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A BRIEF REVIEW: NASAL THERMOREVERSIBLE *IN SITU* GEL LOADED WITH ANTI-DIABETIC DRUG AND NATURAL ANTIOXIDANT TO TREAT DIABETES-INDUCED ALZ HEIMER DISEASE

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ABSTRACT

The present review was focused on study of Nasal thermoreversible *In situ* gel which contains the combination of antidiabetic agent with natural antioxidant to manage diabetes-induced Alzheimer disease in elderly population with Type-2 diabetes. The *in situ* gelling systems for nasal administration were developed to improve patient compliance, nasal bioavailability of drugs by increasing its nasal retention time and for improvement of drug safety and efficacy in our body system. Antidiabetic drug which is used is Metformin, a biguanide antihyperglycemic agent used for the treatment of type-2 diabetes is well known for its major role as neuroprotective by decreasing the insulin receptor phosphorylation and tau-phosphorylation and increasing neuronal survival. Curcumin has been reported to inhibit amyloid-β-protein (Aβ) aggregation, and Aβ-induced inflammation, as well as the activities of β-secretase and acetylcholinesterase and has resulted in the inhibition of Aβ deposition, Aβ oligomerization, and tau phosphorylation in the brains of AD patients. The present review also focused on the formulation of *in situ* gelling system with respect to physiological temperature and the study of various other approaches. The different kind of polymers used and their role in preparation of gelling system and absorption of drugs by various ways are also discussed in this review. Various evaluation parameters also considered in preparation of nasal *in situ* gels.

KEYWORDS: Metformin, Curcumin, Diabetes-induced Alzheimer's, Thermoreversible polymers, Approaches.

INTRODUCTION

Diabetes is a significant health problem and represents a growing prevalent chronic disease. Diabetic patients are expected to rise from 400 million to 640 million by 2040.[1] The main cause of dementia in elderly people is Alzheimer's disease, which affects a large worldwide aged population and continues to increase in prevalence with age.[2] Both the conditions show an interesting link to each other which are related to age.[3,4] Diabetes-induced Alzheimer's disease was described as "Type 3 diabetes".[5] Many epidemiological data shows that diabetics have a high risk of getting AD compared to non-diabetic patients.[6] The two main points identified to show the pathophysiological links between Type 2 diabetes and AD were insulin resistance and inflammatory signalling pathways.[7]

Alzheimer's Disease Pathogenesis

Hyperinsulinemia and insulin resistance of Type II DM (T2DM) cause substantial risks to elderly cognitive decline.[8] The brain glucose level is reduced due to the production of insulin degrading enzyme (IDE), by Insulin signalling which induces brain to take up glucose and produces which reduces brain glucose level. IDE is involved in degradation of both insulin and amyloid beta (Aβ) protein.[9] Alteration of insulin signalling, and hyperinsulinemia are major cause of diabetes that leads to less production of IDE which reduces the Aβ degradation that resulted in accumulation of Aβ protein in the brain. Therefore, proper insulin signalling reduces accumulation of Aβ and enhances the complete clearance of Aβ protein from the brain.[10] Moreover, soluble Aβ oligomers, identified as amyloid beta-derived diffusible ligands (ADDLs), increase insulin sensitivity in AD by reconfiguring synapse conformation that is responsible for the reduction affinity of synaptic insulin receptor for its ligand. Furthermore, abnormal Aβ induces hyperphosphorylation of the tau protein, the key element of intracellular neurofibrillary tangles.[11] Glycogen synthase kinase-3 (GSK-3) is the enzyme which is produced due to altered pathways associated with the phosphorylation of tau protein leading to development of AD neurofibrillary tangles, which has shown to be suppressed in response to insulin.[12] Synaptic loss is characterized as neuropathology of AD, while insulin receptor signalling increases the synaptic density.[13]

