

Studies on Intranasal Drug Delivery Using a Novel Thermosetting Vehicle

FINAL REPORT

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Introduction:

Nasal drug delivery is an attractive alternative for systemic delivery of drugs with low oral bioavailability due to first pass metabolism and / or enzymatic degradation. Additionally, features such as rich vasculature, rapid absorption and ease of administration make it a convenient route for systemic drug delivery. It also offers the possibility of directing drug delivery to the brain via olfactory pathway bypassing the blood brain barrier ⁽¹⁾.

Thermosensitive polymers are a new generation of polymers, which, in solution, under suitable conditions, exist as liquid at ambient temperatures and form a gel on warming. These polymers gel at or near physiological temperature. They offer specific benefits for intranasal drug delivery which include ease of administration due to fluidity at room temperature followed by prolonged retention due to gelling at body temperature. They are generally based on synthetic polymers including poloxamers, *N*-Isopropylacrylamide copolymers and PEG/PLGA block copolymers.

Chitosan is the deacetylated derivative of chitin. It is a biocompatible, pH-dependant cationic polymer, which is soluble in acidified water up to pH 6.2. Basification of chitosan aqueous solutions above this pH leads to the formation of a hydrated gel-like precipitate. pH-gelling, cationic chitosan solutions can be transformed into thermally sensitive, pH-dependent, gel-forming systems by the addition of polyol salts such as glycerolphosphate (GP). These formulations possess a neutral pH, remain liquid at or below room temperature, and form monolithic gels at body temperature. Upon warming, these solutions quickly transform into a hydrogel structure. The molecular mechanism of gelation may involve multiple interactions between chitosan, glycerolphosphate, and water, several of which may be thermally modulated.

Rizatriptan benzoate is a selective 5HT_{1B/1D} agonist widely prescribed for migraine with an oral bioavailability of 40% due to extensive first pass. Doxepin is an antidepressant with norepinephrine and serotonin uptake inhibitor action and is used in the treatment of mild to moderate depressive disorder. It also has a poor oral bioavailability of 25% due to extensive first pass metabolism.

The present study was designed to explore the potential of a thermosensitive polymeric system based on the natural polymer chitosan for the development of intranasal formulations for systemic delivery of rizatriptan and doxepin; both drugs



with activity in the CNS. The formulations were intended for intranasal administration to bypass the oral bioavailability problems associated with the selected molecules coupled with the possibility of an added advantage of direct delivery to the brain via the olfactory shunt pathway. Also, the formulations were designed to exist as liquids at room temperature allowing easy instillation into the nose and to convert to gel at physiological temperature and provide a sustained release of drugs thereby improving contact with nasal tissues and also reducing the dosing frequency.

The prepared gels were evaluated for gelling properties, rheological behaviour, *in vitro* drug release, *ex-vivo* nasal permeation, *ex-vivo* effects on mucociliary function.

MATERIALS: Chitosan(90% deacetylated) was obtained as gift sample from CIFT, Cochin, India, rizatriptan and doxepin HCl was provided as gift sample from Cipla, Mumbai and Torrent Pharma, Gujarat respectively.

Equipments and apparatus

- Single pan balance (CB-Series, Contech precision balance).
- UV-visible spectrophotometer (Jasco V-530)
- Cyclomixer (Remi)
- Rotamantle (Galaxy Scientific)
- pH meter (Universal Enterprises)
- Digital camera (Canon)
- Capillary viscometer (J-SIL)
- Melting point apparatus (Superfit)
- Electrically heated water bath (Superfit)
- Permeation apparatus (DBK Magna)
- Franz Diffusion cells (Labglass)

EXPERIMENTAL:

1. Determination of molecular weight of chitosan:

Preliminary preformulation studies were undertaken for choosing a suitable grade of chitosan and an appropriate proportion of additives for preparing the thermoreversibly gelling systems. A series of dilute chitosan solutions ranging from 0.02%-0.1%w/v of the different chitosan grades were prepared in 0.1N HCl. The time taken by a definite volume of



these solutions to flow through a capillary viscometer was measured using a U-tube viscometer. The molecular weight of different grades of chitosan was determined from the intrinsic viscosity using the Mark-Houwink equation ⁽²⁾.

