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DESIGN, DEVELOPMENT AND EVALUATION OF INTRANASAL HERBAL AEROSOL FORMULATION FOR MITIGATION OF ASTHMA

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ABSTRACT

Asthma is a chronic pulmonary disease that affects both children and adults in both developed and developing countries. It is becoming more common and serious, particularly among allergic patients. Herbs have been paid more attention since they have very little side effects. *Tylophora indica* (Asclepiadaceae) was used in traditional medicine for the treatment of asthma. The primary goal of this study is to develop a novel herbal spray using *Tylophora indica*. To achieve this goal, various techniques such as extraction, spray drying, pre-formulation studies such as solubility of herbal extracts, and Fourier transmission infrared spectroscopy (FT-IR) studies for herbal extract were investigated. Their quality control, stability, and efficacy studies were carried out in accordance with the guidelines of the United States Pharmacopoeia (USP) and the International Conference on Harmonization (ICH). Histamine-induced bronchial hyper reactivity in Guinea pigs was used to assess anti-asthmatic activity. The quality control test results were in accordance with USP standards. The particle size of the normal formulation (13.9µ) was approximately 4–5 times that of the spray-dried formulation (2.9µ). When the spray-dried test formulation was tested using the histamine-induced bronchial hyper reactivity method, a significant p value was found. The percent protection of PCD (pre convulsion dysponea) in seconds was measured after 1hour, 3hours, and 24hours of administration of histamine aerosol for the test group.

The current study attempted to design and develop a novel drug delivery system using herbal medicine to treat Asthma. An FT-IR compatibility study for the herbal formulation was performed, as well as behavioral studies.

Keywords: Asthma, *Tylophora indica*, Spray drying

INTRODUCTION

Asthma is derived from the Greek word asthma, which means 'panting' or 'breathless.' it is a bronchial tube disease that causes wheezing, shortness of breath, and coughing, especially in children **[1]** asthma is an allergic reaction that causes inflammation and narrowing of the airways, resulting in spasms and breathing difficulties **[2]**.

Asthma is a chronic pulmonary disease that affects both children and adults in both developed and developing countries. It is becoming more common and serious, particularly among allergic patients **[3]**. Asthma prevalence (the number of people who have ever been diagnosed with asthma and still have it) rises from 7.3% in 2001 to 8.4% in 2010. An estimated 25.7 million People, 18.7 million adults aged 18 and up, and 7.0 million children aged 0–17 years, were reported to have asthma **[4]**. Eosinophils, macrophages, mast cells, epithelial cells, and activated lymphocytes are among the airway inflammatory cells that release cytokines, adhesion molecules, and other mediators in asthma. Inflammation causes an acute, sub-acute, or chronic process that affects airway tone, vascular permeability, stimulates neurons, increases mucus secretion, and changes the structure of the airway in a reversible or permanent way **[5]**. The majority of asthma drugs currently available operate by calming bronchospasm (bronchodilators) or reducing inflammation (corticosteroids). While these medications are unsuccessful in treating asthma fully because they have many toxic and side effects, they are available **[6]**.

According to Ayurveda, herbal plants are less harmful and more effective than other plants. In contrast to available synthetic drug therapies, they also have a lower risk of side effects and complications for patients. According to India's Materia Medica, the herbal medication Tylophora indica has anti-inflammatory and antihistaminic properties. Histamine-induced bronchospasm and inflammation, mast cell degranulation, and inflammatory cells including leucocytes and eosinophils were all affected **[7]**. Herbal extract was used in the current analysis. A novel technique called spray drying was used to prepare the formulation for this. Preformulation experiments were carried

out prior to formulation, as well as behavioral studies.

Spray-drying is a method of generating powders by spraying the feed into a hot drying medium and processing it from a liquid state to a dried particulate form. A solution, suspension, dispersion, or emulsion would be used as the feed. The drying solution is sprayed into a hot air stream and circulated in a chamber. The dried product may be transported to cyclone or bag separators, or it may fall to the drying chamber's bottom and be expelled through a valve.

