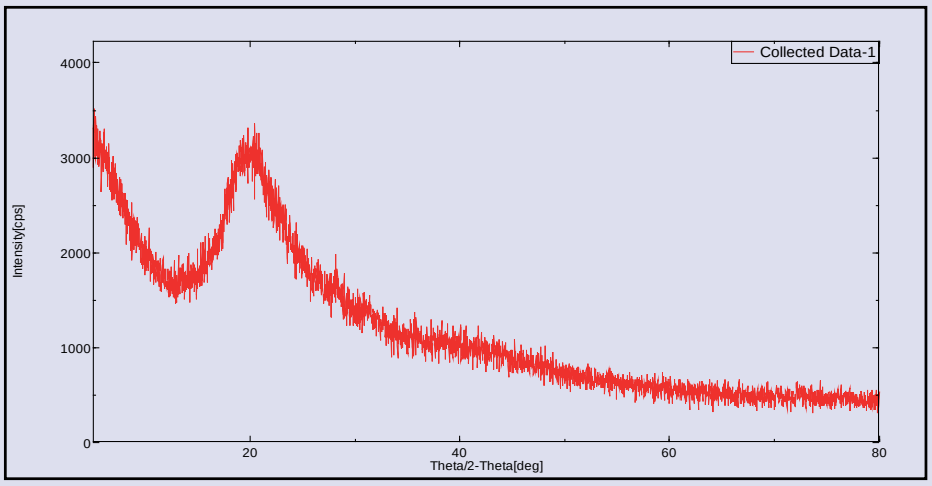

Figure 21. Batch F2 XRD.

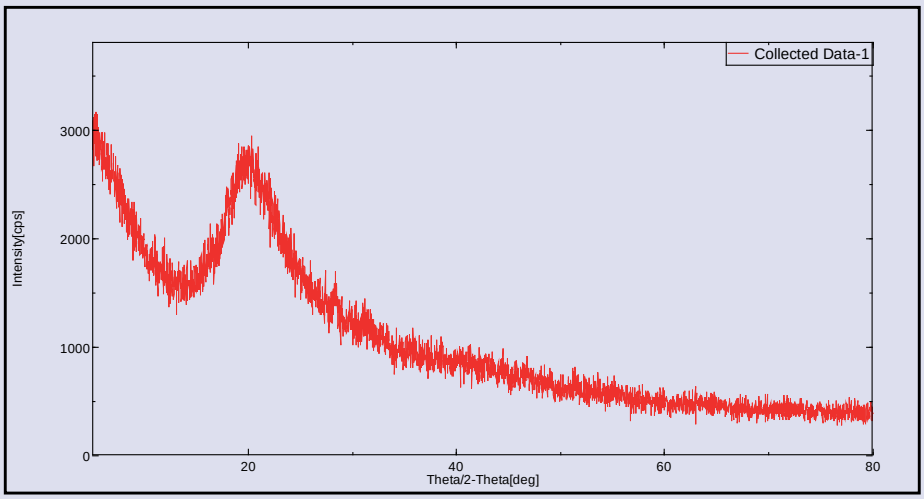

Figure 22. Batch F3 XRD.

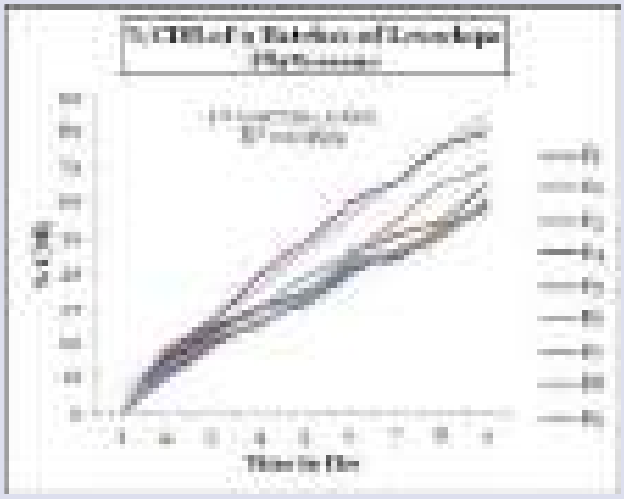

Figure 23. In vitro drug release profile Batches F1-F9.

