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Original Article

Pharmacokinetics of Dual Amino Acids for Dry Powder Inhalation Therapy in Pulmonary Drug Delivery: An In-Vivo Study

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Abstract

Background: Smoking cigarettes leads to serious health problems, and addiction is a major concern. De-addiction therapy includes e-cigarettes and counseling, and the success rate is poor in these approaches, warranting alternate therapeutic interventions. The present study evaluates dry powder inhalation using amino acids as a new method.

Methods: A novel formulation was prepared using nanospray drying and ball milling techniques. SEM analysis was conducted to ascertain particle size, and pharmacokinetic assessment was done to evaluate how rapidly the drug is released from the formulation. The optimized ratio of ingredients revealed the best formula, L-tyrosine, L-tryptophan, and lactose in the ratio of 5 mg:1 mg: 4 mg, which led to the dry powder preparation. SEM analysis revealed that the dry powder comprising L-tryptophan and L-tyrosine had undergone micronization to 2–4 µm.

Findings: An innovative treatment, such as an inhalation powder therapy comprising amino acids, can help reduce mood changes. Depletion of tryptophan to kynurenine controls mood changes, and maintaining a steady tryptophan concentration is expected to help overcome mood changes that trigger smoking recurrence. The microparticles produced by spray-drying were confirmed to include loose agglomerates, which are amenable to inhalation and free dispersion. In-vivo studies revealed that drug action is quick with drug delivery and retention at the site of action.

Conclusion: The synergistic effect of the novel formulation's sustained concentration of tryptophan and tyrosine and inhibition of AChE could diminish the recurrence of smoking. Dry powder inhalation of the formulated drug offers a new and strong method of drug delivery to the alveolus, making it a convenient route of administration that could be superior to other modes of administration in smoking cessation.

Keywords: Lung, Inhalation, Smoking cessation, Dual amino acid

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Introduction

Cigarette smoking causes serious health issues, and smokers get addicted to it.[1] In a few seconds, smoking raises nicotine concentration,[2] leading to dopamine release.[3] Thereby, libido and food consumption are triggered.[4] The subsequent drop in nicotine concentration leads to a condition called withdrawal, making the person resort to the next cigarette, which could lead to repeated stimulation and recovery.[5,6] This phenomenon is the basis on which nicotine replacement therapy (NRT) products have been designed, aiming to release nicotine in a sustained manner, maintaining a low concentration of nicotine levels so as to avoid causing addiction.[7]

Several forms of NRT products are available, including chewable products, films, etc, while other types, like inhalation products, require a prescription. Therapeutic interventions that favor the immediate effects of smoking withdrawal are needed. Agents useful for curbing withdrawal symptoms are mostly neurotransmitter agonists like tryptophan. High-calorie diets are also used.[8]

Munafò et al identified the relevance of tyrosine-reducing composition with amino acid intervening withdrawal-related symptoms.[9,10] Dowlati et al employed a formulation using a combination of amino acids along with blueberries and found that the product had no appreciable impact on recovery from mood suppression.[11] Venugopalan et al reported the importance of a reduction in phenylalanine/tyrosine in triggering smoking,[12] emphasizing the requirement of sufficient tyrosine levels to arrest the desire for smoking. Hughes et al evaluated the possible role of medicines used in depressive disorder in arresting smoking, and the results were negative. The

effects of Bupraset[13] and acute tryptophan depletion (ATD) in reducing smoking recurrence have been studied[14]. Furthermore, ATD was found to augment the desire to start smoking, indicating the relevance of keeping tryptophan concentration adequate for success in smoking cessation.[15-19]

As precursors[20-23] for neurotransmitters norepinephrine, dopamine, and serotonin, tyrosine and tryptophan are important in managing mood variations.

Pulmonary root administration containing dry powder has garnered attention because of its novelty, absence of propellants, and the achievement of higher drug loading.[24,25] The advantage of DPIs is derived from the particle size of less than 10 μm and the newer excipients used[26, 27].

Fast delivery of rivastigmine (RHT) microparticles reduced drug loading[28] using DPI.[29] L-leucine was found to reduce hygroscopicity and further minimize the nephrotoxicity observed in the parenteral route of administration.[30] DPIs for tiotropium bromide[31] and surface-modified lactose (SML) particles of fluticasone propionate[32] have been reported. Mice lung delivery with other DPI formulations and their drug-release properties have been reported.[33-35]

Mechanistic aspects of smoking cessation

L-tryptophan, an essential amino acid, is crucial for organ growth and activity, and its main role is to get converted into 5-HTP (5-hydroxytryptophan). Serotonin, a neurotransmitter, also narrows blood vessels, and its concentration in the brain causes mood changes. Although tryptophan at 50 mg/kg has been used for smoking cessation, it has resulted in toxicity.[36] Tyrosine is a stimulator in the CNS and provides recovery from depression. It has an FDA GRAS status,[37] but safety data on pregnant women is unavailable.

