IJPSR (2017), Volume 8, Issue 8 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 January, 2017; received in revised form, 29 March, 2017; accepted, 16 April, 2017; published 01 August, 2017

INTRANASAL DRUG DELIVERY - NEW CONCEPT OF THERAPEUTIC IMPLICATIONS FOR EFFECTIVE TREATMENT OF CNS DISORDERS

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Keywords:

Blood brain barrier, Bioavailability, Mucocilliary clearance, Nasal cavity, Biopharmaceuticals, Nasal drug absorption

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ABSTRACT: Intra nasal drug delivery system (INDDS) has emerged as a non-invasive alternative for delivering peptides, proteins and other therapeutic agents, specifically to CNS (Central nervous system). This route is well suitable for systemic delivery because of high vasculature, permeability, elimination of hepatic first pass metabolism and low enzymatic environment of the nasal cavity. This route of delivery is especially suited to treat CNS disorders like - neurodegenerative diseases, mood disorders, cognitive dysfunctioning etc., due to drugs ability to directly reach the brain by passing blood brain barrier (BBB). It is also a well suited delivery route for the biotechnological products like DNA plasmids, hormone, proteins, peptides, DNA vaccines to give enhanced bioavailability. A wide variety of therapeutic compounds may be administered intra-nasally for topical, systemic and CNS disorders. In this review, we have focussed on the nasal passage anatomical pathway leading to CNS drug delivery, different factors affecting nasal absorption, bioavailability barriers, vaccines and other therapeutic, biologically active molecules delivered via intra nasal route and strategies to improve nasal absorption. We have also outlined various *in vitro* and *in vivo* cellular models for nasal drug absorption and permeability studies.

INTRODUCTION: Intra nasal drug delivery (INDD) system was used for intake of various medicinal compounds in Indian Ayurvedic and yogic medicinal practices by referring them as "*Nasya karma*" or "*Nasya rasayana*" from last thousands of years. And in today's modern medicine concept also, in response to the insufficiency of conventional delivery mechanisms, nasal drug delivery (NDD) route has been extensively explored as an alternative system to promote more effective delivery of drug molecules [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(8).3194-05

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (8).3194-05

Due to the ever-increasing pharmaceutical technology and diverse medicinal opportunities, intranasal administration has proven to be a reliable delivery system for several peptides, proteins and biopharmaceuticals like - insulin, VEGF, Nerve growth factor (NGF), activity derived neurotrophic factor (ADNF) and Vasoactive intestinal protein (VIP), Neuropeptide Y (NPY) etc [3].

Nasal products have been used for several therapeutic indications, which includes pain management, treatment of erectile dysfunction, migraine headaches etc. But, if we specifically discuss the treatment of central nervous system (CNS) disorders, the clinical failure of much potentially effective therapeutics is often not due to lack of drug potency rather, due to shortcomings in the method by which the drug is delivered and hence, here INDD system has proven to be a

landmark strategy in improving the drug absorption and bioavailability with respect to neuro therapeutics [4]. Many essential therapeutic products are successfully administered via this route due to supportive anatomical, physiological and histological characteristics of nasal cavity; [5] it encourages fast drug absorption, extensive vascular supply and quick onset of action of these agents [6] with benefit of by passing the first pass metabolism of drugs.

This route is simple, effective, painless and non-invasive hence, making it more patient complaint. Due to the direct targeted approach of drug administration through INDDS, it enhances the bioavailability of drug molecules, accomplishing them to be a suitable mode for replacement of many parenteral drugs and long term therapies. Besides, all these gains, intranasal drugs are targeted for local, systemic as well as olfactory administration. Likewise, for various local nasal symptoms treatment like –delivery of steroidal agents, anti- histamines, corticosteroids etc. and for systemic action, it includes treatment for migraine headaches, calcium supplementation, Vitamin B_{12} deficiency, pain management [7]. Whereas, targeting brain, for the treatment of various CNS disorders like brain tumours, Alzheimer's disease; epilepsy is an important application of intranasal drug delivery system [8, 9].

