A NEW NASAL DRUG DELIVERY SYSTEM FOR DIAZEPAM USING NATURAL MUCOADHESIVE POLYSACCHARIDE OBTAINED FROM TAMARIND SEEDS

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تم تطوير نظام جديد لتوصيل دواء ديازيبام عن طريق الأنف باستخدام لواصق مخاطية طبيعية مأخوذة من نبات *Tamarindus indica L.* وقد تبين أن قوة ولزوجة وخاصية تكوين الهلام لهذه اللواصق الهلامية الطبيعية كانت أعلى بالمقارنة مع البوليمرات التخليقية مثل هيدروكسي بروبيل ميثيل سليولوز وكاربوبول 934 اللذان يستخدمان لهذا الغرض. وتبين أن خواص إطلاق الدواء معملياً باستخدام خلية فرانز للانتشار وباستخدام الغشاء الأنفي المستأصل من البقر أفضل بالمقارنة مع البوليمرات التخليقية السابق ذكرها وقد يحل هذا الشكل الدوائي السهل على المريض محل حقن دواء ديازيبام مستقبلاً.

A new nasal drug delivery system of diazepam has been developed with a natural mucoadhesive agent from *Tamarindus indica* L. The mucoadhesive strength, viscosity and gelling property of this natural mucoadhesive agent was found to be higher in comparison to synthetic polymers, hydroxy propyl methyl cellulose (HPMC) and carbopol 934 which are conventionally used for similar purpose. *In vitro* drug release characteristic through franz-diffusion cell using excised bovine nasal membrane was also found to be better in comparison to the above synthetic polymers. This patient friendly, needle free dosage form may replace the diazepam injections in future.

Key words: Nasal drug delivery system, natural mucoadhesive agent, diazepam and *Tamarindus indica* L.

Introduction

Nonparenteral routes for drug delivery include nasal, buccal, pulmonary and transdermal routes. In particular, drug administration by the nasal route allows for high absorption of small molecular weight hydrophobic drugs, avoidance of first pass effects and ease in administration by patients (1). The nasal cavity has a highly vascularized mucosa. Drugs absorbed by the rich network of blood vessels pass directly into the systemic circulation, thereby avoiding first pass metabolism. Despite the potential of the nasal route, a number of factors like mucus and epithelial barrier, mucociliary clearance and enzymatic activity limit the intranasal absorption of drug.

Rapid mucociliary clearance of drug formulations that are deposited in the nasal cavity is thought to be an important factor underlying the low bioavailability of drugs administered intranasally. However, due to rapid mucocilliary clearance, a platform for drug delivery is most essential. Highly swellable mucoadhesive gels exhibiting mucoadhesive behaviour could be extremely useful in nasal delivery applications. Mucoadhesive agents in their molecular form make intimate contact with mucin of mucosa and then make adhesion with the nasal membrane and finally the mucoadhesive carriers allow the release of drug through nasal membrane in a continuous fashion.

In vitro permeability studies offer advantages over *in vivo* studies in that they can be performed more rapidly, involve fewer animals and simpler analytical procedures can be followed since the presence of plasma proteins in the samples is avoided (2). Additionally, since pre- and post mucosal factors are eliminated with *in vitro* techniques, the systems are more standardized (3-6).

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The aim of this study was to develop a nasal delivery system of diazepam with a natural mucoadhesive agent extracted from tamarind seeds. This drug is a benzodiazepine derivative which is widely used in anxiety, tension skeletal muscle spasm, psychosomatic and behaviour disorders, eclampsia status epilepticus, upper neuron spasicity, preanaesthetic medication and in labor. Diazepam produces brief initial phase of strong action followed by milder effect due to two-phase plasma concentration. It is routinely given in the form of injection to many patients in hospitals and nursing homes. Injection is one of the most hazardous dosage forms due to various reasons. This nasal drug delivery system may replace the conventional diazepam injection. This work will definitely add a new dimension in the field of newer drug delivery systems.