It is known that some of the amino acids act as neurotransmitters or precursors whose BBB transport is essential for CNS action. The two amino acids used here are precursors of a catecholamine precursor (tyrosine) and a serotonin precursor (tryptophan). Their entry to the brain through the BBB is controlled by concentration gradient through LAT1, Na^+-LNAA, $ATB^{0,+}$ transporters.[38] Hence, dual amino acid dry powder inhalation would be beneficial to increase craving for smoking through their ability to cross the BBB, considering they are precursors to neurotransmitters.

L-tryptophan and L-tyrosine are monoamine precursors, and it is well established that neurotransmitter serotonin concentration in the brain influences mood changes.[39] Furthermore, Carhart-Harris and Nutt evaluated mood activity and sensation of pain with the use of tryptophan and tyrosine at a 1:2 ratio (50 mg/kg and 100 mg/kg) in a double-blind, placebo-controlled study, which attributed alertness to tryptophan.[40]

Administering a dietary supplement with the monoamine precursors tryptophan (2 g) and tyrosine (10 g) has been shown to influence depressed mood positively.[41,42] Use of tryptophan at 50 mg/kg for smoking cessation showed toxicity.[36] On the other hand, tyrosine, credited with FDA GRAS status, is a CNS stimulator that has shown beneficial effects in recovering from depression.[37]

Considering these factors, the two amino acids were chosen for the intranasal delivery, with a larger concentration of L-tyrosin than L-tryptophan.

The present work describes the development of a convenient and better dosage form in order to suppress the temptation and recurrence of smoking. Micronized L-tryptophan and L-tyrosine of μm size were prepared by the nano spray drying technique to target the lungs and produce an effective therapy for altering mood during smoking cessation and synergistically facilitating smoking cessation. The Andersen cascade impactor provided aerosolization properties, and diffusion studies showed the percentage of drug release in vitro. Drug accumulation necessary for lung release and subsequent pharmacokinetics in plasma were obtained using in-vivo methods.

Materials and Methods

Chemicals used

L-tryptophan and L-tyrosine were procured from Nice Chemicals Pvt. Ltd. Lactose anhydrous (sterile), HPLC water, disodium phosphate, di-potassium hydrogen phosphate, NaCl, N-octanol, and diethyl ether were purchased from HiMedia Laboratories Pvt. Ltd. Diazepam was purchased locally under the brand names Diastat and Valium.

List of instruments used

Shimadzu balance (Digital AY-120), Buchi nanospray dryer (Buchi 90) RETSCH ball mill, Shakthi Pharmatech double cone blender, Tescan – Mira3 XMU field 2 emission scanning electron microscope with EDS, X-Pert Pro XRD machine, 8400S Shimadzu FT-IR, Mettler Toledo differential scanning colorimetry, zoom Yamayo Vernier caliper, Shimadzu UV spectrophotometer (UV-1800), SITRA ASTMF2101 cascade impactor, REMI centrifuge (R8C), deluxe digital pH meter (101, Deep Vision), Labman scientific bath sonicator and ACG Pam Pharma Technologies gelatin capsule size were used.

Methods

Preformulation studies

a) L-tryptophan and L-tyrosine-preparation of standard curve: The stock solution for the two amino acids[43] was prepared by individually dissolving L-tryptophan or L-tyrosine (used quantity = 100 mg) in phosphate buffer 7.4 pH (100 mL) as solvent. Aliquots (10 mL) were taken

from the above stock, and 100 mL dilution was made using buffer solvent. The resultant solution was 100 µg/mL for further dilutions to solve 10, 20, 30, 40, and 50 up to 100 µg/mL. An estimation curve was produced using wavelength at 220 and 283 nm, respectively.

b) Studies on compatibility: The medication and carrier were combined before being filled into a previously cleaned container. After the container was sealed, they were kept in a stability chamber for 28 days at 37 ± 0.5 °C. FTIR and DSC drug excipient compatibility investigations were carried out after removal from the stability chamber.

c) Fourier-transform infrared spectroscopy (FT-IR): FTIR spectral data were obtained for the carrier anhydrous lactose, L-tryptophan, L-tyrosine, and pure amino acids. Similar information was gathered for the physical combination of amino acids and carriers at a 1:1:1 ratio.

d) Differential scanning colorimetric analysis (DSC): DSC was used to determine whether the drug and lactose were compatible, using a 5–10 mg sample of the individual components and a 75–150 mg sample of the drug-excipient combinations.