Anatomical layout of INDD route: The nasal cavity has a total volume of about 16 to19 ml and has extensive surface area. It is divided in to two nasal cavities by the nasal septum and is maintained by high degree of vascularity. After the drug administration into the nasal cavity, a solute can be deposited at one or more anatomically distinct locations i.e. may be in the vestibular, respiratory or olfactory regions as shown (**Fig. 1a**). The vestibular region is present at the opening of the nasal passage and its main role is to prevent the entry of air borne particles (dust, microbes etc.), further it extends into three turbinates: superior, middle and inferior. So, if the drug is delivered/ deposited into the superior turbinate, it takes up the olfactory pathway, finally, delivering the drug molecule into the cerebral cortex, curing the CNS disorders (brain tumours, Alzheimer's disease, epilepsy). Similarly, the depositions in the middle turbinate leads to the local treatment by the drug for the conditions like (migraine headaches, calcium supplementation, and Vitamin B12 deficiency). And finally, if the drug molecule is deposited in the inferior turbinate then, it is expected to be taken up in the systemic circulation.

Additionally, the nasal passage consists of four distinct types of cells: ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells (Figure 1b), to facilitate the INDD. Ciliated cells contain fine projections called cilia on the apical surface and are responsible for transportation of mucus towards nasopharynx. [10] The non- ciliated cells are involved in transportation of fluid in and out of the cells. [11] The goblet cells contain a variety of secretory granules. Basal cells are the precursors of ciliated and non- ciliated cells which are poorly differentiated. [12] As little as 5% of the total area of nasal cavity in man is constituted by olfactory epithelium. [13] The interesting thing about INDD is that the drugs can be delivered to brain thereby bypassing the blood brain barrier. [14] The olfactory epithelium, a pseudo stratified epithelium, comprises of sensory neurons and two types of cells; basal cells that have the capacity to differentiate into neuronal receptor cells and sustentacular cells (elongated cells resting on the basement of the epithelium) ensheath neuronal receptor cells thereby providing the support and helps in maintaining the extra cellular potassium level for activity of neurons. [15].

Mechanism of drug absorption through INDD route: The drug molecules may cross the olfactory epithelium through olfactory bulb and circumvent the BBB via various suitable transport systems like - simple diffusion, receptor-mediated transcytosis, cell mediated, and transcellular or paracellular transport, to reach the cerebral cortex [16]. The drug transportation mechanism is expedited by either of the process (transcellular, paracellular and transcytosis).

Transcellular route is responsible for movement of lipophilic drugs, within the cells and show rate dependency on their lipophilicity, on the contrary, the paracellular route is more slow, passive movement of molecules between the cells and there is an inverse log-log relation between molecular weight of the therapeutics and the nasal drug absorption as it's been reported that the compounds

having molecular weight more than ~ 1KDa seem to have low bioavailability [17]. Transcytosis is a type of transcellular transport in which macromolecules are captured in vesicles on one side of the cells, drawn across the cell and ejected on the other side. **Fig. 2** represents different routes of drug transportation through INDD.

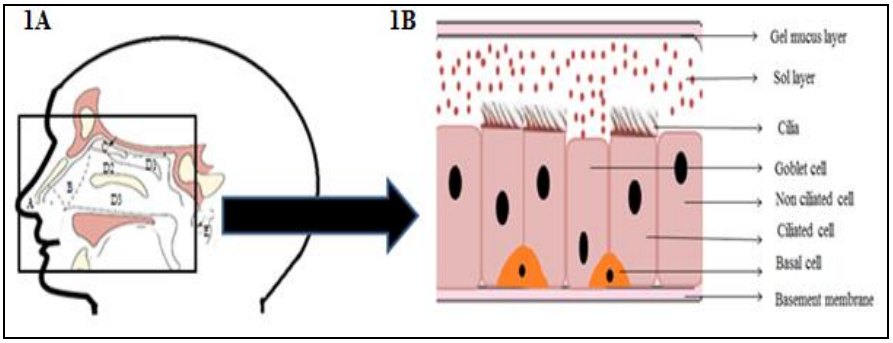

FIG. 1A: SCHEMATIC OF A SAGGITAL SECTION OF THE NASAL CAVITY, SHOWING THE (A): NASAL VESTIBULE (B): ATRIUM (C): RESPIRATORY AREA: OLFACTORY REGION (D1): SUPERIOR TURBINATE (D2): MIDDLE TURBINATE (D3): INFERIOR TURBINATE RESPIRATORY AREA (E): NASOPHARYNX
FIG. 1B: IMAGE SHOWS VARIOUS CELLS PRESENT IN RESPIRATORY REGION