2. Selection of chitosan by gelation studies

Preliminary drug free gels were prepared using 2% w/v concentration of chitosan samples of different molecular weights over a range of 8-12% w/v of disodium salt of β -glycerol phosphate (GP) (Table 1).

3. Preparation of drug free gels and evaluation of drug free gel

The thermoreversible gelling systems (drug free gels) were prepared by simple admixture of the components under appropriate conditions. Two types of systems were prepared.

- I. Based on chitosan and GP (C-GP systems): Chitosan solution in 0.1N HCl (2.5%w/v, 4ml) and aqueous GP (40%w/v-60%w/v, 1ml) were cooled to 4°C. This was followed by dropwise addition of GP to the chitosan solution with vigorous mixing with the aid of a cyclomixer. The final C-GP mixtures contained 8%w/v-12%w/w of GP and 2%w/v chitosan.
- II. Based on chitosan, GP and PEG 4000 (C-GP-PEG systems): The above C-GP gels were extremely hypertonic due to the high proportion of GP. In an attempt to reduce the concentration of GP and thereby reduce the osmolarity of the preparations, the effect of addition of PEG 4000 into the gel was evaluated. Gels containing PEG 4000 (C-GP-PEG gels) were prepared in a similar manner as C-GP gels by keeping the chitosan concentration constant and varying the concentration of aqueous GP. The final mixtures contained 4.5%w/v- 4.8%w/v of GP, 2%w/v chitosan and 1-4% w/v PEG. The gels were cured for an hour at 4°C before checking for gelation.

Physicochemical properties and performance characteristics of the systems like gelation temperature, gelation time, pH, rheological properties and gel strength were determined as follows :

a) Gelation temperature, time and pH ⁽³⁾

The series of C-GP and C-GP-PEG systems prepared were taken into test tubes. The gelling temperature was measured by immersing the sols in a water bath and increasing the temperature gradually from 15 °C to 40 °C at a rate of 0.5 °C/min. The temperature





for doxepin. A validated standard plot of the drugs in PBS 6.4 was used for quantification. Release profile was plotted as cumulative % released vs. time. And the data was subjected to analysis for elucidating the mechanism of release.

b) *Ex-vivo* permeation studies:

Ex vivo permeation study was carried out using sheep nasal mucosa to assess the permeability of rizatriptan and doxepin from formulations and solutions. Phosphate buffered saline (pH 6.4) was used as receptor medium. Sampling was done at time intervals of 1, 2, 3, 4, 5 and 6 hours and samples were analyzed for drug content by UV spectrophotometry. The data was plotted as % drug permeated vs. time. The aliquots from drug free gels were analyzed at both 280 nm as well as 292 nm to check for rizatriptan and doxepin respectively for any interference. Apparent permeability coefficient (Papp) for rizatriptan and doxepin were calculated from the permeation data.

c) Mucoadhesion

Mucoadhesive strength of the prepared gels was measured both at 25°C and 37°C as the force required for detaching two coverslip surfaces coated with porcine mucin solution with the formulation between them using a modified two pan balance.

d) Mucociliary transport rate

The mucociliary transport rate measured as the time taken by an opium poppy seed to move on frog palate treated with excipients and formulations was measured as an indicator of ciliary damage. Data was analyzed by one way ANOVA followed by Bonferroni's test.

5. *In vivo* studies

Drugs that stimulate or depress CNS play an important role in human therapeutics.

Rodent models provide a vital means of assessing efficacy of such drugs and their formulations *in vivo*. The *in vivo* studies for their intranasal formulations developed during the present studies were undertaken as follows

1. Rizatriptan manifests its clinical effect by acting on the serotonergic receptors which are also involved in the regulation of locomotion⁽⁵⁾. Therefore effect of the rizatriptan containing formulations was tested in mice model via measurement of changes in locomotor activity of the treated animals using actophotometer.



2. Doxepin formulations were tested for their antidepressant efficacy in rodent models by forced swim test ⁽⁶⁾. The animals were dosed for 13 consecutive days and the effect on activity counts and immobility duration was studied on the last three days of dosing.

6. Histopathology of nasal tissues

Nasal tissues of animals post the efficacy studies were histopathologically examined for damage/irritation due to the treatment. One Swiss albino mice from each group used in *in vivo* efficacy testing of formulations was randomly selected for histopathological studies. The selected animal was further dosed once daily for three more consecutive days (6 days exposure) or for further two days (15 days exposure) in case of rizatriptan and doxepin treated animals respectively. The sections of the formulation and drug solution treated nasal mucosa were compared with the control group (table 3).