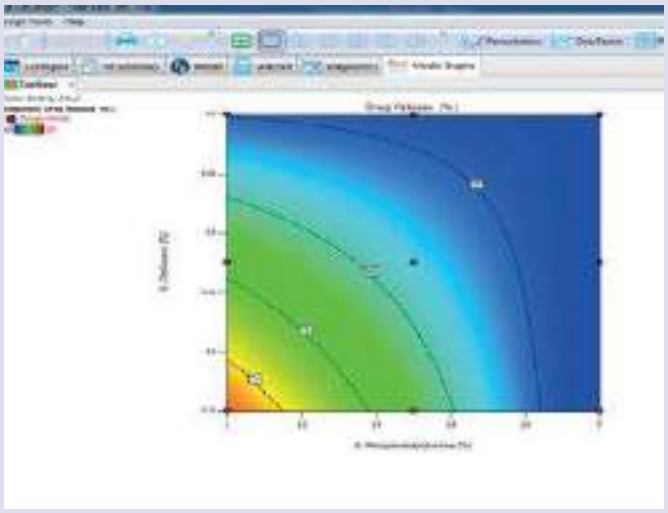

Figure 24. Overlay plot % drug release in 8 Hrs.

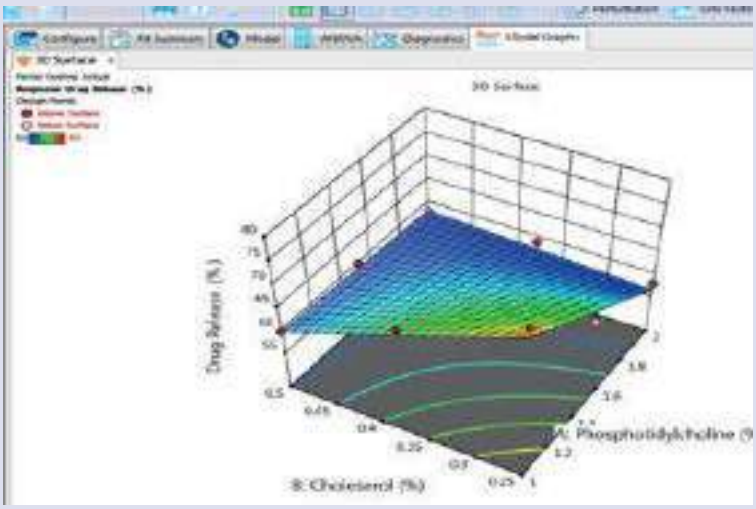

Figure 25. 3D Plot % Drug Release in 8 Hrs.

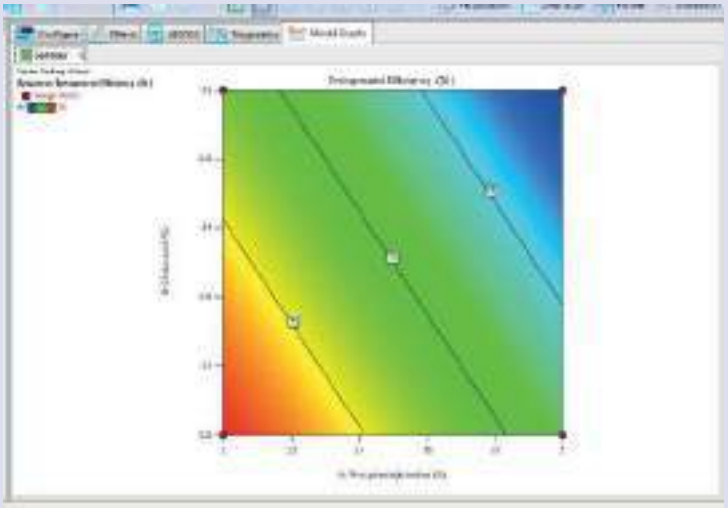

Figure 26. Overlay plot Entrapment Efficiency.

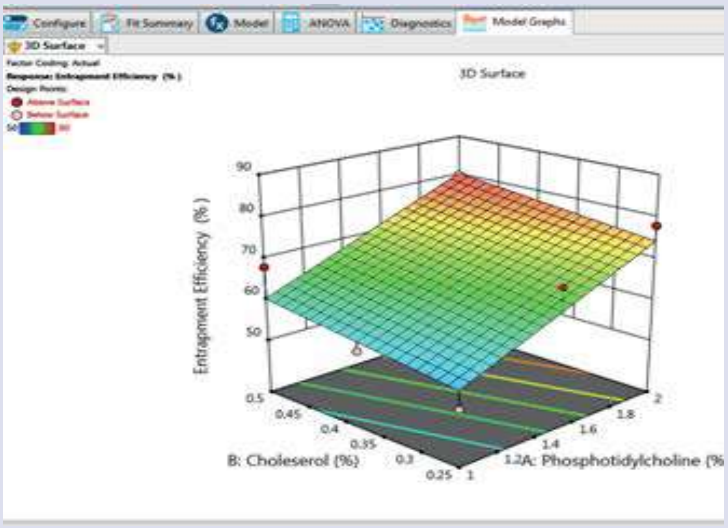

Figure 27. 3D Plot Entrapment Efficiency.