Materials and Methods

Materials:

Diazepam was obtained as a gift sample from M/S East India Pharmaceutical Works Ltd, Kolkata. Tamarind seeds were purchased from local market. Hydroxy propyl methyl cellulose (HPMC) 5 cPs and carbopol 934 were purchased from S.D.Fine-Chemical Ltd, Mumbai. All other chemicals used were of analytical grade.

Methods

Extraction of mucoadhesive agent from tamarind seeds:

Tamarind seed mucilage was extracted following the methods of Rao *et al.,* (7,8) with little modifications. 200g of tamarind seeds were soaked in double distilled water and boiled for 5 hours to remove the outer dark layer. To inner white portion sufficient amount of doubled distilled water was added and boiled under stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in refrigerator overnight so that most of the undissolved portion was settled out. The upper clear solution was decanted off and centrifuged at 500 rpm for 20 minutes. The supernatant was separated and concentrated at 60ºC on a water bath until the volume reduced to one third of its original volume. Solution was cooled down to the room temperature and was poured into thrice the volume of acetone by continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50 –60ºC under vacuum. The dried material was powdered and kept in a desiccator.

Determination of pH and Viscosity:

The pH and viscosity of 1% w/v solution of the tamarind seed extract were measured in Toshcon pH Meter and Toki sangyo Viscometer TV-10, respectively and values were compared with 1% w/v solutions of the synthetic polymers HPMC and carbopol 934.

Characterization of mucoadhesive property of tamarind extract:

The mucoadhesive property of tamarind seed extract was determined by Shear stress method (9) and Park and Robinson Method (10) and results were compared with synthetic polymers. In this study 0.5% w/v solutions of the polymers were used for shear stress method and 1% w/v solutions of the polymers for Park & Robinson method.

Release studies of the drug:

Preparation of excised epithelial tissue from nasal mucosa:

Bovine nasal mucosa was obtained from the local slaughterhouse. After removing the skin, the nose was stored on ice in buffer solution during transport to the laboratory (11). The septum wall was fully exposed by a longitudinal incision through the lateral wall of the nose. The septum mucosa was carefully removed from the underlying bone by cutting along the whole septum and pulling the mucosa off the septum with homeostatic forceps. The cavity mucosa was also carefully removed from the conchae and lower cavity using homeostatic forceps after exposing the cavity each side of the septum. The mucosal tissues were then immediately immersed in Ringer's Solution.

Preparation of diazepam solution:

The diazepam solution was prepared by dissolving the drug in a little amount of chloroform and diluting the solution with the nasal solution (12) (0.65% NaCl, 0.04% KH_2PO_4, 0.09% K_2HPO_4 and 0.02% benzalkonium chloride).

Formation of drug-polymer gel:

Diazepam (5mg) was dissolved in 0.1 ml of chloroform. Nasal solution (5ml) was added to the drug in a constant stirring condition. The required

amount of tamarind extract or synthetic polymer (HPMC or carbopol 934) was added to the drug solution and stirred on a magnetic stirrer until the gel is formed.

Release studies of drug from gel:

The franz diffusion cell (13) was used for the drug release study. The diffusion chamber with an exposed tissue surface area of 2.54cm^2 is filled with 100ml of phosphate buffer solution of pH 6.0. The excised nasal mucosal membrane was secured over the mouth of the upper tube keeping mucus side exposed to gel. For equilibration the mucosae were preincubated with preheated buffer for ~30 min. Diazepam containing gel (1mg/ml) placed on the membrane were dispersed in 100 ml of phosphate buffer solution of pH 6.0 and stirred at a constant rate by a PTFE-coated magnetic bar at 600 min−1. Cells were kept under a constant oxycarbon flow (95% O_2, 5% CO_2). Throughout the studies the buffer solution in the chamber was maintained at 37°C connecting the franz diffusion cell with water bath. At predetermined period of time 1ml of sample was taken and simultaneously replaced with same volume of prewarmed (37°C) fresh buffer solution. The collected samples were diluted suitably and drug concentration estimated using Jasco V-530 UV/VIS Spectrophotometer at 240 nm wavelength.