7. Stability studies:

Stability studies were carried out at 4°C and 25°C as per ICH guidelines ⁽⁷⁾. Studies were carried out in triplicate. One vial was withdrawn at each time point (0, 30, 60, 90, and 180 day intervals) and tested for gelation time, gelation temperature, pH, gel strength drug content and drug release.

Results and discussion

1. Determination of molecular weight:

The molecular weight of different chitosan grades were found to range from about 28,000 to greater than a lakh (28,886-1, 00,505).

2. Selection of chitosan by gelation studies

Our studies on chitosan solutions in presence of GP reveal that there seems to be an intermediate range of molecular weight values suited to forming thermoreversible systems. Systems prepared using medium molecular weight of chitosan seemed to favour sol-to-gel transition and chitosan with molecular weight of 73,339 was selected for the present studies (Table 1).

Chitosan sample with an intermediate molecular weight of 73,339 was found to show optimum gelation characters in presence of GP with respect to gelation time,



temperature, thermoreversibility and stability and was selected for further development of thermoreversibly gelling systems

3. Preparation of drug free gels and evaluation of drug free gel

a) Gelation temperature, time and pH

The systems selected were those capable of gelling at or near physiological temperature and having mildly acidic pH (6.5-6.8). The gelation time for all the formulations was between 7-10 min.

b) Gel strength measurement

Gel containing 10% w/v GP was found to have the highest gel strength amongst all those examined. Further increase in GP concentration resulted in marginal reduction in the gel strength. C-GP-PEG gels showed comparatively lower gel strength than C-GP gels.

Systems containing 2% w/v chitosan, 10% w/v GP for C-GP gels and those containing 2% w/v chitosan, 4.5% w/v GP and 1% PEG for C-GP-PEG gels were selected for preparation of drug containing formulations since these showed optimum gelation characteristics.

c) Rheological evaluation

The formulations at both ambient and body temperature showed pseudoplastic behaviour with mild thixotropy and the viscosity of C-GP-PEG gels were higher than C-GP gels at both the temperatures at which determination was carried out (*Fig 1.1 and 1.2*).

4. Preparation of drug containing gels

Results of evaluation of formulations containing rizatriptan and doxepin for pH, gelling temperature, gelling time, gel strength and drug content are as shown in (*Table 2*). All the properties of the gels were found to be almost superimposed on those of drug free gel indicating no alteration in the properties due to presence of rizatriptan or doxepin.

a) In- vitro drug release

Both C-GP and C-GP-PEG gels were able to sustain release of the incorporated drugs. The release began with an initial burst followed by sustained release following Higuchi's kinetics. At the end of 8 hrs about 70-75% of both incorporated drugs were released from the C-GP gels. Inclusion of 1% w/v PEG into gels containing 4.5% GP and 2%w/v chitosan resulted in a further sustainment of drug release (40-50% in the same time



period). Identical release profiles for both the water soluble drugs indicate an inherent ability of the gel to sustain release of incorporated agents (*Fig 2*).

b) *Ex-vivo* permeation studies:

The *ex-vivo* permeation studies revealed an improved permeation of drugs across sheep nasal mucosa from the C-GP formulations in comparison to C-GP-PEG gels and also drug solutions which is in accordance with literature reports that chitosan possesses permeation enhancing properties across biological membranes (*Fig 3*). Such effects however were less conspicuous in case of the C-GP-PEG gels although they contained the same concentration of chitosan, possibly due to interaction between PEG and chitosan.

c) Mucoadhesion

At 25°C, both types of formulations were more mucoadhesive than chitosan solution with PEG containing drug free gels showing the highest mucoadhesive strength. PEG gels have higher chain flexibility and viscosity and therefore at 25 °C the interaction of the polymeric chains with the mucin is higher. Addition of drug reduces mucoadhesion in all instances. Unfortunately, mucoadhesive force for formulations at 37°C was found to be low than when measured at 25°C (*Fig 4*). This is attributed to the interaction of the polymer chains amongst themselves during gelation rather than with mucin.

e) Mucociliary transport rate

The studies showed significant reduction the transport rate of seed on the palate treated with formulations and excipients when compared with control (*Fig 5*). Treatment with C-GP formulations resulted in a nearly complete arrest in movement of poppy seed indicating ciliary damage whereas the PEG containing formulations revealed a significantly lower severity of mucociliary damage. Transient or reversible arrest of mucociliary function may be beneficial in reducing mucociliary clearance and increasing the contact time of the formulations ⁽⁴⁾.