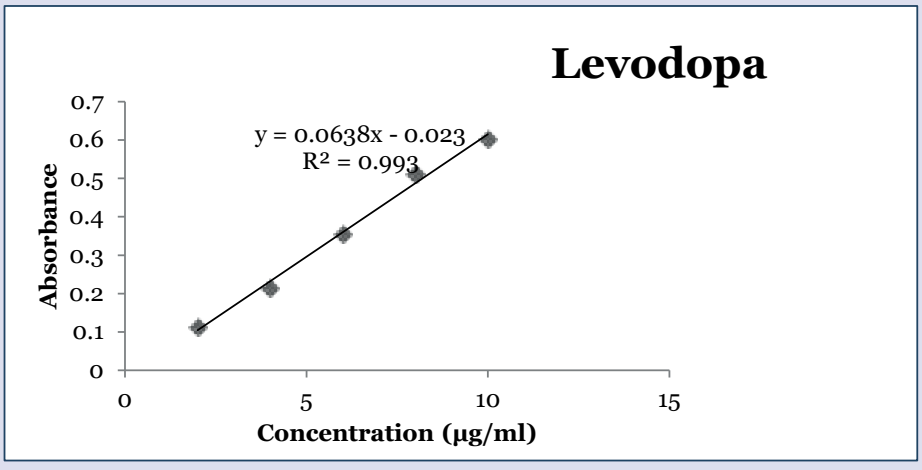

Figure 28. Calibration curve of the Drug.

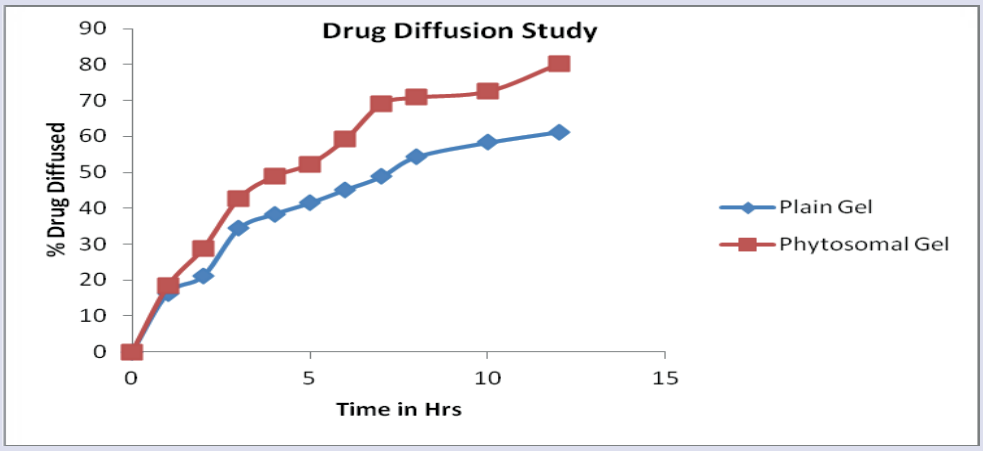

Figure 29. *In-vitro* diffusion profile of Plain gel and Phytosomal gel.