Spray-drying process consists of various steps:

1. Atomization
2. Spray-hot air contact
3. Evaporation
4. Separation

MATERIALS AND METHODS

Animals, Chemicals and Reagents

Guinea pigs, Tylophora indica plants from Bharat herb care, methanol, span 80, Chlorphenaramine maleate, Histamine hydrochloride. All the chemicals and reagents used were of analytical grade.

Instruments

Spray dryer (CRONIMACH), centrifuge (REMI), Fourier transmission infrared spectroscopy (FT-IR) (BRUKER), optical microscope (OLYMPUS), High performance thin layer chromatography (HPTLC) (CAMAG), and weighing balance (Essae) were used.

Plant material extraction and authentification

Tylophora indica was dried in the sun as a whole plant. In the mixer grinder, dried whole plants were ground and the powder was weighed. In a soxhlet apparatus at temperatures below 50 ˚C, 200 gm of dried powder was extracted with ethanol. The extraction took around 18 to 20 hours. The plant material was then removed from the soxhlet apparatus and dried until the ethanol evaporated. The ethanolic extract was refrigerated until it was used. The Department of Botany at Acharya Nagarjuna University (ANU), Guntur, authenticated the entire plant of *Tylophora indica*.

EXPERIMENTAL METHODOLOGY

Compatibility studies

FT-IR study was carried out using FT-IR (BRUKER), where the spectra of herbal extract, spray dried herbal extract, and the final formulation were taken for the study.

Design of formulation

Drug delivery system containing herbal extracts was developed by the following steps.

By spray-drying technique

1gm of herbal extract was weighed and dissolved in a 1:1 mixture of ethanol and water. The formed solution was stirred for 5 minutes at 2800–3000 rpm with ultra-

Turrax. The resulting solution was spray dried at 115°C inlet air temperature and 1.5-2 psig pressure **(Figure 1) [8]**.

Preparation of Intranasal Herbal Aerosol Formulation

The product concentrate (2 percent w/v extract of *Tylophora indica* containing 0.02 percent w/v sodium benzoate and the propellant HFA-134a) was used to develop an aerosol dosage form. The product concentrate was quantitatively packed into a cold sterilized aerosol container, and the valve assembly was inserted and crimped using a semiautomatic aerosol filling machine (twin tech, India). The propellant was then dispensed into the container via an automatic aerosol filling machine. Packed containers were subjected to a leakage test before being labeled and stamped with the batch number and manufacturing date.

Evaluation:

Measurement of particle size:

Using an Olympus CX 21i biological microscope, the particle sizes of all formulations were determined. The formulations were placed on the glass slide and suspended with glycerin-water before being covered with a cover slip and measured. The images were captured with a microscope, and the particle size was calculated to determine the average minimum radius and average maximum radius.

Quality Control of Intranasal Herbal aerosol Formulation:

The flammability test, physico-chemical characterization, performance, and biological evaluation for the finished topical herbal aerosol spray formulation were performed in accordance with the procedure described in Lachman *et* al and USP **[9]**.

Zeta Potential: -

Zeta potential is used to determine the particle size distribution by laser diffraction in the formulation and to access its degree of flocculation. The technique of laser diffraction is based on the principle that particles passing through a laser beam will scatter light at an angle that is directly related to their size where the large particles scatter at low angles and small particles scatter at high angles.

Zeta Potential	Inference
0±5	Rapid coagulation of flocculation
10-30	Incipient stability
30-40	Moderate stability
40-50	Good stability
>60	Excellent stability

High Performance Thin Layer Chromatography [HPTLC]:

HPTLC analysis was carried out by taking 10 mg of spray dried formulations. It was dissolved in 1 ml methanol and centrifuged at 3000 rpm for 5 minutes and used for HPTLC analysis as test sample. The aliquot of 2 µl of the samples were loaded as 6 mm band length at a 15.0 mm application position in a 10 × 10 cm silica gel 60 F 254 TLCplate using a CAMAG automatic TLC sampler IV (CAMAG, Switzerland). Distance between the tracks was maintained at 23.3 mm.