Effect of the enhancers:

Several enhancers like sodium taurocholate, sodium tauroglycocholate, bile salt and sodium thioglycollate were used in 0.5% w/v concentration to evaluate the effect of these materials on drug release characteristics from natural and synthetic nasal gels.

Results and Discussion

pH and Viscosity measurement studies

The pH of tamarind extract, HPMC and carbopol 934 (1% w/v) were determined. The pH of the natural mucoadhesive material and synthetic polymers (HPMC and Carbopol 934) ranged between 3.0-7.2. The pH of tamarind extract varied from 6.0 to 7.2 ,whereas pH of HPMC and carbopol 934 were around 6.5 and 3.0 respectively. As the pH of nasal mucosa varied between 5.5-6.5, all the materials tested were found to be suitable for preparing nasal gels.

Viscosities of tamarind extract, HPMC and carbopol 934 (1% w/v) were determined and the results are given in Fig. 1.

It was found that the viscosities of 1% w/v; tamarind extract, HPMC and Carbopol 934 ranged between 38-40 cp, 3-6cp and 14-17cp respectively. From the above experimental observations it was found that natural mucoadhesive tamarind extract exhibited a higher viscosity than synthetic polymers (HPMC and carbopol 934) at the same concentration.

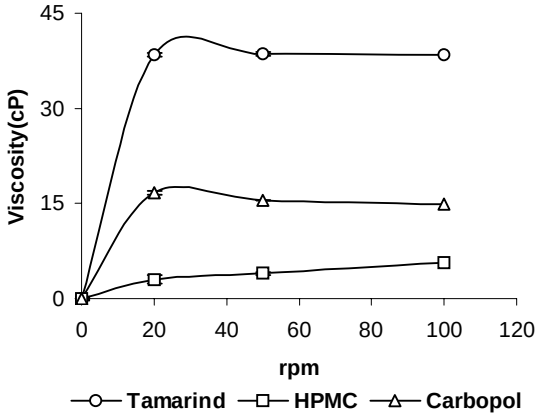

Fig. 1. Comparative evaluation of viscosity of the natural mucoadhesive extract of Tamarind with synthetic polymers HPMC and carbopol 934 .All the polymers used in 1% w/v solution. Experimental temperature was maintained at 37°C±1. Values are expressed as the mean of 6 observations.

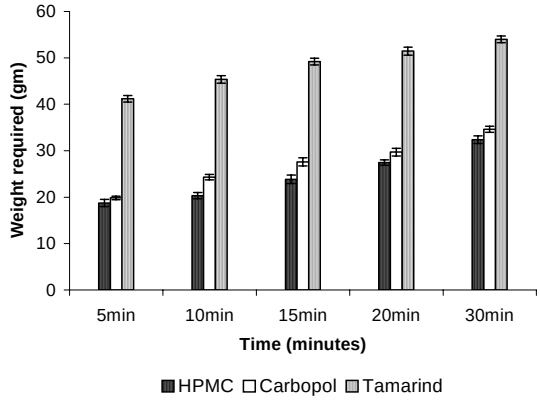

Fig. 2. Comparative result of bioadhesive property of the natural mucoadhesive extract of Tamarind with synthetic polymers HPMC and carbopol 934 by shear stress method. All the polymers used in 0.5% w/v solution. Experimental temperature was maintained at 37°C±1. Values are expressed as the mean of 6 observations.