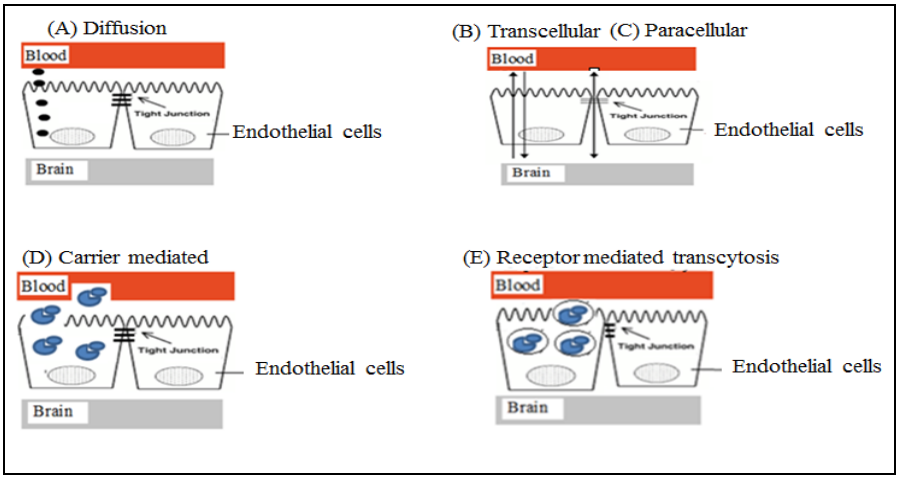

FIG. 2: DIFFERENT ROUTE OF DRUG TRANSPORTATION THROUGH INDD SYSTEM. IMAGE (A) SHOWS THE PROCESS OF DIFFUSION; (B) REPRESENTS TRANSCELLULAR; (C) PARACELLULAR ROUTE OF TRANSPORT; (D): DESCRIBES CARRIER MEDIATED PROCESS AND (E) SHOWS RECEPTOR MEDIATED TRANSCYTOSIS

Barriers to INDD absorption: Polar molecules generally have low bioavailability as they are unable to pass through the nasal membrane but lipophilic molecules can pass through the membrane easily. Also, the molecules having molecular weight, more than 1 KDa (such as proteins and peptides) have difficulty in crossing the membrane, because the pore size of almost all of the biological membranes are less, thus, exhibiting low bioavailability [2]. Another important factor for low absorption of therapeutic molecules is low membrane transport; which may be accounted due to the process of mucocilliary clearance (MCC), especially in case of drugs which do not get absorbed across the nasal membrane. MCC is an important function of the upper respiratory tract and the role is to prevent toxic substances (bacteria, toxins, viruses, allergens etc.) from reaching the lungs. When these toxins get adhered or dissolved in the mucus lining of the cavity, they are eventually transported towards nasopharynx for clearance from the body through gastrointestinal tract (GIT). [18] Removal of mucus and toxins which are either adsorbed or deposited from the nasal cavity into the GIT is called MCC. This mechanism of MCC influences the process of absorption because of the dissolved drugs in the nasal cavity which is released by both cilia and mucus [19]. It has also been observed in many studies that deposition of molecules in the anterior end of

the cavity decreases MCC, leading to increase in absorption and bioavailability [20, 21]. Likewise, also due to the presence of enzymes such as peptidases - Exo- peptidases (mono and diamino-peptidases), which cleave the peptides at N and C terminal and endo- peptidases (serine and cysteine) cleaves the internal peptide bond, in the lumen of the nasal cavity, causes low transport of these biomolecules leading to low absorption of therapeutic molecules. Many approaches are been adopted to overcome this physiological barriers either by using various bio adhesive excipients (cellulose derivatives, poly acrylates, starch, chitosan) to decrease MCC [22] or by using enzyme inhibitors and also by inciting saturation of enzymes to inhibit peptidases [23].

As discussed above**,** the molecular size of the drug influences the absorption through the nasal route [24]. Further, the nature of the molecule (lipophilic and hydrophilic) also affects the absorption process, for example on increasing the lipophilicity the permeation through the cavity increases [25]. Despite, there is some hydrophilic character found in the nasal mucosae but it is assumed that they are basically lipophilic and the function of the lipid domain is to act as a barrier for these membranes.[26].