Chromatographic conditions: Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase.

Stationary phase: - Aluminum back coated silica gel of 60F and 254

Mobile phase: - Toulene: Chloroform: Methanol: Ammonia [4.5: 2.5: 0.5: 0.5]

Flow rate: - 20 µl / sec

Plate size: - 5×5 mm

Detection wavelength: - 254 nm & 366 nm

Dosing speed: - 20 µl / sec

Injection volume: - 20 µ

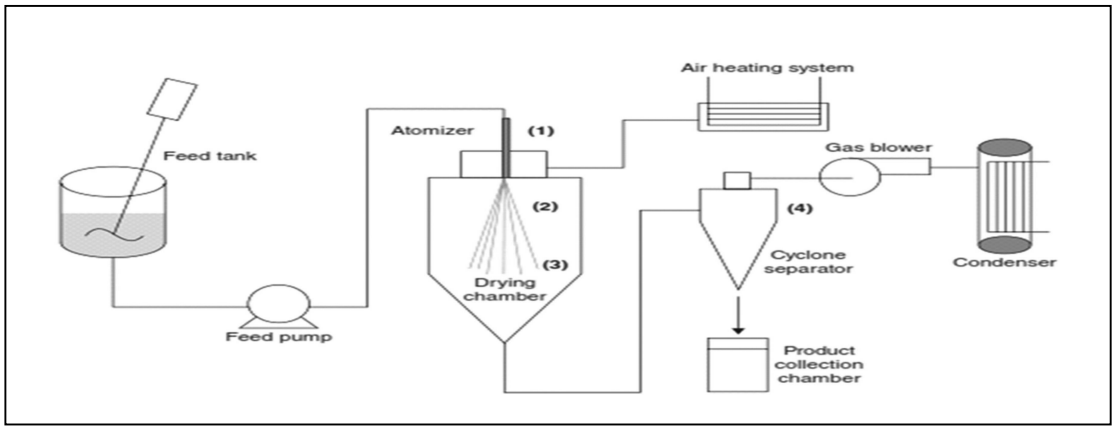


Fig. 1: Process steps of spray drying.
(1) Atomization, (2) Spray-hot air contact, (3) Evaporation, (4) Separation

In-vivo Studies:

Guinea pig weighing 350-500 gm the animals were housed in under well-controlled conditions of temperature (22 ± 2˚C), humidity (55 ± 5%) and 12h/12h light-dark cycle. Animals had received standard pellet diet and drinking water. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. Each group contains four animals.

Ethical approval

All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to Committee for the Purpose of Control and

Supervision of Experimental Animals (CPCSEA) registered no: 14/IAEC/CLPT/2020-21 at the Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur.

Experimental design:-

Histamine induced bronchoconstriction in guinea pig.

Symptom like asphyctic convulsion resembling bronchial asthma can be induced by inhalation of histamine or other bronchospasmogen in guinea pig. The occurrence of these symptoms can be delayed by bronchodilator drugs **[10]**.

Procedure: - [11]

- Overnight fasted guinea pigs were randomly divided into four groups [n=4]. Prior to drug treatment each animal was placed in histamine chamber and exposed to 0.2% histamine aerosol.
- The pre-convulsion dysponea time [PCD] was noted for each animal. The pre-convulsion dysponea time is the time of aerosol exposure to the onset of dysponea leading to the appearance of convulsion
- As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover from the dysponea from 24 hrs.
- This time of dysponea was recorded as basal value.
- Group 1:- After 24 hrs animals are served as control
- Group 2:- Animals were administered with Chlorphenaramine maleate [1mg/kg ip].
- Group 3 & 4: - Animals were received respective doses of *Tylophora indica.*
- These animals were again subjected to histamine aerosol. Later at an interval of 1hr, 3hrs, and 24hrs and to determine PCD.
- The protection offered by the treatment was calculated by using the formula:

$$\% \text{ Protection} = [1 - T_1/T_2] \times 100$$

T_1 – the means of PCT before administration of test drugs

T_2 – the means of PCT after administration of test drugs at 1hr, 3hrs, and 24 hrs.