Drug solubility measurement:

Shake flask saturation solubility was measured with water, pH 4.0, 6.8, and 7.4. Absorption was measured with a UV spectrophotometer at 220 nm and 283 nm for L-tryptophan and L-tyrosine, respectively. Saturation solubility was ascertained using the calibration curve produced using the absorbance value.

a) Calculating the partition coefficient and pH: A digital pH meter measured the pH of the drug solution. Then, using the formula, the partition coefficient was calculated.

$$LogP = \frac{Conc.\ of\ the\ drug\ in\ the\ oil\ phase}{Conc.\ of\ the\ drug\ in\ the\ aqueous\ phase}$$

b) Development of formulations and optimization

c) Amino acid micronization using the nano spray drying method

Each unique 20% concentration of the amino acid solution was created and spray dried using a nanospray dryer that was set to start at 100 °C, end at 48 °C, and pass the solution at 40 mL/min for feed, 100% for aspirator rate, and 700 l/h for atomizing airflow. Table S1 displays the parameters used for optimization.

d) Lactose particle size reduction through milling: Anhydrous lactose was fed into the ball mill through a 60° cone on the left, and the result was discharged through a 30° cone on the right. The impact of the balls caused the solid particles to undergo size reduction.

e) Blending and filling: The improved formulas were produced as a homogenous mixture using a shaker mixer set to 42 revolutions per minute for an hour. The resulting mixture was precisely weighed, and capsules in three sizes were filled with it.

Formulation assessment

a) Physical description: Determining the moisture content of the dry powder involved placing it inside a desiccator with a lowered pressure and running a vacuum for a full day. The moisture content was ascertained using the formula below:

$$\text{Moisture content [\%W/W]} = \frac{Initial\ weight\ -\ Final\ weight}{Initial\ weight} \times 100$$

b) Solid state stability studies: The enhanced formulation's stability was tested by filling the capsules with dry powder for inhalation and sealing them in a 30-cc container made of high-density polyethylene. The 90-day stability experiments used storage at 25 °C and 60% relative humidity and 40 °C and 75% relative humidity. Drug content changes after the 30th, 60th, and 90th day were examined.[38]

Micrometric description

a) Analysis using a scanning electron microscope (SEM): At 5 kV, the morphology of the milled lactose and spray-dried amino acids (L-tryptophan and L-tyrosine) was determined. The optimized particle sizes for lactose and amino acids were 50–150 µm and 1–4 µm, respectively.

Counting the elements in the medicinal molecule using energy dispersive spectroscopy (EDAX)

b) Powder X-ray diffraction: Using a step size of 0.017/s, an X-ray powder diffraction investigation was carried out on the 2θ scale between 5 °C and 40 °C. Every sample underwent a duplicate measurement.

v) Flow characteristics

Bulk and tapped density: Bulk and tapped density were measured using standard procedures in triplicate.[39]

Carr's index: Carr's index was determined using the formula

$$Carr's\ index = \frac{Tapped\ density\ -\ Bulk\ density}{Tapped\ density} \times 100$$

Hausner ratio: Hausner ratio is calculated using the formula below

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{Bulk\ density}$$

The nature of flow is inferred by comparing the data given below.[40]

Capsule evaluation

a) Physical characteristics: We examined the capsules' dimensions, form, and integrity. We measured their locking lengths using Vernier calipers and noted the

results.

The fill weight was averaged for each capsule. The twenty capsules were opened to extract the contents as thoroughly as possible, avoiding destroying any shell component.