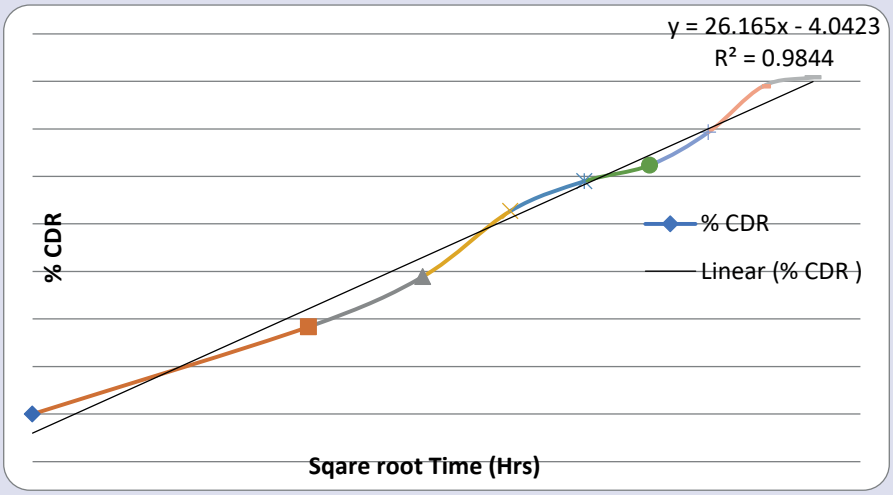

Figure 30. Higuchi's plot.

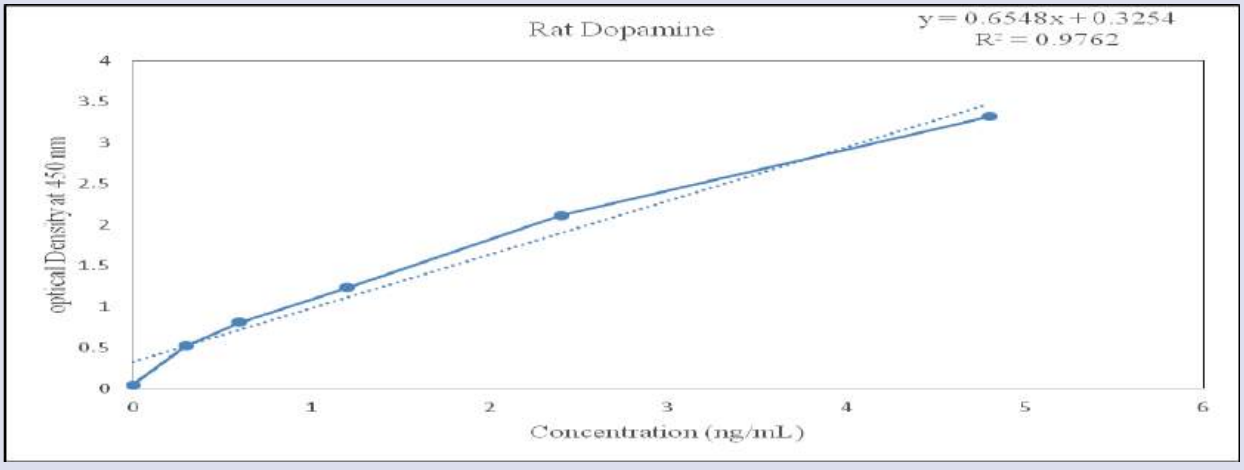

Figure 31. Standard Curve for Concentration Vs Optical density of Dopamine in rats.

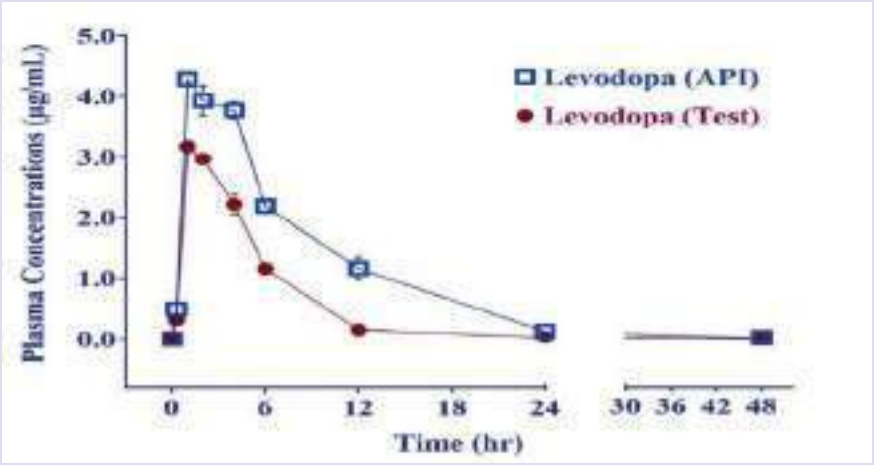

Figure 32. plasma concentration vs time profile for the levodopa test and reference standard in nasal gel.