Statistical Analysis:-

The results of various studies were expressed as mean ± SEM and analyzed statistically using one way ANOVA followed by student t-test to find out the level of significance. Data were considered statistically significant at minimum level of $P< 0.05$.

RESULTS AND DISCUSSION

FT-IR studies:

The significant peaks that were present in herbal extract were also existed in spray-

dried herbal extract, and final formulation, indicating that there were no incompatibilities in the extracts of study **(Figure 2, 3 & 4)**.

Particle size:

The particle size determination was carried out using Olympus CX 21i Biological Microscope. The optical microscopy images are shown in **(Figure 5)**. The average minimum radius and average maximum radius of product was tabulated **(Table 1)**.

Quality Control Test for Intranasal Herbal Aerosol Formulation:

The flame extended for 6 cm in a flammability test after spraying the formulation in an open flame, indicating that the product is not extremely flammable. The physicochemical characterization effects, i.e. Vapour pressure, density, ph, viscosity, pr **(Table 2)**. Opellant compatibility of product concentrates, discharge rate, spray pattern, net content, particle size, leakage test, and spray angle were in accordance with USP standards.

High Performance Thin Layer Chromatography [HPTLC]: (Figure 6).

Effect of methanolic extract of *Tylophora indica* on histamine induced bronchoconstriction in guinea pig:

The guinea pigs when exposed to 0.2%w/v histamine aerosol showed signs of progressive Dysponea leading to convulsions. Chlorphenaramine maleate (1mg/kg, ip) significantly prolonged ($p<0.05$) the preconvulsive dysponea in 1st, 4th and 24th hr as compared to test and the percent % protection observed was respectively **(Table 3)**.

The ethanolic extract of leaves of *Tylophora indica* at doses of 200 mg/kg ($p<0.05$) and at the dose of 400mg/kg nasal spray. ($p<0.02$) significantly prolonged the preconvulsive dysponea at 1st, 4th hr and 24 hr as compared to control. Thus showed more protection against preconvulsive dysponea as compared to control, following exposure to histamine aerosol.

The percent protection observed for *Tylophora indica* at the dose of 200 mg/kg was 38.10, 52.95 & 26.62 in 1st, 4th and 24th hr respectively. The percent protection observed for at the dose of 400 mg/kg was 61.17, 69.63 & 30.74 in 1st, 4th and 24th hr respectively **(Figure 7)**.

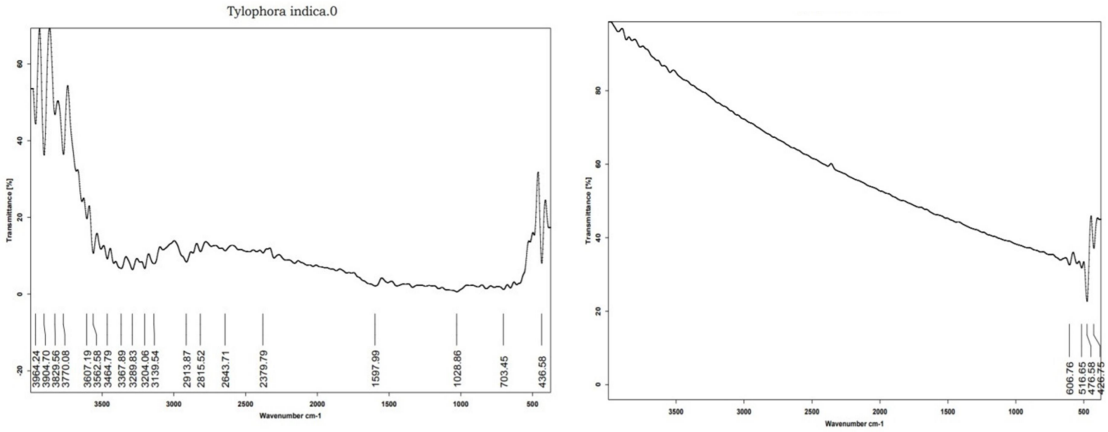


Figure 2 & 3: FT-IR Spectrum of *Tylophora indica*, spray dried *Tylophora indica*