Moreover**,** the pH of the formulation and nasal surface, can affect the drug's permeation through nasal epithelium [27]. The pH of the nasal formulation should lie in the range of 4.5–6.5, as presence of lysozyme is found in nasal secretions within these limits, which is responsible for destroying certain bacteria at acidic pH. Above this limit the enzyme is inactivated and the tissue is susceptible to microbial infection. Equivalently, osmolarity of the formulation too affects the nasal absorption of the drug and it was reported that higher the concentration more will be the bioavailability but above, 0.462 M sodium chloride concentration, it may lead to the toxicity in nasal epithelium [28]. Viscosity of the nasal formulation influences permeability across the epithelium. Higher the viscosity of the nasal formulation more will be the contact time between the nasal mucosa and formulation thus, increasing the absorption and permeation through the cavity. Further, it was observed that highly viscous nasal formulations interfere with MCC or the ciliary beating thus, altering the absorption of the drugs.

Strategies to improve nasal absorption: Recently many research studies along with various strategical drug delivery approaches, are been focussed to increase the bioavailability, absorption through nasal cavity and residence time of the therapeutics, and to modify their structures according to the physicochemical properties in the nasal mucosa. INDD is used to target a variety of drug molecules to the Central nervous system. [29] Here are some examples of drugs administered intranasally to treat CNS disorders: neurotrophins [30] (NGF [31] and insulin-like growth factor [IGF]-1[32]); neuropeptides (hypocretin-1 [33] and exendin [34]; cytokines (interferon β-1b [35] and erythropoietin [36]); polynucleotides (DNA plasmids [37] and genes [38]); and small molecules (chemotherapeutics [39] and carbamazepine [40]) etc.

INDD is best delivery route for the therapeutics which are active in nanomolar concentration. [41] Even therapeutic substrates like - P-glycoprotein efflux transporter protein, which are known to work in the epithelia of nasal, is investigated to reach the CNS in effective concentration. [42] The fascinating thing about INDD is that the drugs can be delivered to the brain bypassing the blood brain barrier and helps in the treatment of various neurodegenerative diseases. This strategic delivery routes is found to be advantageous in- Alzheimer's disease, Epilepsy, Parkinson's disease, or pain [43] where rapid or/and targeted delivery of therapeutics is required.

The nasal administration of protein, advanced peptide and vaccine research provides an attractive delivery route. [44, 45] Because of the large molecular size of the proteins, the bioavailability of these molecules was low and degradation by enzymes was some of the disadvantages of conventional delivery systems. Proteins and peptides are administered parenterally because of their susceptibility to hepatic first pass effect and their physicochemical instability. It is on this basis that INDD is a promising delivery route. Most of the formulations are made in simple aqueous or saline solution with certain preservatives in it. Nowadays, research is focussed on the development of INDD systems for delivery of peptide/proteins. In the United States only four intranasal products were developed for systemic drug delivery (till 2004) and were marketed.

These are Nafarelin acetate (Synarel), Lypressin (Diapid), desmopressin (DDAVP), Oxytocin (Syntocinon). Proteins and peptides such as arginine vasopressin, insulin, cholecystokinin analog and adrenocorticotropic hormone are delivered through intranasal route directly to brain. [46] For delivering of protein and peptide therapeutic agents to the central nervous system, extra neuronal transport is involved. It was observed from *in vitro* and *in vivo* studies that higher concentration of hexarelin (a growth hormone releasing neuropeptide) was attained in the brain and cerebro spinal fluid when delivered through intranasal route when compared to intravenous administration of the same. [47] Lack of ionization at physiological pH, lipophilicity, low molecular weight favours CNS penetration. The blood brain barrier can be by passed by delivering poorly lipid soluble molecules to the brain. The transient osmotic opening of blood brain barrier, exploitation of transporter proteins, high dosage of chemotherapy are some of the strategies. [48]

Delivering vaccines by nasal route: Chitosan microspheres made with sodium sulfate by the process of ionic gelation have been used for nasal vaccine delivery. [49] On chitosan nanoparticles, a causative agent of Atrophic rhinitis (AR) *i.e.* Bordetella Bronchiseptica Dermonecrotoxin (BBD) was loaded and used for vaccination. With increase of chitosan's molecular weight and pH of media *in vitro* because of weaker interaction between BBD and chitosan, more BBD was released. RAW 264.7 cells when treated with BBD loaded chitosan nanoparticles, released Nitric oxide (NO) and Tumour necrosis factor-alpha (TNF alpha). Thus, concluding that BBD which was released from the chitosan nanoparticles had therapeutic effect against AR.