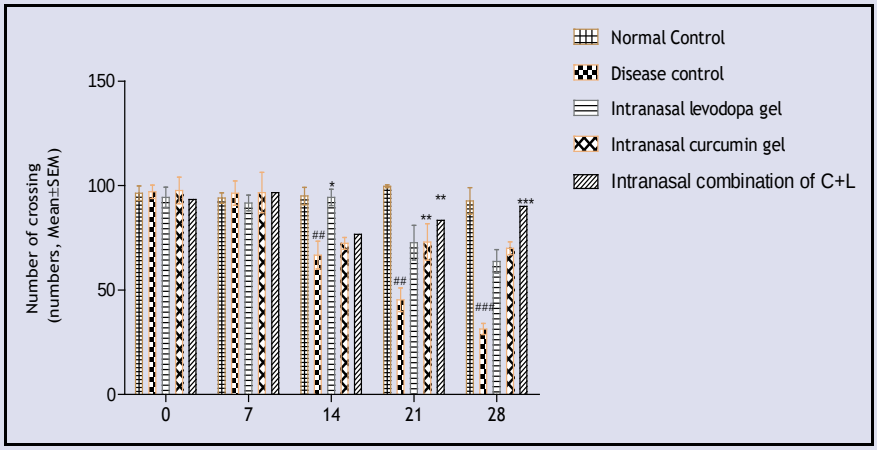

Figure 33. Effects of intranasal administration of gel of levodopa or curcumin or their combination in on number of crossing behaviour in rotenone-induced Parkinson's disease in rats ##p<0.0001 when compared to normal control animals of respective day; ***p<0.0001, **p<0.001, *p<0.01, when compared to Disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test.

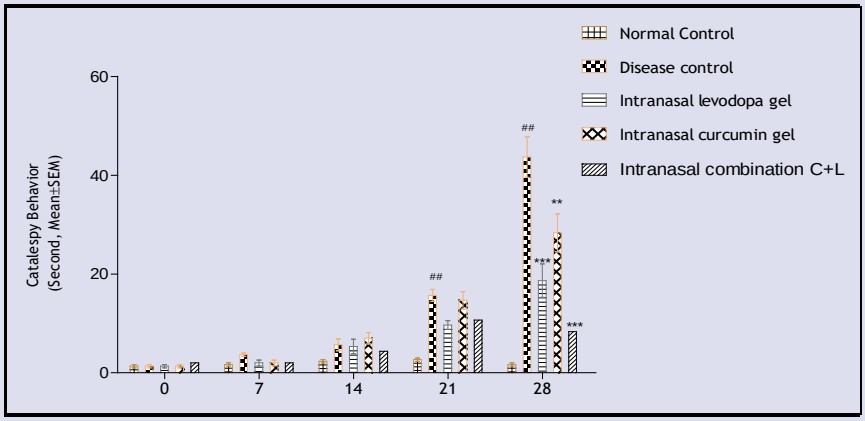

Figure 34. Effects of intranasal administration of gel with of levodopa or curcumin or their combinations on catalepsy behavior in rotenone-induced Parkinsons disease in rats ##p<0.0001 when compared to normal control animals of respective day; ***p<0.0001, **p<0.001, *p<0.01, when compared to disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test.

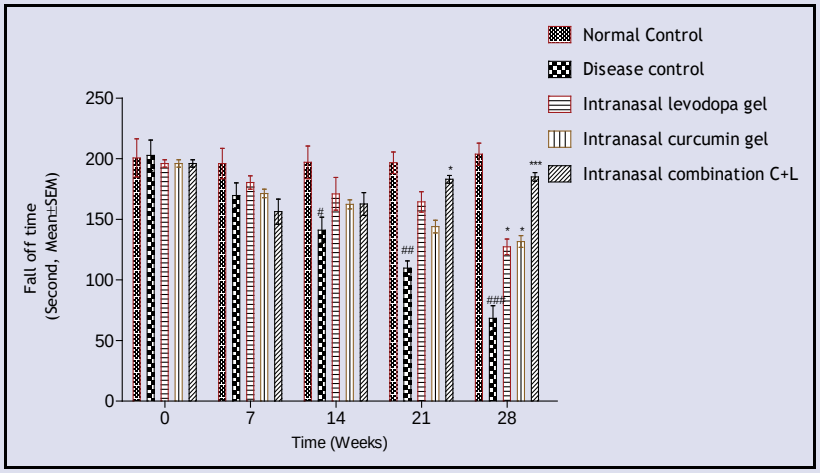

Figure 35. Effects of intranasal administration of gel with of levodopa or curcumin or their combinations on fall off latency in rotenone-induced Parkinsons disease in rats; ##p<0.0001 when compared to normal control animals of respective day; ***p<0.0001, **p<0.001, *p<0.01, when compared to disease control of respective day, one-way ANOVA followed by Bonferroni post hoc test.