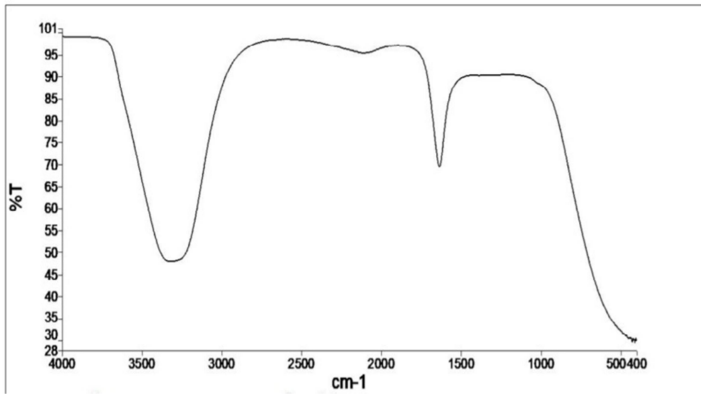


Figure 4: FT-IR Spectrum of final formulation

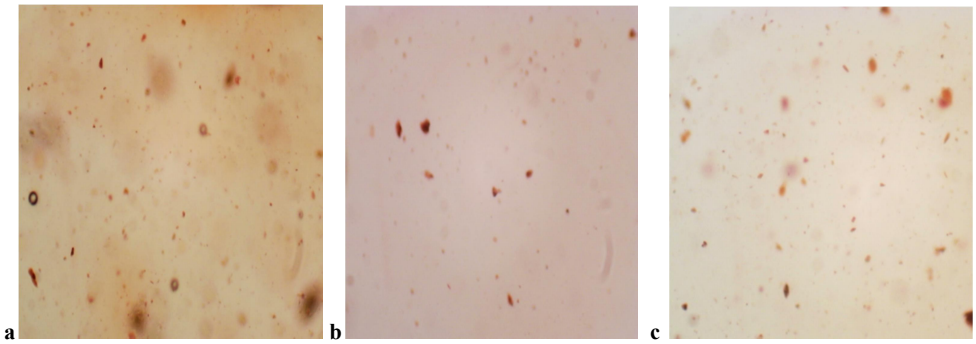


Figure 5: Optical microscopy images of formulations. (a) Herbal extract, (b) Spray dried -herbal extract, (c) Final formulation

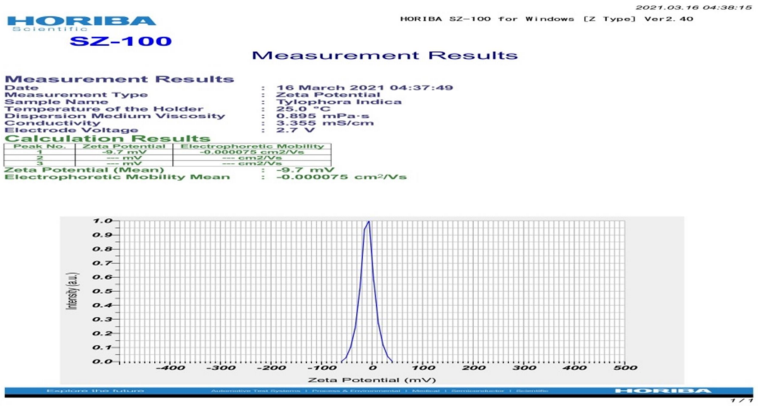
Table 1: Particle size analysis

S. No.	Product	Number of particles	Average minimum radius (µm)	Average maximum radius (µm)
01	Extract	29	13.381	25.789
02	Spray dried extract	35	3.679	8.567
03	Final formulation	35	2.931	7.871