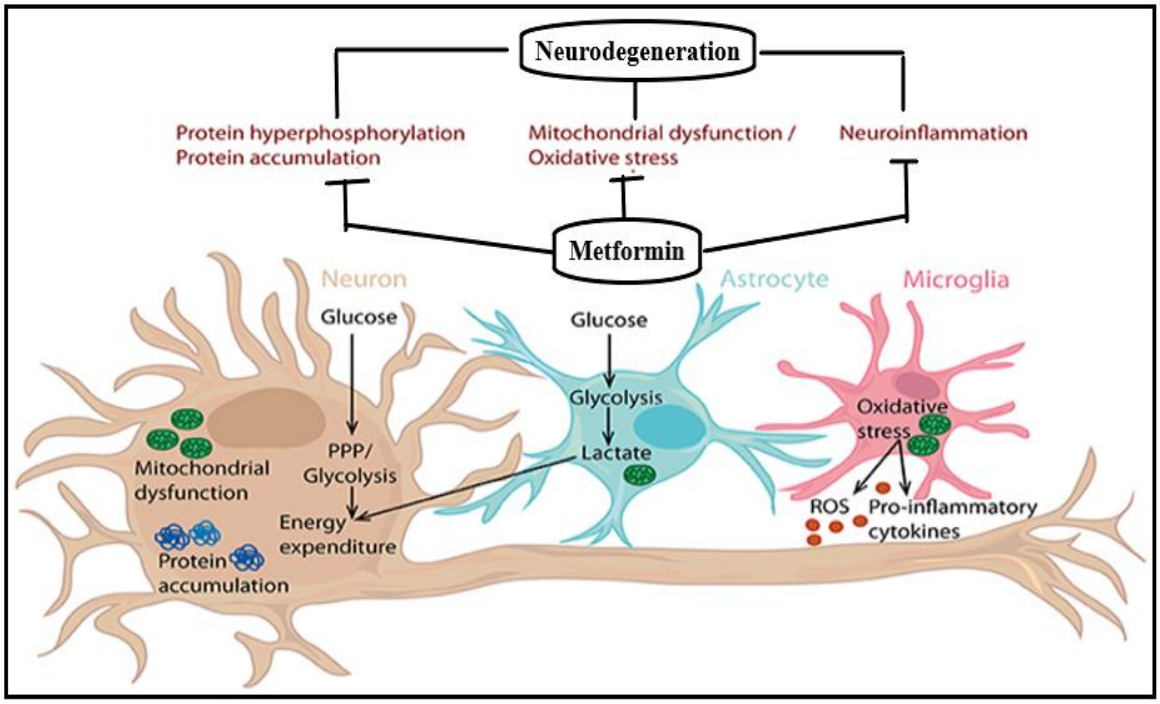

Fig. 1: Mechanism of action of Antidiabetic drug.

Inflammatory processes are majorly related to both T2DM and AD. Insulin resistance cause increased level of proinflammatory cytokines namely, C-reactive protein, tumour necrosis factor-α (TNF-α), interleukin- (IL-1), and IL-6 which are led to synaptic dysfunction and accumulation and progression of Aβ plaque.[14] Insulin has anti-inflammatory effects which suppresses these cytokines and induces antiphlogistic mediators.[15] Altered insulin receptor signalling pathway by increased inflammatory response results in dyslipidaemia and hypertension, prelude of T2DM.

The risk of AD is correlated with many abnormalities like insulin resistance, inflammation, oxidative stress, advanced glycation end products (AGEs), autophagic dysfunction and hyperglycaemia. In addition, PET studies showed that greater insulin resistance is associated with an AD-like pattern of reduced cerebral glucose metabolic rate in some regions of brain of adults with T2DM.[16]

A major qualitative abnormality in lipids is dyslipidaemia and hypercholesterolemia that contribute to both DM and AD development. Apolipoprotein E4 (APOE4), mainly expressed in liver and brain, has a higher Aβ plaque deposition and oxidative stress susceptibility and an increased rate of tau phosphorylation.[17,18,19] Sugar with protein-amino groups, nucleotides and lipids has irreversible and non-enzymatic reactions which produce heterogeneous

composites called AGE's, which are usually accelerated in elderly patients during ageing because of increased reactive oxygen formation. In this connection, Sasaki and the collaborators described an improved immunosuppression of AGE in patients with AD, particularly in Aβ plaques and Hippocampal Neuron's NFT.[20]

Reactive oxygen overproduction causes oxidative stress in diabetes patients that induce oxidised protein accumulation in the brain of patients with MCI (mild cognitive impairment. Mitochondria plays a key role in oxidative stress reduction. Oxidative phosphorylation uncoupling and respiratory chain alteration which are major mitochondrial disorders results in the development of neurodegeneration and uncontained metabolism in elderly people with type-2 diabetes mellitus.[21]

Anti-diabetes drugs for Alzheimer's therapy

As AD and DM are pathophysiological allied disease, controlling on conditions insulin resistance and hyperglycaemia helps in decreasing the AD progression rate. Also, the clinical trials have been done on FDA approved anti diabetic drugs for treating AD. Some of the FDA approved anti diabetic therapeutic agents assessed against AD are mentioned below.

1. Intranasal insulin: Clinical trials have shown that dispensation of insulin through nasal route directly delivers it to the brain by bypassing the BBB via trigeminal and olfactory nerves pathway which improves cognition which have caused due to insulin resistance induced by Aβ plaques by enhancing insulin signalling.[3,48]

2. Dipeptidyl peptidase IV inhibitors: Dipeptidyl peptidase-4 inhibitors such as Saxagliptin or Sitagliptin helps in recuperating cognitive brain by precluding mitochondrial dysfunction caused in the hippocampus region. These agents also decrease oxidative stress, reduces nitrosative stress and neuroinflammation in the brain and recuperate insulin signalling which reduces the Aβ plaques.[48]

3. Thiazolidinediones: Thiazolidinediones also known as Glitazones are a class of antidiabetic agents which decreases hepatic gluconeogenesis, acts on peroxisome proliferator-activated receptors specifically on PPARγ (PPAR-gamma) and improves hypoglycaemia. Clinical trials of this drug have shown that these agents are act as an anti-amyloidogenic and anti-neuroinflammatory by reducing amyloid peptides deposition, oxidative stress and neuroinflammation (inhibiting TNF and interleukins) in the brain which improved cognition.[3,48]

4. Metformin: It is the most extensively used biguanide class of antidiabetic agents for the treatment of diabetes. Metformin controls blood insulin levels and abate the hepatic gluconeogenesis which reduces hyperglycaemia in diabetic individuals. It also increases the liver and muscle cell's sensitivity to insulin through AMP-mediated route. It is also demonstrated to be neuroprotective by decreasing the phosphorylation of the insulin receptors and increasing neuronal survival, apart from its anti-hyperglycaemic activity.[3,48]

Combination of Metformin and Curcumin[36]

Combination of Curcumin and Metformin act synergistically on oxidative stress and dyslipidaemia, and enhanced PON 1 level which are major cause of AD. Therefore, it is a promising strategy for combating diabetic complications.