For L1 sample- 353.577/3404.497x0.5=0.50% (Method 1 for extraction)

For L2 Sample -3660.380/3404.497x0.5=5.37% (Method 2 for extraction)

Based on the amount of Levodopa in the extract the second method was further selected for extraction

High-performance thin layer chromatography (HPTLC)

To identify presence of drug in the extract of Macuna pruriens HPTLC method was found suitable where comparison of chromatogram of standard with sample was done. Comparison of standard L-Dopa with Extract at peak apex and peak base was done, which confirmed that method was selective.

Fourier Transformed Infrared Spectroscopy FTIR

To ensure the functioned group and their detection FTIR study of extract was done that gives following results. Based on the functional group present it was confirmed that L-dopa was present in the given samples of extract.

Formulation and Evaluation of Phytosomal formulation

Four different methods were tried to form phytosome. And method selection was done based on the results obtained for SEM and Entrapment efficiency as follows:

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy confirmed the formation of vesicles (Phytosomes) with methods one (Solvent evaporation method) and two (Salting out anti solvent precipitation method) only.

Entrapment efficacy and Drug loading results

The entrapment efficiency was found better in the Phytosome prepared by using salting out and antisolvent precipitation technique **68.30±0.7%** and Drug loading was found to be **12.25±0.25%** this is higher than obtained by solvent evaporation technique.

Based on this salting out and antisolvent precipitation technique was further explored for the preparation of L-Dopa Extract Phytosome. HPTLC study of drug, Extract and Phytosome confirmed that extract get entrapped well in the Phytosome.

Phytosome prepared by using salting out and antisolvent precipitation technique were evaluated for various parameters and following results were obtained

Optimization of Rotating speed and Temperature Based on results obtained for

Scanning Electron Microscopy (SEM) temperature of 60^0C.and Magnetic stirrer rotating speed of 500 rpm was selected further for the preparation of phytosome.

Optimization of Temperature

Optimization of Rotating Speed

Particle size determination:

Formulation F3 Showed best result with minimum average particle size of 12 micrometre.

Particle size determination of all three batches was done.

Zeta Potential:

Zeta Potential of the batch F3 was found to be -55.1mv. It showed good stability for the Phytosomal formulations.

Powder X-ray diffractometry

In diffraction pattern the intense and sharp crystalline peaks at 2θ of 20 & 28 is observed. With Batch F3 crystallinity of 48.67% .It is possible that the extract is molecularly distributed in the phospholipid matrix and exists in an amorphous state.

Drug 5.3.7 Entrapment Efficiency

The maximum entrapment percentage of 67.23% was found for L-Dopa extract in the formulation with 2% (w/w) Lipid (Batch F3) . It was observed that Phytosome with higher lipid content gives better results.

Optimization study

Selected method was further optimized after phytosome were considered ready for formulation. 3D Plot showed that with increase in concentration of Phosphotidylcholine and Cholesterol there is decrease in the release of drug at 8 hours. It may be due to increase in the bond strength (Extract and Lipid) with increase in the concentration of both excipients. 3D Plot showed that with increase in concentration of Phosphotidylcholine and Cholesterol there is increase in the drug entrapment efficiency. This may be due to more availability of sites for bonding with lipid and increase in the stability due to cholesterol. Therefore formulation (Batch F5) containing 2 %w/v of Phosphatidylcholine and 0.5% w/v of Cholesterol were selected as optimized formulation.

As seen from **Fig.24 and 25** the surface response plot and 3D Plot revealed that a corresponding decrease in the drug release at 8 hours was observed with increase in concentration of Phosphatidylcholines and Cholesterol. This may be due to increase in the bond strength (Extract and Lipid) with increase in the concentration of both excipients.

As seen from **Fig.26 and 27** the surface response plot and 3D Plot revealed that a corresponding increase in the drug entrapment efficiency was observed with increase in concentration of Phosphotidylcholine and Cholesterol. This may be due to more availability of sites for bonding with lipid and increase in the stability due to cholesterol.

Therefore formulation (Batch F5) containing 2 %w/v of Phosphotidylcholine and 0.5% w/v of Cholesterol was selected as optimized formulation.