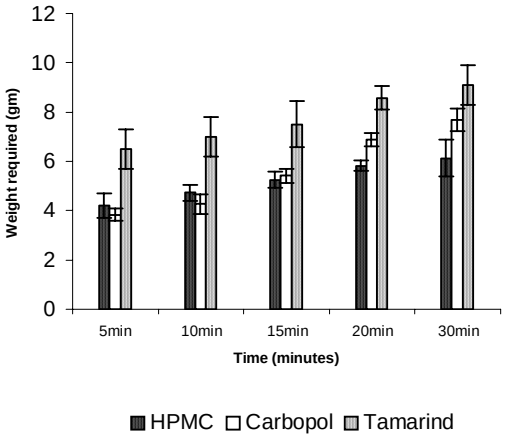

Fig-3. Comparative result of mucoadhesive property of the natural mucoadhesive extract of Tamarind with synthetic polymers HPMC and carbopol 934 by Park & Robinson method. All the polymers used in 1% w/v solution. Experimental temperature was maintained at 37°C±1. Values are expressed as the mean of 6 observations.

Mucoadhesive property measurement studies:

The results of the mucoadhesive property measured by shear stress (9) and Park & Robinson methods (10) in which weights required to break the adhesion, recorded for various polymers like tamarind extract, HPMC and carbopol 934 with different contact times (14,15) are shown in Fig 2 & 3. From the figures it is observed that increased contact time increased the adhesion strength, allowing for greater adhesion. Probably increasing contact time might be reducing hydration due to evaporation facilitating higher adhesion.

From the results of both methods it was found that natural mucoadhesive tamarind extract having high molecular weight and high viscosity exhibited higher adhesion and better mucoadhesive property in comparison to synthetic polymers (HPMC and carbopol 934) at the same concentration. This may be due to presence of numerous carboxyl and hydroxyl groups, which adopts more favorable macro molecular confirmation and accessibility of its hydrogen-binding group, while compared with other polymers. HPMC may have formed weaker bondage with mucus, which is due to decrease available hydrogen binding sites or an unfavorable contamination for entanglement to occur.

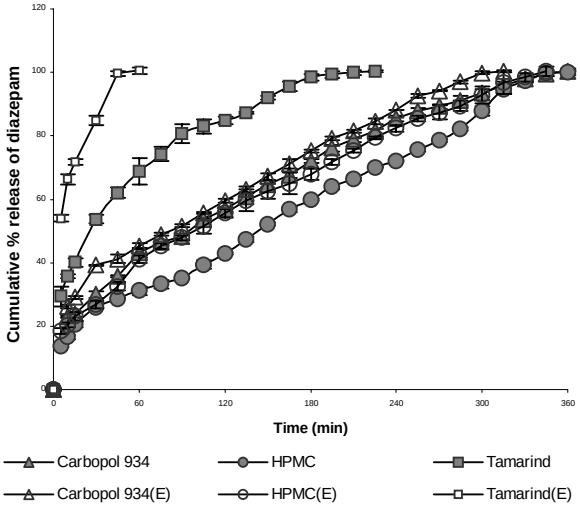

Fig. 4. Comparative result of cumulative percentage release of diazepam from the natural mucoadhesive extract of Tamarind and synthetic polymers HPMC and carbopol 934 with and without enhancer (E) Sodium taurocholate 0.5% w/v. Values are expressed as the mean of 6 observations.

In-vitro release study:

It was observed that 100% drug was released in case of synthetic polymers (HPMC and carbopol 934) containing diazepam between 5-6 hours, whereas in case of natural mucoadhesive agent (tamarind) containing diazepam 100 % drug was released within 3 hours. (Fig. 4).

Effect of the enhancers:

Fig 4 shows that 100% diazepam was released from tamarind after 3 hours and 45 minutes. However, when sodium taurocholate was used as an enhancer 100% drug was released within 45 minutes. In case of other enhancers like sodium thioglycollate, sodium tauroglycocholate and bile salts 100% drug was released within 2 hrs, 2 hrs15 minutes and 2hrs 30 minutes respectively.

In case of carbopol 934 and HPMC 100% drug was released in about 6 hrs when no enhancer was used. However for carbopol 934 when sodium taurocholate was used as an enhancer 100% drug was released within 5 hrs. With other enhancers used, no noticeable change of drug release pattern was observed in case of carbopol 934 . In case of HPMC, all enhancers did not increase the drug release.