Therapeutic nanoparticles: Nanoparticles serve as a vehicle for loading the drug and it plays an integral role in targeting brain. [50, 51] These carrier systems can be used to maintain desirable range of drug in the plasma. Further they can be utilized to increase stability, half-lives, permeability, and solubility of drugs. These nanoparticles can be adapted structurally so as to deliver a wide range of drugs, improve drug releasing efficiency, and further, reducing side effects by targeted drug delivery strategy. [52] Dendrimers, polymeric micelles and liposomes are some of the examples nanoparticles which are used for constructing nanomedicines.

All these kinds of delivery vehicles have found applications in treating CNS disorders. [53] Recently, anti - EGFR antibody-carrying immuno liposomes were used to administer BSH (Sodium boro captate) for treatment of neuron. [54] A large amount of boron is delivered via this delivery system to glioma cells. Dendrimers can be used as a carrier system to deliver therapeutics and they are reported to cross the physiologic barriers *e.g.* - Polyanionic PAMAM dendrimers (polyamidoamine) has showed rapid serosal transfer rates in crossing adult rat intestine and showed low tissue deposition. [55, 56] Angiopep, a peptide with a high brain penetration, which is known to target LRP1 (Low-density lipoprotein receptor-related protein-1), was linked through the distal end of PEG to PEGylated PAMAM dendrimer generation 5 (G5.0). Thus, it was used to deliver pEGFP-N2 plasmid to the brain. [57] For targeted drug delivery to the brain: the architecture and size of dendritic nanoparticles are taken into account because they affect efficiency of drug delivery to brain. PEPE dendrimer (poly ether co polyester) with various types of branching structures was synthesised and the *in vitro* studies reflected that the cellular upkate, absorption and permeation of PEPE across the BBB was affected by the architecture of dendrimers. [58]

The PEPE dendrimers were internalized by the process of Clathrin- and caveolin mediated endocytosis (raft dependent endocytosis) and were shown to cross the blood brain barrier without disturbing the tight junctions suggesting further validation for brain targetting. The challenge is to design the drugs which are able to reach the site of tumor and cross the BBB. Dendrimer nanoparticles with the range of 11.7 to 11.9 nm crossed BBB tumor pores of RG-2 malignant gliomas. [59] The transport of carbamazepine to cerebral cortex was studied on endothelial cells of cultured brain of a rat (rBMEC) and ABC transporter efflux proteins (ATP-binding cassette) were reported to transport carbamazepine across the blood brain barrier. [60] Drugs are released in endothelium cells which are further transported to the brain (after the process of endocytosis) by transcytosis or diffusion. [61]

For example, for effective treatment of Alzheimer's disease (characterized by disbalance in homeostatis of iron level in brain) chelator-nanoparticle (NP) system conjugated with iron and chelator-NP system was used. [62] The *in vitro* studies reported preferential adsorption of apolipoprotein A-I and apolipoprotein E, thus implying transportation of chelator- nanoparticle system and chelator nanoparticle system complexed with iron across the blood brain barrier. [63]

These findings were further supplemented by a study in which PBCA NPs (Poly butyl cyanoacrylate nanoparticles) coated with poloxamer 188 (Pluronic® F68) and bounded to doxorubicin were synthesised and PBCA synthesised NPs resulted in enhancement of anti-tumour effect of doxorubicin when compared to intracranial glioblastoma in rats. [64] The hypothesis behind increase in anti-tumour effect was the interaction of (Apo A-1) apolipoprotein A-I present on the surface of PBCA NPs, with the SCRAB 1 (scavenger receptor class B, type I), which is the receptor for high density lipoprotein/ ApoA-I which is expressed on BCEC (Brain capillary endothelial cells). However, more focussed R & D is needed to know the roles of Apo-A1 and SCRAB-1 to know the mechanism in increasing the drug delivery by receptor mediated endocytosis and mechanisms of interaction between the two. [65] In a novel approach, the design of a delivery sytem to brain called Angiopeps (a family of Kunitz domain-derived peptides) was reported, using *in situ* brain perfusion system and an *in vitro* model of the blood brain barrier. The peptides especially Angiopep 2 showed higher parenchymal accumulation and transcytosis when compared to other receptors, avidin, transferrin and lactoferrin. It was demonstrated that these peptides, and in particular Angiopep - 2, exhibited higher transcytosis capacity and parenchymal accumulation than other receptors such as transferrin, lactoferrin, and avidin, suggesting that endocytosis of Angiopep-2 is mediated by LRP 1 (Low-density lipoprotein receptor-related protein-1).[66]