The beneficial effects of treatment on diabetes by combining Metformin and Curcumin can be attained via two approaches.

a) The best effects of isolated therapeutic measures (Curcumin and Metformin) include a decreased level of glycosuria and plasma AGEs, increased PON 1 activity and reduced glycemia were retained (metformin effect).

b) Additive effects in comparison with isolated therapy were achieved, mainly due to additional reduction in plasma, triacylglycerol, cholesterol and TBARS.

Curcumin and AD[39]

It has been reported that curcumin inhibits the aggregation of amyloid-β-protein (Aβ) and inflammation induced by Aβ as well as β-secretase and acetyl-cholinesterase activities. The inhibition of Aß, Aß oligomerization and tau phosphorylation in the brains of AD animal models, together with improvements in behavioural impairment for animal models, were the result of oral administration of curcumin in *in vivo* studies. These results suggest that curcumin can be one of the most promising compounds for AD therapy development.

Nasal drug absorptions

The delivery of drug through nasal route is an important mode for systemic and local administration which is attained by the absorption of drug through mucosal membrane. It is difficult for large and charged drugs/particles to cross this layer as compare to small uncharged particles. Additionally, some structural changes caused due to environmental factors in the layer and principal protein of mucus called mucin have a tendency to create

hindrance in absorption or diffusion process. There are several mechanisms of drug absorption has been proposed, only two mechanisms are predominantly considered.[22,23]

Mechanism of drug absorptions

First mechanism – This mechanism is passive and slow and known as paracellular process which involves an aqueous path of conveyance of drug.[22,23]

Second mechanism- This method called as transcellular route which involves a lipoidal path of conveyance of drug. It is a carrier mediated or an active transport.[22,23]

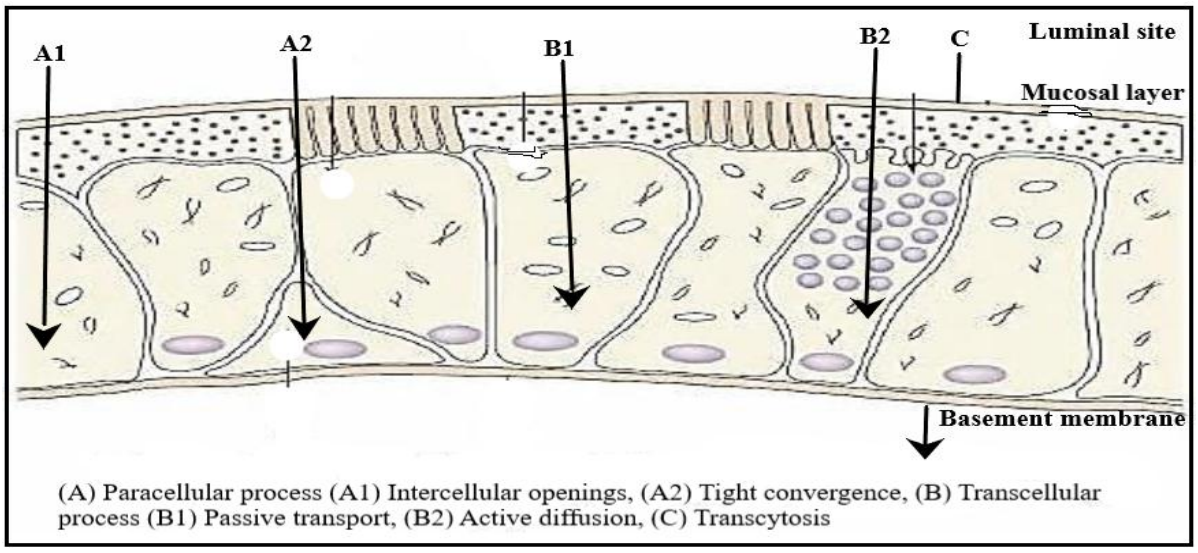

Fig. 2: Mechanism of Nasal drug absorption.

Mechanism of drug delivery through nose to brain

The methods of transport of drugs from the nasal cavity to the brain involved the combination of CSF, vasculature, and a lymphatic system (Illustrated in Figure 3). The therapeutic agents are absorbed into the systematic circulation and reaches the CNS when deposited in the respiratory epithelium. These agents are transported by paracellular or transcellular processes to CNS when it is deposited on the olfactory epithelium. Another pathway to deliver the bioactive agents directly from nose to brain by circumventing BBB is through trigeminal nerves.[24]

The olfactory nerve pathway is the main intranasal route of administration through which the drug is delivered through the axons of the olfactory nerve via olfactory bulbs to the brain. The above noted administration of therapeutic agents via olfactory nerve can be further categorized as.[24]

(i) A transcellular path which involves passive transport of drug and receptor-mediated endocytosis.

(ii) A paracellular path via tight convergence present between the olfactory neurons and sustentacular cells of the olfactory epithelium.

(iii) The olfactory nerve route via which the drug is engrossed by the mechanisms called endocytic or pinocytotic and the drugs are carried into the olfactory bulb by intracellular axons.