The 20 capsules were filled, and the average fill weight was determined using the formula below[41]:

$$Average\ fill\ weight\ (mg) = \frac{20\ Capsules\ content\ in\ mg}{20}$$

b) Assay (drug content determination): After the weight was measured, 10 capsules were dissolved in a 100 mL volumetric flask. Then, 10 ml of water was added for sonication to dissolve the capsules further. After adding phosphate buffer (pH 7.4) in the prescribed amounts, the mixture was subjected to a 10-minute sonication. After being filtered over a 0.45 µm membrane, the drug content was measured with a UV spectrometer at 220 nm for L-tryptophan and 283 nm for L-tyrosine. The formula was used to calculate the drug content.

$$\text{Quantity of drug} = A_{Sam} \times C_{Std} / A_{Std}$$

Where,

A_{Sam} = absorbance of sample solution

C_{Std} = concentration of drug in standard solution

A_{Std} = absorbance of standard drug solution

C_{Std} and A_{Std} were obtained from the standard graph.[41]

c) Using in-vitro research: Anderson cascade impactor: The experiment used an Anderson cascade impactor (ACI) with a 60 L/min air flow rate. UV was used to analyze wash solutions for drug concentration following normal practice.[43] Fractions observed at respirable (RF) and emission dose (ED) were used to derive the inhalation properties of DPIs. Six measurement repeats were carried out.[44]

d) Bag diffusion study: A dialysis bag diffusion technique was used to measure the amino acid release in vitro. Using phosphate buffer (pH 7.4) as the diffusion medium and a dialysis membrane (HiMedia Mumbai, India) with a molecular weight cutoff of 14 kDa, the in-vitro release was performed according to established protocols. An in-vivo investigation was conducted, and a plot of the drug's percentage over time provided the overall drug percentage.[45]

e) In-vivo examination: The Institutional Animal Ethical Committee (Reg. No. 1762/PO/R/S/14/CPCSEA) and the IAEC (KFMSR/M.Pharm/01/2019-20) approved this study.

Before beginning the study, the animals were checked for animal activity for one week at the Animal House, Karpagam Faculty of Medical Sciences, Coimbatore, after being obtained from Kerala Veterinary and Animal Sciences University, Thiruvazhamkunnu, Kerala.

Animals: This investigation used Wistar rats weighing between 150 and 200 g of both sexes.

Animals: 150–200 g Wistar rats of both sexes were employed for this investigation.

Experimental methodology

Calculation of % dosage administered: The device's supplied dry powder (DP) dosage was computed using weights[46,47] as a baseline based on empty weights taken for the empty cannula tube.

$$\%\ \textbf{Delivered dose} = \frac{\textbf{Loaded dose} - \textbf{Remaining dose in cannula tube}}{\textbf{Loaded dose}} \times 100$$

Assessment of drug concentration in lung tissue

Grouping for assessment of drug concentration in lung tissue with a total number of 30 animals is shown in Table 1.

The animals were sacrificed at intervals of 1/2, 1, 2, 3, and 4 hours. The lung tissues that were extracted at predetermined intervals were twice rinsed with regular saline (0.9% w/v). During homogenization, 20 µL of methylparaben (100 µg/mL) was used as an internal standard. After applying 20 µL of NaOH (1 N) treatment, this was combined with 120 µL of lung or plasma sample and vortexed for ten minutes. After centrifugation, the supernatant was removed for UV analysis.[48,49]

Plasma pharmacokinetic study was estimated with collected data using oral, intravenous and intratracheal administration, as shown in Table 2.

The 150–200 g animals were weighed, numbered, and split into six units for test groups I, II, III, and control. The medication was not administered to the control group. Test groups II, III, and IV received intravenous, intratracheal, and oral doses. Animals were given an injection of 0.1 mL of diazepam to induce anesthesia prior to intratracheal therapy. The animals were then fastened to a 45°-angled platform that had self-adaptation. Using an otoscope and blunt, non-metallic tipped forceps, a gentle tug on each animal's tongue made the tracheal aperture easier to see so that a 20 G tubular cannula could be inserted.

Animal lungs were filled with formulations rich with air and DP. Three identical air bursts, separated by five minutes, were released. The animals were released and placed inside the cage. Following a seven-day period of physical behavior assessment for mouth or nose bleeding, changes in locomotion, and variations in body weight, the animals were rehabilitated. The medication was prepared as an injectable solution with sterile water and given orally and intravenously. Centrifugation was used to separate the plasma from the 0.5 mL of blood extracted from the retro-orbital vein at different intervals: 30 minutes, 1 hour,

Table 1. Grouping for assessment of drug concentration in lung tissue

Category	Drug	Dose	Route of administration	Time interval (hrs)	No. of Animals
Group I	DPI formulation	10 mg/kg	Intratracheal	½	6
Group II				1	6
Group III				2	6
Group IV				3	6
Group V				4	6
	Total number of animals				30