Conclusion

It can be concluded from the present study that tamarind is a better mucoadhesive agent than conventionally used synthetic mucoadhesive agents HPMC and carbopol 934. When sodium taurocholate is used as an enhancer the drug release is increased to a significant extent in case of tamarind. Since this natural mucoadhesive agent is edible, it is easily biodegradable and non-allergic like many synthetic agents used for similar purpose. Diazepam is routinely given in the form of injection to many patients in hospitals and nursing homes. Injection is a hazardous dosage form due to various reasons. This nasal drug delivery system may replace the conventional diazepam injection. This work will definitely add a new dimension to newer drug delivery systems. However, further *in vivo* study is necessary to elucidate the safety of the extracted natural material on nasal mucosa and its self cleaning mechanism.

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References

1. Nakamura K, Maitani Y, Lowman AM, Takayama K, Peppas NA and Nagai T. Uptake and release of budesonide from mucoadhesive, pH sensitive copolymers and their application to nasal delivery. Journal of controlled release. 1999;61:329-35.
2. Lee CP, de Vrueh RLA and Smith PL. Selection of development candidates based on in vitro permeability measurements. Adv. Drug Del. Rev. 1997;23:47– 62.
3. Jorgensen L and Bechgaard E. Intranasal permeation of thyrotropin releasing hormone: in vitro study of permeation and enzymatic degradation. Int. J. Pharm. 1994;107:231–37.
4. Kubo H, Hosoya KI, Natsume H, Sugibayashi K and Morimoto Y. In vitro permeation of several model drugs across rabbit nasal mucosa. Int. J. Pharm. 1994;103:27–36.
5. Wheatley MA, Dent J, Wheeldon EB and Smith PL. Nasal drug delivery: an in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. J.Control. Rel. 1988;8:167–77.
6. Hosoya KI, Kubo H, Natsume H, Sugibayashi K, Morimoto Y and Yamashita S. The structural barrier of absorptive mucosae: site difference of the permeability of fluoroscein isothiocyanate-labelled dextran in rabbits. Biopharm. Drug Disp. 1993;14:685–96.
7. Khullar P, Khar RK and Agarwal SP.Evaluation of guar gum in the preparation of sustained-release matrix tablets. Drug. Dev. Ind. Pharm. 1998; 24:1095-99.
8. Rao PS, and Srivastava HC, Tamarind in Whistler RL (ed), Industrial Gums. 2nd ed. New York; Academic Press, 1973;369-411.
9. Rao YM, Vani G and Bala Ramesha Chary R. Design and evaluation of mucoadhesive drug delivery systems. Ind. Drugs. 1998;35:558-565.
10. Park K & Robinson R, Bioadhesive platforms for oral-controlled drug delivery: method to study bioadhesion, Int. J. Pharm, 1984;19:107-27.
11. Schmidt MC, Simmen D, Hilbe M, Boderke P, Ditzinger GN, Sandow JR, Lang S, Rubas W and Merkle HP. Validation of Excised Bovine Nasal Mucosa as In Vitro Model to Study Drug Transport and Metabolic Pathways in Nasal Epithelium. J. Pharm. Sci. 2000;89:396-407.
12. Loyd V, Allen Jr. Compounding Nasal Preparations. Secundum Artem. 1998;7:1-6.
13. Frantz SW. Instrumentation and methodology for in vitro skin diffusion cells in methodology for skin absorption. In: Methods for Skin Absorption (Kemppainen BW,Reifenrath WG, Eds), CRC Press, Florida, 1990:35-59.
14. Gandhi RB, Robinson JR. Bioadhesion in Drug Delivery. Ind. J. Pharm. Sci. 1988;50:145-52.
15. Ahuja A, Khar RK and Ali J. Mucoadhesive Drug Delivery Systems. Drug Dev. Ind. Pharm. 1997; 23:489-515.