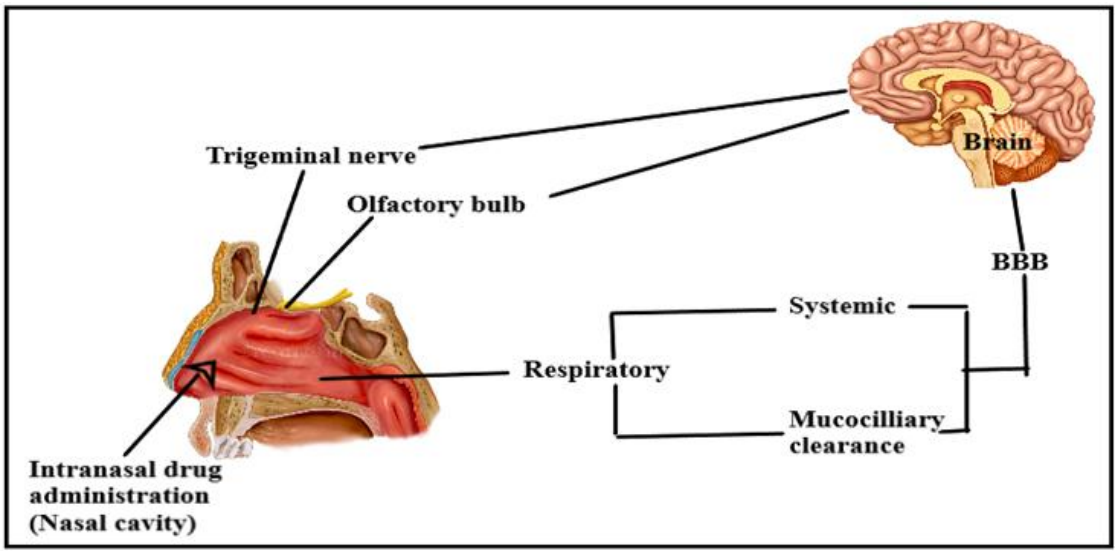

Fig. 3: Schematic illustration of the mechanisms of drug delivery through nose to brain

Merits of drug delivery by nasal route.[54]

a) This route is non-invasive, Easy administration of drug, rapid and convenient.

b) Easily accessible to blood capillaries and side effects namely, nausea and vomiting which are observed in conventional route of administration can be avoided.

c) Avoids enzymatic and chemical degradation of therapeutic agents in the GI tract, hepatic "first pass" metabolism.

d) Low bioavailable Drugs can be delivered directly to the systemic circulation through nasal drug delivery.

e) When long term therapy is considered, the nasal route is an alternative to parenteral route, particularly in case of proteins & Peptides.

f) Through nasal mucosa the penetration of lipophilic and low molecular weight drugs is suitable.

g) Nose has relatively high vascularization and large absorption surface area due to which quick absorption and rapid onset of action can be observed.

h) Delivery of drug from nasal cavity directly to brain by circumventing BBB via the olfactory region is seen.

Different approaches of *in situ* gelling systems[46]

To trigger sol to gel phase transition on the surface of nasal mucosal membrane the undermentioned approaches of gelling systems are identified.

1. Stimuli Receptive *In Situ* Gelling System

a. Thermal dependent system.

b. pH triggered system.

2. Osmotically Persuaded *In Situ* Gelling System

3. Chemically Persuaded *In Situ* Gelling System

a. Ionic responsive.

b. Enzymatic responsive.

1. Stimuli Receptive Gelling System

In response to changes in external environmental factor there is physical and chemical changes in polymer.

pH activated gelling system[49,56]

In this system the sol-gel transition takes place with respect to change in environmental pH. Polymers which are pH sensitive contains either acidic or basic groups that in response to environmental pH either accept or release proton. When pH is increases then swelling of gel increases in polymer which contains anionic groups but decreases with presence of cationic groups in polymer.

Physiological Temperature dependent system[56]

The most commonly used stimuli receptive *in situ* gelling system is temperature. The variation in temperature can be easily controlled, and relatively applicable in both *in vivo* and *in vitro* studies. These dosage forms are in liquid state at room temperature (19˚C-25˚C) and endures gelation when comes in body contact, due to rise in temperature (35˚- 37˚C) which a body temperature. Critical solution temperature (CST) is the temperature at which gelation occurs. Hydrogels which are sensitive to temperature are classified into two types.

1 Negatively thermo sensitive- Lower critical solution temperature (LCST)

2 Positively thermo sensitive- Upper critical solution temperature (UCST).

Table-1: Types of Thermo-responsive polymers.

Types of Thermoresponsive sol-gel polymeric system	Properties of gel system	Polymers
Negatively thermo sensitive	Having lower LCST and contract upon heating above LCST	poly[N isopropylacrylamide] [PNIPAAm]
Positively thermo sensitive	Having UCST and contracts upon cooling below UCST	poly[acrylic acid][PAA] and polyacrylamide [PAAm]
Thermally reversible	Polymer solution is a free-flowing liquid at an ambient temperature and gels at body temperature	poly [ethylene oxide]-b-poly [propylene oxide]-b-poly[ethylene oxide] Pluronics®, Tetronics®, Poloxamers

2. Osmotically Persuaded *In Situ* Gelling System[58]

Hydrogels occur in this system as a result of ion strength changes. The rate of gelation depends upon the ionic gradient around the surface of the gel. In the presence of osmotic ions that is mono and divalent ions, the aqueous polymer solution forms a clear gel.