Quantum Dots (QDs): QDs comprises of a core made up of metal crystal and a shell which shields the core. QDs possess properties which makes them a useful candidate in biomedical applications including some in CNS. These properties include long-term photo stability, high brightness and emission spectra with size tuning. The use of QDs has risen because of imaging technology and has been shown to have various biomedical applications. To EGF or anti-EGFR (Her1) was coupled to QDs which further was applied to monoclonal antibodies (MAbs) (mouse MAb 528, H-11, and H199.12) to map human glioblastoma multiforme by the technique of fluorescence microscopy. [67] Different glioblastoma cell line derived from humans like – G28, G120, G44, G112, G28, and U87 were tested to evaluate its therapeutic and diagnostic effects.

In contrast, On the contrary, the wild-type and mutant EGFR expression was consistent to that of QDs coupled to EGF which could be taken up and the level of their uptake varied in the reported cell line. Glioblastomas often express regulated Her 1 and mutations. Additional classes of glioblastomas can be stained using the scan of anti-EGFR MAb coupled to QDs. QDs when conjugated with anti-EGFR antibodies were studied and it was found that they can be used in the diagnosis of brain tumors. QDs were used to deliver MMP 9-siRNA in to micro vascular endothelial cells of brain. [68] The reason behind low endothelial permeability was the increase in expression of extra cellular matrix proteins including collagens I, IV, and V as a result of inhibition of MMP-9 expression revealed by i*n vitro* experiments. The decrease in permeability can potentially fortify the blood brain barrier against invasion of inflammatory cells.

It was observed that QDs coupled with transferrin can easily cross BBB. [69] In order to increase the stability of QDs in aqueous media, an experiment was performed QDs were encapsulated into polyethylene glycol- polylactic acid (PEG-PLA) nanoparticles functionalized with wheat germ agglutinin which resulted in increase in the stability of QDs. [70] Thus, QDs can be utilized for both both imaging properties and brain targeting following intranasal administration. The tight junction opening of BBB can be caused by nanoparticle toxicity. An experiment was performed in which Polyamidoamine nanoparticles (PBCA NPs) was synthesised and they induced permeabilization of the blood brain barrier at 10 μg/mL which was attributed to the toxicity caused by NPs. [71]

TABLE 1: LIST OF NASAL PRODUCTS AVAILABLE IN THE MARKET

Drugs formulated	Delivery vehicle	Product name	Usage
Beclomethasone dipropionate monohydrate	Nasal Spray	Beconase AQ®	Seasonal or perennial allergic and non-allergicrhinitis [73]
Calcitonin-salmon	Nasal Spray	Miacalcin®	Post-menopausal Osteoporosis
Budesonide	Nasal Inhaler	Rhinocort Aqua	Treatment of seasonal or perennial Allergic Rhinitis
Mometasonefuroate and formoterolfumaratedihydrate	Inhalation aerosol	Dulera®	asthma
Nafarelin Acetate	Nasal Spray	Synarel®	Endometriosis and Central precocious puberty
Formoterolfumarate	Inhalation Powder	Foradil aerolizer	Asthma and Bronchospasm [74]
Tiotropium bromide	Inhalation Powder and Capsules	Spiriva® handihaler®	Bronchospasm, Chronic obstructive pulmonary disease (COPD), Chronic bronchitis, Emphysema [74]
Fluticasone propionate and Salmeterol	Inhalation Aerosol	ADVAIR HFA 45/21	Asthma
Zanamivir	Nasal Spray	Relenza	Influenza A and B
Desmopressin acetate Injection	Nasal spray	DDAVP® injection	Diabetes insipidus

Models for Nasal Drug Transport and Absorption Studies: Various *in vitro, in vivo* and *ex vivo* models are used to evaluate and analyse the transport and absorption of biomolecules or drug particles *via* intranasal route (**Fig. 3**). The development of *in vitro* models, in order to evaluate the toxicity and therapeutic effects of drugs, proves to be less expensive, as compared to animal testing. It is easier to obtain replication and quantification of results in case of *in vitro* models. The exact molecular mechanism of the drugs cannot be predicted with *in vivo* studies. Moreover, animals, involved in biological testing experience pain and sufferings, which ultimately leads to reduction in their number, in contrast, *in vitro* experiments can be performed more rapidly and easily. Several *in vivo* and *in vitro* models are used for the purpose of nasal drug absorption studies as well.