Table 2. Grouping for plasma pharmacokinetic Study

Group	Sample	Dose	Route of administration	No. of animals
Group 1	Control	-	-	6
Group 2	Test 1	10 mg/kg	Oral	6
Group 3	Test 2	10 mg/kg	Intravenous	6
Group 4	Test 3	10 mg/kg	Intratracheal	6
Total number of animals				24

2 hours, and 4 hours. Drug concentration was determined using the separated plasma.[48,49]

Results

Preformulation studies

A standard curve was created with phosphate buffer at a pH of 7.4 and a range of drug solution concentrations from 10 to 100 µg/mL. Based on Figure S1, the regression value (R^2) was determined to be 0.997 which shows th standard curve of L-tryptophan

The standard curve was created using phosphate buffer with a pH of 7.449 and drug doses ranging from 10 to 100 mcg/ml. Based on the data from Figure S2, the regression value (R^2) was determined to be 0.998 for the standard curve of L-tyrosine

Standard curves for L-tyrosine and L-tryptophan were constructed to understand the change in concentration during the formulation studies.

FT-IR analysis of L-tryptophan and L-tyrosine are shown in Figure S3 and Figure S4 respectively.

FT-IR analysis of lactose is captured in Figure S5.

Figure S6 depicts FT-IR analysis of DPI formulation which compares their formulation and compatibility analysis.

DSC analysis of L-tryptophan and L-tyrosine are shown in Figure S7 and Figure S8, respectively (Supplementary file 1). Simialrly, DSC spectrum of lactose and DSC analysis of DPI formulation are captured in Figure S9 and Figure S10, respectively (supplementary information). These DSC analyses of the individual chemicals employed in the formulation and the finished formulation were assessed to evaluate the effect of the combination.

Determination of solubility of drugs was carried out and the data is captured in Table S2, while the pH and partition coefficient values are shown in Table S2. From values in Table S2 the logP values for L-tryptophan and L-tyrosine can be found at -0.99 and -1.71, respectively.

Optimization & formulation development

Ball milling decreased the medications' size, and the carrier was micronized using nano spray drying technology.

In the DPI formulation, the drug-to-carrier ratio is as provided in Table S3 that indicates the values as L-tyrosine: 5, L-tryptophan: 1 and Lactose: 4

Determination of moisture content indicates 2% for 0.98 g weight and 1.0 % for 0.99 g weight for the formulations studied, as shown in Table S4 the average percentage moisture content which according to IP, was within the limit.

Following ICH recommendations, stability studies of the optimized formulation were conducted for 90 days. The results provided information on periodic drug concentration variations and physical changes as shown in Table S5. Table S6 displays the values of logP for L-tryptophan and L-tyrosine at -0.99 and -1.71, respectively. This suggests that the medications had a hydrophilic composition.

Micromeritic characterization was carried out using scanning electron microscopic analysis with data from L-tryptophan and L-tyrosine.

Scanning electron microscope photograph of L-tryptophan and L-tyrosine are shown in Figure S11 and Figure S12, respectively while that of Lactose is shown in Figure S13.

Energy dispersive spectroscopy (EDAX0-graph) of L-tryptophan is shown in Figure S14 while the elemental analysis of L-tryptophan is shown in Figure S15:

EDAX graph of L-tyrosine and EDAX-graph of L-tyrosine is shown in Figure S16. Elemental analysis of L-tyrosine is captured in Figure S17.

Powder X-ray diffraction image of L-tryptophan and L-tyrosine are shown in Figures S18 and Figure S19,

respectively.

Flow characteristics

The bulk density of the DPI formulation was found to be 0.53 g/cm³ while the tapped density of DPI formulation was 0.66 g/cm³. Carr's index and Hausner ratio were determined with evaluation of the Capsule using physical appearance, locking length and weight uniformity which indicated the stable nature of the capsules of good integrity as shown in Table S7.

Assay for drug content determination was conducted and Table S8 captures percentage of drug content of L-tryptophan and L-tyrosine.

In-vitro study was used to get Anderson cascade impactor (ACI) from the data in Table S9. ACI results for DPI formulation ($n=6$) with the parameters such as Emitted dose (%) 88.56 ± 2.96, Respiratory fraction (%) 19.6 ± 3.53 and Total recovery 95.5 ± 0.92 having a Mass median aerodynamic diameter of (μm) 1.92 ± 0.01

Figure S20 indicates the ACI histogram for percentage deposition profiles in all seven stages of DPI formulation.