3. Chemically Persuaded *In Situ* Gelling System[57]

The chemical reaction which are responsible for forming nasal *in situ* gels are ionic responsive, enzymatic responsive and photopolymerization.

Ionic responsive

In the presence of different ions such as k+, Ca2+, Na+, ion-sensitive polymer endures phase transition. For instance, in case of divalent/multi-valent cations such as Ca2+ alginic acid is subject to gelation.

Enzymatic responsive

Natural enzyme catalysed enzymatic responsive polymer to form *in situ* gel has not been widely examined but appears to be beneficial in chemical and physicochemical ways. For example, an enzymatic process works effectively without requiring potentially harmful chemicals, such as monomers and initiators, under physiological conditions.

Method of preparation of nasal *in situ* gel

Typically, two methods are worked out for the formulation of nasal *in situ* gel.

Cold Method[45]

This technique uses sufficient distilled water to solubilize the drug and to keep this drug solution overnight at 4 °C in a refrigerator. With the continuous stirring the polymers used for *in situ* gel formation is added slowly in the drug solution. The solution is then again stored in a refrigerator at 4˚C until a clear dispersion is obtained, and then final volume is adjusted with the distilled water. This process is most preferred for the formulation of nasal *in situ* gel when poloxamer or Carbopol is utilised as a gelling polymer. This polymeric solution containing poloxamer remains as liquid at a lower temperature and gets transformed into a gel at higher nasal temperature.

Hot Method[45]

This process is preferred when gellan gum or pectin is utilized as gelling. Gellan gum at higher temperature, dissolves in distilled water and form a random-coil conformation with a high segmental mobility and remain as a solution. The transition of sol-gel phase occurs on cooling of gellan gum polymeric solution in the occurrence of ions like K+ or Ca2+.

Table-2: Different polymers used in nasal *in situ* gelling systems.

S. No.	Approaches	Polymers
1	pH responsive	Polymethacrylic acid and polyethylene glycol (P(MAA-g-EG)), Polyvinylacetal diethylamino acetate, Carbopol (poly acrylic acid), Chitosan
2	Temperature responsive	Poloxamer 407, Poloxamer 188, Carbomer and Pluronic F127, chitosan-PVA, Poly(N-isopropylacrylamide) (PNiPAAm), Ethyl (Hydroxyethyl) Cellulose, HPMC, MC, Xyloglucan, Gelatin
3	Osmotically responsive	Gellan gum, Sodium alginate
4	Ionic responsive	Gellan gum, fentanyl citrate, Pectin
5	Enzymatic responsive	Dextran, Gelatin

EVALUATION PARAMETERS OF DRUG NASAL IN-SITU GEL[26,33]

Clarity[53]

To check the clarity of product can be done by visual inspection.

Rheological behaviour[33]

With the application of an appropriate spindle at fixed rpm at temperatures range from 4°C to 37°C, the viscous nature of a formulation can be determined with the Brookfield RV-E

viscometer. The spindle remained constant for each batch to increase the temperature by putting the solution into the water bath. The temperature-viscosity graph can be drawn.

Determination of pH of Formulation[53]

Digital pH meter is used for the determination of pH of each formulation. 1 ml quantity of each formulation was transferred to 25 ml beaker and diluted with distilled water and make up to 20ml and then pH is measured.

Drug Content of preparation[33]

1ml of preparation was withdrawn in 10ml volumetric flask and then diluted with 6.4 pH phosphate buffer and made up to volume 10ml. 1 ml of this primary solution was again diluted with 6.4 phosphate buffer and volume adjusted to 5ml. Absorbance of the final solution was determined using UV-Vis Spectrophotometer at wavelength of drugs.

Gelation Temperature[33]

The formulation was first kept at 4°C to evaluate the temperature of the gelation. Then a 25 ml beaker contained 20 ml of the solution with inserted magnetic bead was placed on magnetic stirrer with hot plate. With a temperature increase of 1°C/min, the solution should constantly agitate at 100 rpm. As to determine the gelation temperature of specific formulation, the temperature at which the magnetic bead ceased its rotation was observed.

Mucoadhesion potency[53]

A modified laboratory analytical balance was used to determine the adhesive potency of each formulated solution by quantifying the strength or force needed to detach the formulation of nasal mucosal tissues. The fresh goat's nasal mucosa was acquired from the slaughterhouse and cut from its nasal cavity and stored in Tyrode solution for longer use. The mucosal layer was cut in square shape and about 2.5 cm in diameter was tied on secured end of each glass vial using thread by keeping the mucosal side out and keep it stored for 5 min at 37ºC. First vial with part of the mucosa was attached to the balance in an inverted position while another vial was fixed on the adjustable pan. A fixed quantity of sample of each formulation was placed on the nasal mucosa of the second vial on pan. Then, the height of the first inverted vial was acclimatised so that the mucosal surfaces of the two vials come in close contact for about two minutes which reassure the intimate contact between the membrane and the sample. Gradually, the weight is increased on the opposite pan. The weight which detaches

the vials is note in grams. The adhesive strength, articulates as detachment stress(dyne/cm²), was determined using following equation,

$$Detachment\ stress = m \times g/a$$

Where,

m = mass required for separation of two vials(g),

g = Acceleration due to gravity [980 cm/s²],

a = Exposed membrane area [πr², r= radius of exposed tissue or vial]