Reparation and evaluation of gel of optimized Phytosomal formulation of extract

Evaluation of Nasal Gel Formulation:

Different batches were prepared based on the varying concentration of carbopol (trial and error method) and final formulation was set ready by using predetermined experimental method.

Physical evaluation:

The prepared Phytosomal gel of Macuna pruriens formulation were white viscous creamy with smooth and homogeneous appearance.

Measurement of pH

L-Dopa extract: All the formulation was evaluated for its pH and the pH of the formulation was in range 6.3-6.5. It was acceptable to avoid the risk of irritation upon application to the mucosa with pH is in the range 5.5-6.5

Spreadability:

The values of spreadability indicates that the gel is easily spreadable by small amount of shear

L-Dopa: spreadability values of various gel formulations were calculated. the spreadability of Batch *LF5G3* was found to be 22.22± 0.31 (g.c.m/sec).

Homogeneity:

Visual Inspection of the developed formulation was done for homogeneity. The formulated herbal gel produced a white creamy. Gel formulations were found well-homogeneous and lump-free.

Extrudability:

An important parameter during the application is the extrusion of the gel from the tube for better patient compliance. Extrudability values of Batch LF5G3 (L-Dopa) was found to be **91.81%**.

Viscosity Study

Determination of drug content (Macuna pruriens extract-Phytosomal Gel)

Determination of drug content in all herbal gel formulation spectrophotometer assays for the quantitative determination of Drug content of the herbal gel formulation was calculated by calibration curve concentration (2-10µg/ml) was confirmed by Beer's Law at 624 nm. The different concentration absorbance shown in **Table no 12** with a regression co-efficient (R2) = 0.993. The equation of standard curve is y = 0.0638x-0.023 (**Fig. 29**). The drug content of gel formulation shown in **Table no. 13.**

Drug diffusion study:

Gel formulation of Phytosome of Macuna pruriens extract (Batch LF5G3)

The % CDD (% Cumulative Drug Diffused) of gel formulation shown in **Table no. 14** and graph shows in **Fig no.30** the cumulative amount of drug release was found to be 61.27±0.74 and 80.23±0.28 for plain and Phytosomal gel respectively after period of 12 hr which proved that Phytosomal formulation could enhance bioavailability of extract.

Drug release kinetic:

The drug release was analysed by Disso calculator software to study the kinetics of the drug release mechanism. On comparison of the kinetic modelling and release profile data it was evident that the Phytosome formulations were found to release the drug in accordance to Higuchi kinetics, the regression coefficient was not found to be exactly near to 1 ,which could be due to influence of some other factors.

Gel formulation of Phytosome of Macuna pruriens extract (Batch LF5G3)

The study of the drug release showed that the formulation is governed by Higuchi's model. The curve was obtained after plotting the cumulative amount of the drug released from each formulation .*i.e* % CDR v/s **square root of time** given in the **Fig.No. 31**

Animal Studies:

Estimation of Dopamine content:

The animal study results showed that there is considerable enhancement of dopamine level after administration of nasal gel formulation containing phytosome of L-dopa (Three times more as compared with normal).

Pharmacokinetic study:

The mean plasma concentration-time profiles of the investigated components were studied The Cmax of curcumin in rat plasma was 3.16±0.08 ug/ml and the Tmax was 2 h (3.22±0.08ug/ml and 2 hr for Standard L-Dopa). The Cmax of L-Dopa in rat plasma was 3.24 ug/ml and the Tmax was 1 h (4.28±0.08 ug/ml and 1hr for Standard L-Dopa).

RESULTS

The area of peak was used for the estimation of the concentration

Antiparkinson's study

DISCUSSION

The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners. Natural products are a source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential properties. The Indian traditional system of medicine described several drugs of plant, mineral, and animal origin Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda. Since time immemorial, man has used various parts of plants in the treatment and prevention of many ailments. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts.

After the procurement of the plant parts it was subjected to authentication. The macroscopic characters were studied. The plants were then subjected to preliminary evaluation which included the

determination of physical constants and preliminary phytochemical evaluation. *Macuna pruriens* is used as antiparkinson's drug. It is given in tablet or capsule However it has low permeability which leads to inadequate therapeutics effect. Recent work was done to develop vesicular system having enhanced penetration power. As a result novel vesicular system like phytosmes were developed.