Human nasal specimens are less commonly used for such studies as various methodological and ethical limitations are associated with them. The main problem is that, species differentiation and differences in anatomical features of nasal cavities of animals and humans can affect the study results. Among the human cell line, the one from the nasal epithelium (RPMI2650) are being extensively used for studying drug permeability and absorption. Hence, safety, efficacy, toxicity and therapeutic properties of intranasal drugs can be studied by designing of *in vitro* experiments. Although *in vitro* information cannot replaced by *in vivo* data, but it can often provide insight into questions regarding the human metabolism of a drug prior to the initiation of clinical studies and finally, leading to less number of animals required to confirm the predicted safety and efficacy of various intranasal drugs [75].

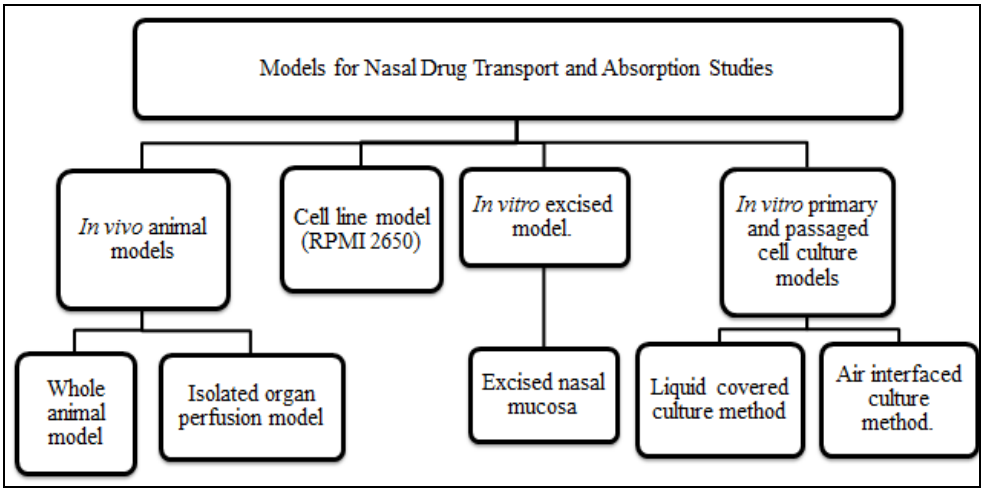

FIG. 3: VARIOUS MODELS FOR INTRA NASAL DRUG TRANSPORT AND ABSORPTION STUDIES

***In vivo* Models:** The whole animal model and the organ perfusion model are the forms of animal models which are utilized for detecting the absorption and permeability studies. [75] The animal models using sheep [76], rat [77, 78], rabbit [79, 80], dog [81] and monkey [82] are reported for estimation of nasal absorption. Various studies have reported the usage of e*x vivo* nasal perfusion models (using sheep and rat). Because of the small size of rat and low cost of maintenance it is highly advantageous but, the limitation of blood sampling is an issue with it. So, it is useful only for the fundamental studies to check the nasal drug absorption.

***In vitro* Cell Line Model:** RPMI 2650 and CaCo-2 cell lines are used to assess nasal absorption and permeability studies. RPMI 2650 a cell line derived from human nasal epithelia (from a spontaneously formed tumor). This cell line grows to multilayer and does not form confluent monolayers; therefore this cell line is used for metabolism studies. [83, 84] CaCo-2 is another cell line used to evaluate the nasal absorption of the formulation. The cell line is originally obtained from human colon carcinoma. The cell line differentiates slowly to various monolayers with a differentiated phenotype with various functions of the epithelium of small intestinal villus.Caco-2 is one of the valuable cell lines to assess the drug absorption and permeability across the intestinal epithelia. [85-87]

***In vitro* Excised Models:** Nasal mucosae are excised from various animals and are important tools to study nasal metabolism and transport. [88] While conducting the experiments the viability of excised nasal mucosae should be maintained. Nasal epithelia are excised from sheep, rabbits, bovine and dog's tissue [89] and have been ideal for the experiments on nasal permeability and metabolic studies. The variation in the enzyme active and that of the excised nasal mucosae from these animals is an important issue. [90-93].

***In vitro* Primary and Passaged Cell Culture Models:** A controlled environment for the study of epithelial cell differentiation and growth, assessment of drug transportation and permeability mechanisms, and minimum use of animals are some of the advantages of *in vitro* cell culture models. [94] Some hurdles need to be solved involving *in vitro* cell culture models these are: small amount of obtainable cells and differentiation issues with epithelial cells although advancement in cell culture techniques appear are promising.