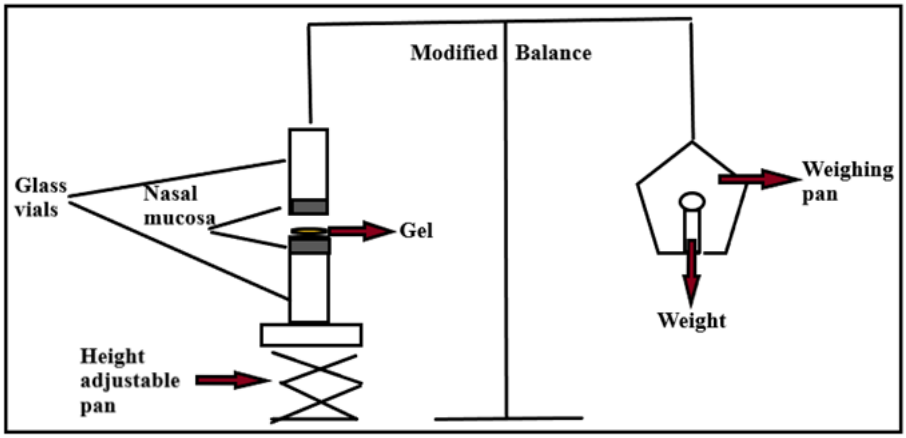

Fig. 4: Modified Analytical balance for Mucoadhesive force study.

In vitro drug diffusion Studies[53]

In vitro drug diffusion test is key evaluation parameter of post formulation evaluation of thermoreversible *in situ* gel. The test was done with the application of Franz diffusion cell inserted with any tissue which can be used as diffusion membrane. The diffusion membrane was installed in between donor and receptor compartment. The receptor compartment was filled with buffer (6.4-7.4). The cell was then kept over the magnetic stirrer with hot plate and at sustained temperature of 37°C and incessant stirring of content of receptor membrane by magnetic stirrer. Specific amount of formulation was taken in the donor compartment. At a scheduled time, sample was withdrawn from the receptor compartment, substituted with fresh buffer to the sampled volume after each sampling periodically. The absorbance was analysed spectrophotometrically at given λmax.

In vitro Permeation Study[56]

To assess the drug permeability through the nasal mucosa and capability of permeation enhancer which was used in formulation. Fresh nasal mucosal portion of goat from slaughterhouse is acquired. Mucosa was then incorporated in the diffusion cell. 2ml of

preparation was stationed in donor slot, at scheduled time, 2.5ml sample was removed from receptor slot and substitute the sampled volume with equal amount of buffer periodically, after each sampling. Sample were diluted further, and absorbance was checked spectrophotometrically at certain wavelength of drug.

$$\text{Permeability coefficient} = \text{Slope} \times \frac{\text{Volume of donor solution}}{\text{Surface area of tissue}}$$

Histopathological Analysis[57]

The histopathological analysis was done for optimized formulation. Two mucosal membrane pieces having surface area equals to 2 cm^2 were inserted in *in vitro* Franz diffusion cell. One mucosa was used as control (treated with 1 ml of 6.4 pH phosphate buffer) and the other was treated with 1 ml of formula and kept in incubator. The mucosal tissues were marked with eosin and haematoxylin. The sections of both control and test mucosa under microscope were checked. This histopathological analysis will indicate that the optimized formulation should not have significant effect on the cellular structure of the mucosal layer. The cells of mucosa are perfectly intact. There should be no changes are found in the structure after *in vitro* test.[21]

Stability Studies[33]

According to ICH guidelines the accelerated stability trials are conducted for an optimised *in situ* gel formulation. An adequate quantity of formulated *in situ* gel taken in screw-capped vials, were stored in desiccators containing NaCl which gives relative humidity of 75±5% at a temperature of 45 ± 5°C for 3 months. Samples were withdrawn monthly for 3 months. The various parameters like appearance, pH, drug content and drug release were determined.

CONCLUSION

In the present study it is aimed to study the various approaches for Nasal *in situ* gel preparation and to attain targeted delivery of Metformin and Curcumin to the brain of the patients with Diabetes-induced Alzheimer's disease. Delivery of therapeutic agents through nose is a promising route for direct delivery to CNS via olfactory region as this route bypasses the Blood brain barrier which gives early onset of action. The thermoreversible nasal in situ gel of anti-diabetic drug with natural antioxidant can be a new perspective on future treatment as it has several benefits as it overcomes the oral dispensing route limitations like side effects like diarrhoea, vomiting, by bypassing the liver first pass metabolism, avoids stomach gastric environment etc. This review provides the first testimony of a promising

strategy for the therapeutic treatment of diabetes-induced Alzheimer's disease in the elderly population by combining metformin with curcumin to not only treat hyperglycaemia and dyslipidaemia but also targets various other abnormalities in the brain namely, oxidative stress, insulin resistance, Aβ protein plaque, neuroinflammation and tau phosphorylation that leads to neurodegeneration.