Bag diffusion study for L-tryptophan in DPI formulation was carried out with dose at 10 mg at a Wavelength of 220 nm as shown in Table S10.

In-vitro drug release of L-tryptophan in DPI formulation is shown in Figure S21.

An in vitro diffusion study was conducted at pH 7.4 using phosphate buffer, as shown in Table S11 and Table S12 and Figure S21 and Figure S22.

Bag diffusion study for L-tyrosine in DPI formulation was carried out with a dose of 50 mg at a Wavelength of 283 nm as shown in Table S13.

In-vivo study was conducted to determine percentage delivered dose employing the equation given below:

$$Percentage\ of\ dose = \frac{Dose\ loaded\ -\ Dose\ remaining\ in\ cannula\ tube}{Dose\ loaded} \times 100$$

The percentage delivered dose which ranged from 84 % to 95 % for the 6 groups of animals tested with a loaded dose of 10 mg.

Lung tissue concentration dug – Assessment was carried out at 30, 60, 120, 180 and 240 minutes intervals showing the relationship between Figure 1 the lung concentration –time profile for the DPI formulation.

Plasma pharmacokinetic study revealed the plasma drug concentration at different time intervals with respect to route of drug administration (Table 3).

Plasma concentration-time profile for different routes of drug administration is compared in the graph (Figure 2).

Pharmacokinetic parameters for the formulations under different dosage forms have been determined and shown in Table 4.

The drug's distinctive peaks do not alter, as seen in the FT-IR spectra of the drug and carrier mixture in Figure s6. Thus, there was no interaction between the medication and the carrier.

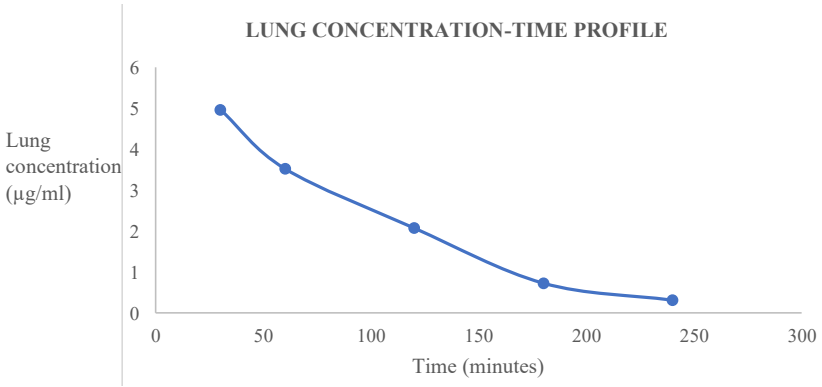

Figure 1. Lung concentration-time profile of DPI formulation

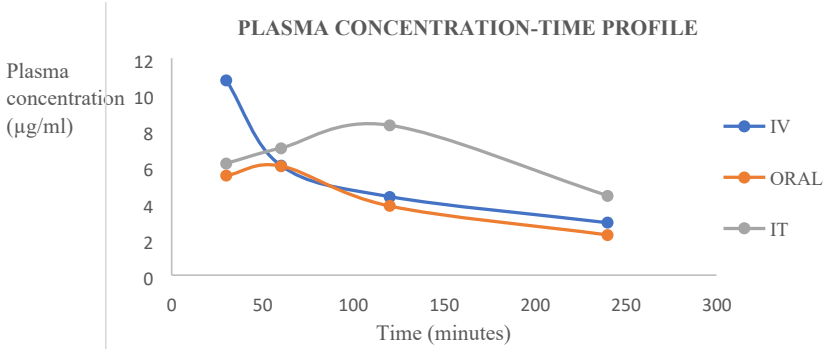

Figure 2. Plasma drug concentration at various time intervals for oral, IV, and IT routes of administration

Table 3. Plasma drug concentration at different time intervals with respect to route of drug administration

Route of drug administration		Oral	Intravenous	Intratracheal
		Plasma drug concentration (µg/mL)		
Time (min)	30	10.78 ± 0.71	5.498 ± 0.29	6.179 ± 0.09
	60	6.069 ± 0.10	6.042 ± 0.10	7.024 ± 0.05
	120	4.34 ± 0.04	3.823 ± 0.33	8.29 ± 1.56
	240	2.90 ± 0.04	2.225 ± 0.06	4.399 ± 0.05