Liquid covered culture method: Werner *et al.*, studied *in vitro* drug transportation using Transwell insert onto which human nasal epithelial cell were grown. [84, 95] This method is used for evaluating the transportation and metabolism of peptides including insulin. [96-98] In spite of the advantages of this method, there is a difficulty in obtaining adequate amount of epithelial cells because biopsies yield limited amount of nasal epithelial cells. Therefore, the limited quantity of cells is a major problem while conducting various experiments, and high-throughput screening studies have not been possible. The contamination of cells with pathogens is another problem in this method.[99]

A serially passaged culture system is considered as an alternative to overcome these limitations of primary cell culture of human nasal epithelial cells. There are not reports on serially passaged cell culture system for studying transportation of the formulation, although studies on the advancement of a passaged culture of primary respiratory epithelial cells of human yielded information on some physiological characteristics (*e.g.* mucin secretion) [100-103]. The most important factor to be considered while studying transportation studies is the formation of tight junctions across the epithelial cell layer derived from serially passaged cells. Epithelial cells which are cultured up to 4 passages can be grown as a confluent monolayer on a Transwell insert. Each monolayer forms tight junction epithelia with a TEER (trans epithelial electrical resistance) value of upto 3,000 ohm cm^2 enabling drug transportation.

Air interfaced culture method: In the cultured epithelial cells, the important properties include the formation of confluent cell monolayer which is interconnected by tight junction proteins, mucin production, expression of apical cilia. An important role in physiological function (MCC) is played by the ciliated cells of nasal epithelium and it is relevant to INDD. [104] And, the presence of enzymes and the expression of various transporters proteins in *in vitro* culture will increase the potential of *in vitro* models. When the passaged culture model is compared to *in vivo* nasal epithelia, it was observed

that the passaged culture method showed few ciliated cells and undifferentiated ciliated cells when they are cultured with LCC method. [74] Specific culture conditions affect the attainment of functional and morphological features of the cells similar to that of *in vivo* setting.

These conditions include the media in which cells are cultivated, the additives that are added for the cellular differentiation (retinoic acid) and air interfaced (AIC) versus liquid-covered culture (LCC) conditions. The culture media which is serum free but is hormone supplemented helps in induction of epithelial cells which is morphologically similar to that of epithelium *in vitro.*[100, 105] Therefore, the coupling of serum free media and AIC culture condition results in the increase in mucin secretion and more ciliated cells. In the Transwell insert the apical side of the For AIC conditions, the apical surface of the epithelial cell is exposed to air whereas the basolateral side is fed with the culture media.

CONCLUSION: INDD system is a promising delivery system for the drugs with poor bioavailability and has an advantage in terms of patient compliance if compared to parentral administration. This This route of administration is especially suited to treat various neurodegenerative diseases for example, Alzheimer's disease, Parkinson's disease [106], [107] as they require specific and rapid targeting of drugs to brain. It is an ideal route for administration of vaccines against diseases influenza, anthrax etc. to produce immune response against them. In near future, it is hoped that intranasal formulations are used for treatments, such as sleep induction, heart attacks, erectile dysfunction, nausea, Parkinson's disease, acute pain (migraine), panic attacks, and for the treatment of long term diseases such as fertility treatment, osteoporosis, diabetes, endometriosis, growth deficiency. These require careful understanding of both the delivery device and the nasal drug and how they effect on each other.

ACKNOWLEDGEMENT: The research group is grateful to the Department of Biotechnology and Bioinformatics of Jaypee Institute of Information Technology, Noida, Uttar Pradesh for providing necessary facilities to execute this work.

CONFLICTS OF INTEREST: All the authors listed in this manuscript have made substantial contributions towards the research work and had given their final approval for the version to be published and hence, there is no conflict of interest between the authors of the manuscript. All the authors (Manisha Singh, Ramneek Kaur, Rashi Rajput, Surya Pratap Singh and Rachana) have contributed equally.

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How to cite this article:
Singh M, Kaur R, Rajput R, Singh SP and Rachana: Intranasal drug delivery - new concept of therapeutic implications for effective treatment of CNS disorders. Int J Pharm Sci Res 2017; 8(8): 3194-05.doi: 10.13040/IJPSR.0975-8232.8(8).3194-05.