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REFERENCES

1. Gao HX, Regier EE, Close KL. International Diabetes Federation World Diabetes Congress 2015. Journal of Diabetes, 2016; 8(3): 300–302.
2. Gaamouch FE, Jing PJ, et al. Alzheimer’s disease risk genes and lipid regulators. J. AD, 2016; 53(1): 15–29.
3. K. Akter, E. A. Lanza, S. A. Martin, et al. Diabetes mellitus and Alzheimer’s disease: shared pathology and treatment? Brit. J. Clin. Pharmaco, 2011; 71(3): 365–76.
4. Biessels GJ, Staekenborg S, Brunner E, et al. Risk of dementia in diabetes mellitus: a systematic review. The Lan. Neuro, 2006; 5(1): 64–74.
5. Monte SM. Type 3 diabetes is sporadic Alzheimer’s disease: mini-review. Euro. Neuro, 2014; 24(12): 1954–60.
6. Haan MN. Therapy insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer’s disease. Nat. Clin. Prac, 2006; 2(3): 159–66.
7. Mittal K, Katare DP. Shared links between type 2 diabetes mellitus and Alzheimer’s disease: a review. Dia. & Meta. Syndr, 2016; 10(2): S144–49.
8. Zhong Y, Miao Y, Jia WP, Yan H, et al. Hyperinsulinemia, insulin resistance and cognitive decline in older cohort. Biomed. En. Sci, 2012; 25(1): 8–14.
9. Edland SD. Insulin-degrading enzyme, apolipoprotein E, and Alzheimer’s disease. J. Mol. Neurosci, 2004; 23(3): 213–17.

10. Vandal M, White PJ, Tremblay C et al. Insulin reverses the high-fat diet-induced increase in brain A beta and improves memory in an animal model of Alzheimer disease. Diab., 2014; 63(12): 4291-301.
11. Robinson CS, Kim B, Rosko A, Feldman EL. How does diabetes accelerate Alzheimer disease pathology?" Nat. Rev. Neuro., 2010; 6(10): 551–59.
12. Miller BC, Sarno PD, Zmijewska AA, Song L, Jope RS. Physiological and pathological changes in glucose regulate brain Akt and glycogen synthase kinase-3. The J. Bio. Chem., 2005; 280(48): 39723-31.
13. Chua LM, Lim ML, Chong PR, Hu ZP, Cheung NS, Wong BS. Impaired neuronal insulin signaling precedes Abeta42 accumulation in female AbetaPPsw/PS1DeltaE9 mice. J. AD., 2012; 29(4): 783-91.
14. Wieser V, Moschen AR, Tilg H. Inflammation, cytokines and insulin resistance: a clinical perspective. Arch. Immuno. Thera. Exp., 2013; 61(2): 119–25.
15. Sun Q, Li J, Gao F. New insights into insulin: the antiinflammatory effect and its clinical relevance. W. J. Diab., 2014; 5(2): 89–96.
16. Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch. Neurol., 2011; 68(1): 51-71.
17. Saunders AM. Apolipoprotein E and Alzheimer disease: an update on genetic and functional analyses. J. Neuropath. Exp. Neuro., 2000; 59(9): 751–58.
18. Martins IJ, Berger T, Sharman MJ, Verdile G, Fuller SJ, Martins RN. Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. J. Neurochem., 2009; 111(6): 1275–308.
19. Brecht WJ, Harris FM, Chang S, et al. Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. The J. Neurosci., 2004; 24(10): 2527–34.
20. Sasaki N, Toki S, Chowei H, et al. Immunohistochemical distribution of the receptor for advanced glycation end products in neurons and astrocytes in Alzheimer's disease. Brain Research, 2001; 888(2): 256–62.
21. Moreira PI, Santos MS, Moreno AM, Seica R, Oliveira CR. Increased vulnerability of brain mitochondria in diabetic (Goto-Kakizaki) rats with aging and amyloid-beta exposure. Diab., 2003; 52(6): 1449–56.

22. Jadhav KR, Gambhire MN, Sheikh IM, Kadam VJ, Pisal SS. Nasal Drug Delivery System: Factors Affecting and Applications. Current Drug Therapy, 2007; 2: 27-38.
23. Dhakar RC. Nasal Drug Delivery: Success through Integrated Device Development. J. Drug Del. Thera., 2011; 1(1): 2-7.
24. Rassu, Soddu G, Cossu E, Gavini M, Giunchedi E, Dalpiaz P. Particulate formulations based on chitosan for nose-to-brain delivery of drugs. A review. J. Drug Deliv. Sci. Technol., 2016; 32: 77–87.
25. Altunas E, Yener G. Formulation and evaluation of thermoreversible in situ nasal gels containing mometasone furoate for allergic rhinitis. AAPS pharma sci-tech, 2017; 18(7): 2673-82.
26. Kaur P, Garg T, Rath G, Goyal A. *In-situ* nasal gel drug delivery: a novel approach for brain targeting through the mucosal membrane. Arti. Cells Nanomed. Biotech., 2016; 44(4): 1167-76.
27. Pardeshi CV, Belgamwar VS. Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood–brain barrier: An excellent platform for brain targeting. Exp. Opi. drug Deliv, 2013; 10(7): 957-72.
28. Farooqui S, Zahid M, Parveen S, Ahmad Z, Singh P, Parveen Z. Alzheimer's disease: delivery of the drug through intranasal route. J. drug deliv. Thera., 2016; 6(6): 60-69.
29. Jaiswal N. Scope of nasal drug delivery: current challenges. W. J. med. Sci., 2017; 14(4): 97-112.
30. Menzel C, Jelkmann M. Nasal drug delivery: design of a novel mucoadhesive and in-situ gelling polymer. Int. J. Pharm., 2017; 517(1-2): 196-202.
31. Vibha B. In-situ gel nasal drug delivery system-a review. Int. J. Pharm. Sci., 2014; 4(3): 577- 80.
32. Khairnar S, Singh AK, Darekar A, Saudagar R. An innovative approach for in-situ gelling system for Nasal drug delivery: an overview. W. J. Pharma. Sci., 2015; 4(1): 334-56.
33. More BA, Mene HR, Pawar RK, Misal NS, Pathak SS, Shivshara KJ. A review on in-situ nasal gel drug delivery system. Int J Pharm Sci Rev Res, 2015; 33: 199-207.
34. Singh S, Kanupriya H, Kumar H. Intranasal thermo reversible mucoadhesive gels: a review. Int J Pharm, 2012; 2(3): 548-56.
35. Remeth J, Kailas K, Jitendra VS, Havaldar VD, Rahul K. Formulation and evaluation of thermoreversible mucoadhesive nasal gels of metoclopramide hydrochloride. Lat. Ameri. J. Pharm., 2010; 29(3): 354-61.