Table 4. Pharmacokinetic parameters

S.No	Pharmacokinetic parameters	Intravenous	Oral	Intratracheal
1	C_{max} (µg/ml)	10.78	6.042	8.29
2	T_{max} (minutes)	30	60	120
3	AUC (µg/min/ml)	1160.905	914.4	1511.49
4	Relative bioavailability (%)	100	60	80
5	Mean resident time (MRT) (minutes)	103.276	108.333	115.985

According to the data in Figures S9–S12, L-tryptophan displayed a distinctive peak at 279.36 °C, which corresponded to its melting point (m. pt.); in contrast, L-tyrosine and lactose displayed similar peaks at 295.95 °C and 233.76 °C, respectively. The peak detected at 227.23 °C in the spray-dried formulation indicated the lactose phase change from solid to liquid. When contrasted with L-tryptophan and L-tyrosine, lactose was found to have a lower m. pt. As a result, there was no discernible rise at or below 227.23 °C, suggesting that the formulation is stable and does not interact until 227.23 °C.

Table 4 provides the data for the solubility investigation. This demonstrates how pH affects the drug's solubility. As pH rises, a drug's solubility increases. It follows that the medication dissolves better in distilled water.

According to IP, the average percentage moisture content was found to be 1.8%, which is within the limit based on the values.

* Mean deviation ($n = 3$)

Table S8 shows that the drug content of L-tryptophan and L-tyrosine ranges from 99.5–98.9% and 99–100%, respectively, at 25 °C / 60% RH and for 40 °C/75% RH over 0–90 days, suggesting that there were no chemical alterations or interactions over the study period.

SEM images (Figures S11–S13) (Supplementary file 1) demonstrated that spray-drying created a dry powder, which resulted in loose agglomerates of microparticles of similar sizes and surfaces. The particles had a rough surface and a good range of morphological features, with sizes ranging from 4 to 13 µm. The particles present in the form of loose agglomerates support the administration of powder dispersion through inhalation.

The EDAX diagrams in Figures S14 and S16 (Supplementary file 1) showed that elements like carbon, nitrogen, and oxygen are exclusively found in the medication, confirming its purity. Figure S15 and Figure S17 show the weights of the components found in the drugs L-tryptophan and L-tyrosine.

The diffraction pattern values with prominent peaks showed that the medication's crystalline nature was maintained even after the micronization procedure, as demonstrated by the crystallinity characteristic shown in Figure S18 and Figure S19.

Flow characteristics quantify the powder's ability to flow freely. For the formulation, the tapped density was 0.66 g/cm^3, but the bulk density was 0.53 g/cm^3. The study's Hausner ratio of 1.24 and Carr's index of 19.6% amply demonstrate strong powder flow potential after preparation.

As Table S10 shows, the percentage drug content was 100% for L-tyrosine and 99.53% for L-tryptophan, indicating that the drug level was within the allowed range.

The main variables that affect aerosolization include bulk flow, the target particle size, and the drug's and carrier's compatibility. The lactose-drug solid-phase interaction during blending resulted in an increase in particle size in the present DPI preparation. Because lung deposition and mass-median aerodynamic diameter (MMAD) inter-related, the Anderson cascade impactor was used to determine the mass-weighed aerodynamic particle size distribution (MMAD).

The in vitro diffusion study (Tables S13 and Figures S21 and S22 shows that the amino acids were released entirely in less than 30 minutes at a pH of 7.4. The investigation was conducted using phosphate buffer. The amino acids were released gradually and took up to an hour to reach a steady state concentration.

The drug released, the impact of the medication remaining in the device, and the amount supplied from the subsequent use of the device for drug delivery computed shows that the average drug dose that was administered

and that was left were found to be 87.83% and 12.16%, respectively. This indicates that 87.83% of the dosage was transmitted from the apparatus.

Since the medication is meant to be deposited in the lungs, determining the drug concentration in the lung region is a crucial criterion for DPI formulation. Figure S23 (Supplementary file 1) displays a temporal profile of lung concentration. The drug concentration was 4.971, 3.524, 2.074, 0.726, and 0.310 µg/mL at 30, 60, 120, 180, and 240 minutes, respectively. Each time interval's decrease in the drug's concentration in the lung tissue showed that the medication diffuses into the bloodstream following deposition displays the plasma concentration-time patterns for various delivery routes at different time intervals that shows how drug concentration drops as the time interval increases due to elimination. The data shows that the intravenous method had the highest plasma concentration at 10.78 µg/mL at a 30-minute interval, whereas the oral and intratracheal routes reached 5.498 and 6.179 µg/mL, respectively. Similarities were seen in the plasma drug concentrations at 60-minute intervals: 6.069 µg/mL for intravenous, 6.042 µg/mL for oral, and 7.024 µg/mL for intratracheal administration. In contrast, oral and intravenous administration reached 3.8 µg/mL for 120 minutes, 2.2 µg/mL for 240 minutes, 4.34 for 120 minutes, and 2.90 µg/mL for 240 minutes. The intratracheal route reached 8.29 µg/mL for 120 minutes and 4.39 µg/mL for 240 minutes. Compared to other methods, the intratracheal route demonstrated a longer duration of action with steady-state drug release by reaching the maximal concentration range.