Table 2: Quality control test for Intranasal herbal aerosol formulation

S. No.		Parameters	Value	Units
1	Flammability	Flame extension	6	Cm
2	Physico - chemical characteristics	• Vapour pressure • Density • P^H • Viscosity • Compatibility of product concentrate with propellants	160 0.854 4.3 0.894 No turbidity	Psig gm - mpa.s
3	Performance	• Aerosol valve discharge rate • Spray pattern • Net content • Leakage test • Particle size range • Total No. of discharges per container • Spray angle	0.342 5 200 No 2-7 210 puff 21	g/3 sec Cm Doses µm 3 sec for each puff

Zeta Potential:



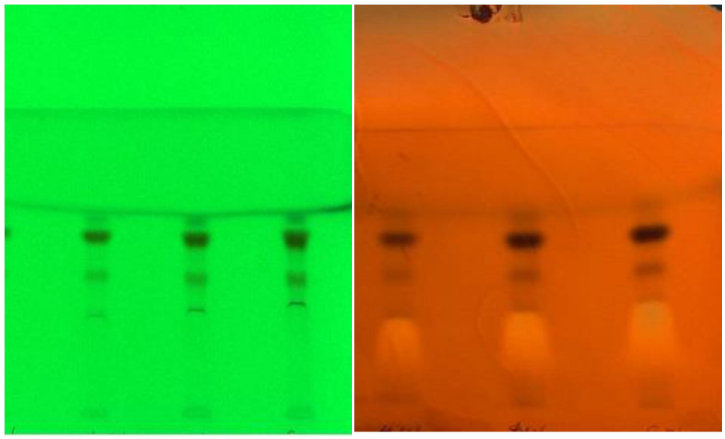


Figure 6: HPTLC Profile of Formulation at 254 nm and 366 nm

Table 3: Effect of ethanolic extracts of *Tylophora indica* on percent protection in histamine induced bronchoconstriction in guinea pigs

Group (n=4)	Before treatment in (sec)	PCT (Mean ± SEM) at After treatment(in sec)			Percent protection [1-T1/T2]×100		
		1hr	4hr	24hrs	1hr	4 hr	24hrs
I.	20.03±1.14	21.02±1.13	20.81±1.12	20.45±1.10	4.70	3.74	2.05
II.	22.6±1.18	60.3±1.15	81.01±1.22	30.07±1.41	62.52	72.10	24.84
III.	19.9±1.03	32.5±1.09	42.3±1.45	27.12±1.76	38.10	52.95	26.62
IV.	21.65±1.5	55.68±1.22	71.3±1.6	31.26±1.76	61.17	69.63	30.74

Data expressed as Mean ± SEM, where n=4,
Statistical analysis done by ANOVA followed by Brown-Forsythe test, where *p<0.05, **p<0.02 when group II, III, IV were compared with group I.
Group 1 (control) = Aerosolized histamine (0.2%)
Group 2 (std) = Aerosolized histamine (0.2%) + Chlorphenaramine maleate (1mg/kg, i.p.)
Group 3 (*Tylophora indica*-200) = Aerosolized histamine (0.2%) + Methanolic extract of *Tylophora indica* (200mg/kg, nasal spray.)
Group 4 (*Tylophora indica*-400) = Aerosolized histamine (0.2%) + Methanolic extract of Tylophora indica (400mg/kg, nasal spray.)