36. Daniela FR, Carlos AA, et al. Curcumin combined with metformin decreases glycemia and dyslipidemia, and increases paraoxonase activity in diabetic rats. Diab. & Meta. Synd., 2019; 11(33).
37. Aaron MK, Dawn MH, et al. Effects of the Insulin Sensitizer Metformin in Alzheimer Disease: Pilot Data from a Randomized Placebo-controlled Crossover Study. Alzheimer Dis Assoc Disord., 2017; 31(2): 107-13.
38. Grazia DF, Leonardo B, et al. Antidiabetic Drugs in Alzheimer's Disease: Mechanisms of Action and Future Perspectives. Hind. J. Diab. Res., 2017; 1-7.
39. Tsuyoshi H, Kenjiro O, Masahito Y. Curcumin and Alzheimer's Disease. CNS Neurosci. Thera., 2010; 16: 285–97.
40. Maha MA, Heba MK, Heba AH, Ossama YA. Formulation, in-vitro characterization, and clinical evaluation of curcumin in-situ gel for treatment of periodontitis. Drug Deliv., 2016; 24(1): 133-142.
41. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimer's Res. Thera., 2014; 6(4): 37-45.
42. Nasare L, Niranjane K, Nagdevte A, Sumedh M. Nasal drug delivery system: an emerging approach for brain targeting. W. J. Pharm. Pharmaceuti. Sci., 2014; 3(4): 539-53.
43. Chaturvedi M, Kumar M, Pathak K. A review on mucoadhesive polymer used in nasal drug delivery system. J. Adv. Pharmaceuti. Technol. Res. 2011; 2(4): 215-22.
44. Mayuri MB, Vijay RC, Gunesh ND, Jeevan RR, Deepak AJ. In-situ gel for nasal drug delivery. Int. J. Dev. Res., 2018; 8(2): 18763-69.
45. Kute JU, Darekar AB, Saudagar RB. In situ Gel-Novel Approach for Nasal Delivery. W. J. Pharm. Pharmaceuti. Sci., 2013; 3(1): 187-203.
46. Kannayiram A. Antidiabetic Drugs and Their Potential Role in Treating Mild Cognitive Impairment and Alzheimer's Disease. Discov Med., 2013; 16(90): 277-86.
47. Li W, Chaudhari K, Shetty R, et al. Metformin Alters Locomotor and Cognitive Function and Brain Metabolism in Normoglycemic Mice. Ag. Dis., 2019; 10(5): 949-63.
48. Nandgude T, Thube R, Jaiswal N, et al. Formulation and evaluation of pH Induced In-situ nasal gel of salbutamol sulphate. Int J Pharm Sci Nanotech., 2008; 1: 177-183.
49. Mrinmoy D, et al. Development, evaluation and characteristics of ophthalmic in situ gel system: a review. Int. J. Curr. Pharm. Res., 2019; 11(4): 47-53.
50. Shaikh RG, Shah SV et al. A Review on Polymers Used in In-Situ Gel Drug Delivery Systems. Int. J. Pharm. Res. Schol., 2012; 1(2): 17-34.

51. Bandyopadhyay SS, Mistry H, Dutta RP. Diabetes and Progression of Alzheimer's: Linking the Two Epidemics. Int. J. Sci. Res., 2014; 3(9): 2238-45.
52. Patil SK, Dhage AN, Patil SV, Patil SS. Formulation and Evaluation of Nasal in situ Gel for Alzheimer Disease. Int. Res. J. Pharm. Biosci., 2015; 2(2): 41- 58.
53. Ankita C, et al. Smart polymers in nasal drug delivery. Ind J Pharm Sci., 2015; 77(4): 367-75.
54. Dibyalochan M, Vasudha B, Nandini S, Haque MA, Sahoo CK. A Review on in situ Gel: A Novel Drug Delivery System. Int. J. Pharm. Sci. Rev. Res., 2018; 50(1): 175-81.
55. Manisha R, Bhavna T, Manjula R. In situ gel -sustained nasal drug delivery. Int. J. Pharm. Sci Res., 2015; 6(12): 4958-66.
56. Lahari L, Bindu EH , Battu S, Abbulu K. Formulation development and evaluation of anti-diabetic oral in situ gel. J Pharma Res., 2019; 8(8): 572-83.
57. Jani RK, Rana P, Baldha RS. Formulation and Evaluation of mucoadhesive in-situ nasal gel of Cyclobenzaprine hydrochloride. Int. J. Pharm. Sci Res., 2019; 10(4): 2054-61.