The pharmacokinetic parameters of DPI for intravenous, oral, and intratracheal administration of amino acids in rats show that the maximum amount of amino acids was reached after intratracheal administration, at 8.29 ± 1.56 µg/mL, and the maximum amount after oral administration, at 6.042 ± 0.10. This suggests that compared to oral treatment, the intratracheal route of administration of amino acids provides a quicker initiation of activity and a longer duration of action. Additionally, the DPI demonstrated greater Cmax and AUC via the intratracheal route than oral administration, with a significant P value (< 0.05). This finding indicates that better dissolving rate and solubility through nanosizing, avoiding first-pass elimination and decreased enzyme activity, improves the bioavailability of the amino acids within the alveoli.

Compared to the oral dose, the intratracheal method demonstrated improved bioavailability of DPI. This indicates that the formulation can reach bronchioles, their thin linings facilitating rapid drug absorption. This offers a chance wherein the lungs serve as a great point of entry for DPI drugs that are appropriate for systemic absorption.[50–52] Low lung metabolic activity provides an additional benefit by mitigating the first-pass effect.[53–54] A relative bioavailability of 80% was reported for the produced DPI formulation administered via the intratracheal route, which is 1.3 times higher than that observed for the oral route. This improved bioavailability was achieved by creating an amino acid dry powder with micronized particles. It is therefore anticipated that this will minimize tryptophan metabolism, which is thought to be a contributing element in memory loss, without the need for additional particle engineering or modification.[49] Tryptophan's plasma half-life for an oral dosage of 10 mg was determined to be just over 4 hours. For the dry powder formulation in the current investigation, we employed 1 mg of tryptophan, 4 mg of tyrosine, and 5 mg of lactose. Compared to intravenous or oral routes of administration, the intra-tracheal route has delayed activation and maintenance of drug concentration for over 115 minutes, which is significantly higher.

This indicates that compared to previous medication formulations, the produced formulation's dry powder inhalation may be more efficacious. It has been underlined that physical and chemical stressors present barriers to protein synthesis, storage, and aerosolization. The proteins are susceptible to proteases and are cleared by alveolar macrophages once they enter the lungs.[55,56] Therefore, our specially designed dry powder for inhalation bypasses first-pass metabolism and delivers the medication straight to the lung.[57,58] The benefits of pulmonary drug administration have been effectively used in various treatments, as reviewed by Komase et al[59] and Geller et al[60] on the use of tobramycin, Nicotine gum, and varenicline[61-63] on smoking cessation has appeared that highlight the need for newer formulation in patient compliant and easy approaches.

Conclusion

Patients who fall over a broad spectrum of neuroticism are known to experience cognitive impairments as a result of serotonin levels being perturbed by ATD. L-tryptophan and L-tyrosine, the amino acids utilized in the current formulation of dry powder for inhalation, have a relaxing and alerting effect on the two neurotransmitters dopamine and norepinephrine. An additional neurotransmitter is serotonin. Therefore, keeping these amino acids at a healthy concentration may be beneficial to frequent mood swings in those who have given up smoking. Thus, by regulating the levels of the neurotransmitters involved in smoking, the current work on creating a dry powder containing these vital amino acids, L-tryptophan and L-tyrosine, for inhalation therapy will assist these individuals in suppressing their desire to smoke. A dry powder formulation for inhalation containing L-tryptophan and L-tyrosine micronized to the 2–4 µm size range with lactose as carrier was created. The designed dry powder, which targets the lungs, offers a synergistic impact. The findings of the in-

vivo investigation demonstrated a rapid onset of action, elevated drug concentration, and drug retention over a period of 115 minutes.

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None.

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All authors consent to the publication of the article.

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Not applicable.

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Supplementary Files
Supplementary file 1 contains Table S1-Table S13 and Figures S1-S23.

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