The *Macuna pruriens* phytosmes was prepared by salting out methods by using Lecthin,Cholestrol,methonao and Distilled water.The prepared phytosmes were characterized for their Entrapment efficiency percent, Zeta potential, Particle size, Vesicle Morphology. Percent Entrapment efficiency was higher in F3 formulation than that of other formulations. The particle size revealed that the F3 formulation gave particle size of 12 micron which gave that there should be little study done for the size of the particles. Zeta potential was more towards the negative charge for the F3 formulation.SEM analysis of the phytosme reveled that the F3 formulation gave a best result in the form of vesicular formation. Further Full Factorial Design: A 2^2 randomized full factorial design was used in this study. In that batch F4 showed 60 % CDR, particles size of 15 (µg) and entrapment efficiency of 70%. The formulation of this batch was further utilized to be converted into gel formulation

The *Macuna pruriens* phytosome gel formulations were prepared by using carbopol 940 and they were characterized for its pH, Spreadability, Viscosity, Drug Content, Extrudability, Homogeneity and washability the result showed fairly acceptable values for all the parameters evaluated. Out of three batches F4G1, F4G2 and F4G3 The batch F3G3 showed better result as compared with other batches. The swelling index of the formulation was found to be acceptable. *In-vitro* drug diffusion studies were in range. *Macuna pruriens* Phytosome gel (Batch F4G3) was found to follow Higuchi's kinetic. This batch was further evaluated to in vivo study for rise in dopamine level in the brain after nasal administration of the same. The animal study result also showed that there is considerable enhancement of dopamine level (Three times more 1.48 (ng/mL) as compared with normal) in the brain after nasal administration of gel of Levodopa Phytosome. The present findings thus, suggested that combination treatment exhibited improvement in Parkinson symptom of motor coordination by reversing the crossing movement in the rats.

Pretreatment with intranasal gel of levodopa and curcumin elicited significant ($p<0.0001$) attenuation of cataleptic behavior compared to disease control. Furthermore, combination of levodopa and curcumin gel treatment exhibited better attenuation in cataleptic behavior compared to vehicle treated disease

The treatment of levodopa and curcumin combination exhibited better effects on muscle coordination disturbance due to repeated rotenone administration observed in the present studies

CONCLUSION

The present study was attempted to formulate the Phytosome of *Macuna pruriens* extract for the nasal delivery with view to target the site of action located in brain. The experimental result exhibits that, the Phytosome prepared by using salting out method are more stable. The gel of the same formulation prepared using carbopol 940 as a base showed satisfactory release. The animal study result also showed that there is considerable enhancement of dopamine level in the brain after nasal administration of gel of Phytosome of L-Dopa extract The finding suggests that the Phytosomal gel formulation can be used to enhance efficacy of L-Dopa extract. Antiparkinsonian activity of test nasal gel formulation showed improvement in behavioral pattern after administration of Phytosomal gel of L-dopa, pharmacokinetic study exhibited significant in vitro nasal mucosa permeation without causing skin irritation Phytosomal gel formulation containing *Macuna pruriens* seed extract and *Curcuma longa* rhizome extract would be the best option to target brain for the treatment of Parkinson's disease.

LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL FORMS
%	Percentage
°C	Degree Centigrade
µg/ml	Microgram per milliter
Mg	Milligram
Gm	grams
ml	Milliliter
pH	Hydrogen ion concentration
UV	Ultraviolet
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
HPTLC	high-performance thin layer chromatography
XRD	X Ray Diffraction
SEM	Scanning Electron Microscopy
L-DOPA	Levodopa
RH	Relative Humidity
rpm	Revolution per minute
min	Minutes
Hrs	Hours
etc	Etceteras
±	Plus, or minus
<	Less than
>	More than
v/s	Versus
λ	Lambda
CDR	Cumulative Drug Release
CADD	Cumulative Amount of Drug Diffuse
ICH	International Conference on Harmonisation
IP	Indian Pharmacopiea
Conc	Concentration
Abs	Absorbance
i.e	That is
FT-IR	Fourier Transform Infrared
R^2	Regression Coefficient
et.al	et alii,'and others'
ANOVA	Analysis of variance
e.g	For example
Fig.No.	Figure Number

DECLARATIONS

Competing Interest

Not Applicable.

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Authors' contributions

Mr. Aniruddha S. Kulkarni: Experimental work Data collection, Data analysis and drafting the article.

Dr. Kishore Gujar: Conception or design of the work.

Dr. Manoj Tare and Dr. Meera Singh: Interpretation of results.

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