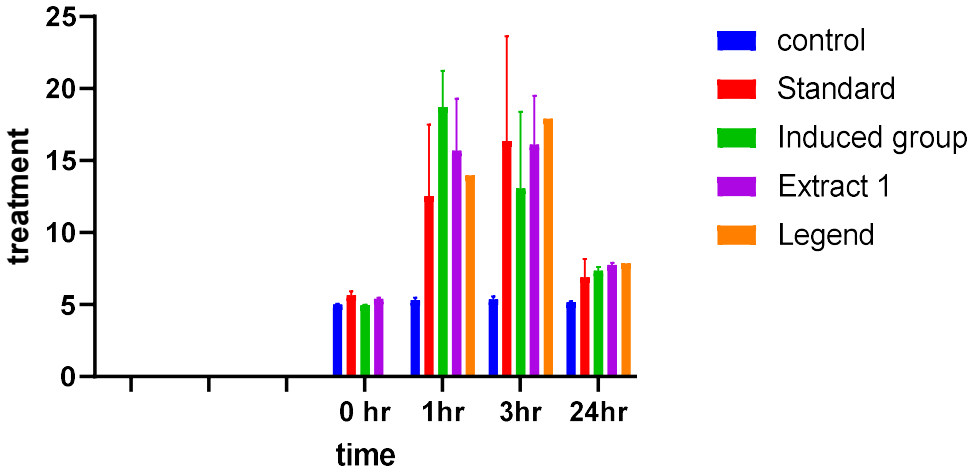


Figure 7: Histamine induced Bronchoconstriction in guinea pig

CONCLUSION

The current study attempted to design and develop a novel drug delivery system for treating ASTHMA using herbal medicine. Spray drying was used to conduct an FT-IR compatibility study for the herbal extract (*Tylophora indica* extract). The formulation was characterized for particle size analysis using an Olympus CX 21i Biological Microscope, a particle size analyzer, and HPTLC, as well as for behavioral testing using histamine-induced bronchial hyperactivity, and it was compared to a formulation that did not use the spray-drying technique. According to Table1, the particle size of normal formulation (13.9 µm) was approximately 4–5 times larger than that of spray-dried formulation (2.9 µm). When the spray-dried test formulation was tested using the histamine induced

bronchial hyperactivity method, a significant p value was obtained (using Graph Pad Prism 6 software). The percent protection for the test groups (III& IV) was 38.10, 52.95 & 26.62 in 1st, 4th and 24th hr respectively and 61.17, 69.63 & 30.74 in 1st, 4th and 24th hr respectively. Further research will be conducted in order to develop an inhalation dosage form.

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REFERENCES

[1] Holgate ST, Polosa R. Treatment strategies for allergy and asthma. Nat Rev Immunol. 2008; 8(3): 218–30.

[2] Del Donno M, Bittesnich D, Chetta A, Olivieri D, Lopez-Vidriero MT. The effect of inflammation on mucociliary clearance asthma: An overview. Chest [Internet]. 2000; 118(4): 1142–9. Available from: http://dx.doi.org/10.1378/chest.118.4.1142

[3] Moorman JE, Akinbami LJ, Bailey CM et al. National Surveillance of Asthma : United States., 2010. 2001–2010 p.

[4] Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, et al. Trends in asthma prevalence, health care use and mortality in the United States, 2001-2010. NCHS data brief, no 94. Hyattsville, MD: National Center for Health Statistics. NCBS Data Brief. 2012; May(94):1–8.

[5] National *Asthma* Education and Prevention Program Expert Panel Report 3 : Guidelines for the Diagnosis and Management of Asthma Full Report 2007. Children. 2007; 11–2.

[6] Thompson Coon J. Goodman and Gilman's the Pharmacological Basis of Therapeutics. Vol. 7, Focus on Alternative and Complementary Therapies. 2010. 207–207 p.

[7] Profile SEE. Evaluation of Antiasthmatic Activity of Dried Whole Plant Extract. 2014;(January 2012).

[8] RAMa Rao Nadendla, Lakshmi Swapna Sai, Nihitha Sanka, Santhi

Priya Nagam. Design and Development of Novel Drug Delivery System Using Herbal Medicine To Treat Alzheimer'S Disease. Asian J Pharm Clin Res. 2019; 12(4): 202–10.

[9] Aiyalu R. Formulation and Evaluation of Novel Herbal Aerosol for Arthritis. J Rheumatol Arthritic Dis. 2017; 2(4): 1–12.

[10] Champeroux P, Guth BD, Markert M, Rast G. Methods in cardiovascular safety pharmacology. Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays, Second Edition. 2013. 73–98 p.

[11] Genwa C, Gilhotra UK, Varma AK, Rajpurohit B. Evaluation of Antiasthmatic Activity of Citrullus Colocynthis Fruit. 2017; 8(2): 716–21.