5. *In-vivo* studies

The *in vivo* efficacies of C-GP-PEG-Riz and C-GP-Riz gels in comparison with solution treated and untreated control showed that both the formulations were equally effective and had prolonged duration of action as compared to rizatriptan solution since they exhibited a marked decrease in locomotor activity.



C-GP-PEG-Dox and C-GP-Dox showed significantly better efficacy in terms of increase in the activity of the animal and reduction in immobility duration in comparison to doxepin solution and control group over a 5 hour study period. It was also observed that the formulations showed prolonged effect on the activities for the observed time period which indicates sustained effects from formulations.

6. Histopathology of nasal tissues

The histopathological examination of the nasal mucosa of mice dosed intranasally once a day with rizatriptan containing formulations for 5 days and doxepin containing formulations for 13 days revealed mild to moderate damage to the nasal tissues when compared to the control (Table 3, Fig 6). Drug solutions were found to cause greater tissue damage than when administered in the formulations.

7. Stability studies

Stability studies carried out at 4°C (Table 4) and 25°C (Table 5) revealed that although both drugs when assessed by UV revealed no signs of instability, the formulations were unable to retain their characteristics beyond two months at either storage condition. Formulations showed an increase in gelling temperature beyond 37°C and a prolongation of gelling time. The release (in triplicates) during the initial 2 months was unaltered (Fig 7). Both rizatriptan and doxepin containing formulations on storage beyond 2 months, showed faster release with about 80-90% of the drug release in 8 hrs in the 6th month. This may be attributed to the changes in gelation characters.

The results presented here showed that chitosan/GP systems have the potential to be used as intranasal *in situ* gelling thermosensitive formulations for delivery of CNS acting agents since the systems possess optimum gelation characteristics, safety and efficacy. In addition, due to the sustained effect the formulations the dosing frequency can be reduced.



Table 1: Observations during preliminary gelation studies on different chitosan grades

Chitosan Sample no.	MW	GP (%w/v)	Gelation Time (min) (n=4)	Gel stability	Thermoreversibility
1	28886	8	12.05 ± 1.09	precipitation on gelling at 37 °C	Irreversible
1	28886	9	11.40 ± 0.8	precipitation on gelling at 37 °C	Irreversible
1	28886	10	11 ± 0.50	precipitation on gelling at 37 °C	irreversible
1	28886	11	10.30 ± 0.78	precipitation on gelling at 37 °C	irreversible
1	28886	12	10 ± 0.34	precipitation on gelling at 37 °C	irreversible
2	39212	8	5 ± 0.55	precipitation on gelling at 37 °C	irreversible
2	39212	9	4.30 ± 0.49	precipitation on gelling at 37 °C	irreversible
2	39212	10	4 ± 0.45	precipitation on gelling at 37 °C	irreversible
2	39212	11	3.45 ± 0.35	precipitation on gelling at 37 °C	irreversible
2	39212	12	3 ± 1.20	precipitation on gelling at 37 °C	irreversible
3	73339	8-12	8 - 10	No precipitation	reversible
4	100505	8-12	No gelation	No gelation	No gelation

Table 2: Physicochemical evaluation of drug loaded C-GP and C-GP-PEG gels

Chitosan %w/v	PEG %w/v	GP %w/v	Drug	Gelation temp. (°C)	Gelation time (min)	pH	Gel strength (sec)	Drug content %
2	-	10	Rizatriptan	37.4±0.42	7.32±0.36	6.93±0.02	9.56±0.92	99.5±1.10
2	-	10	Doxepin	37±0.35	8.15±0.75	6.93±0.03	9.55±0.75	100.4±0.96
2	1	4.5	Rizatriptan	37±0.22	7±0.50	6.5±0.2	5.58±0.46	99.89±0.88
2	1	4.5	Doxepin	38±0.55	7±0.25	6.5±0.15	5.46±0.09	99.43±0.79



Table 3: Histopathological data of nasal tissues

Characteristic features	C-GP-Riz	C-GP-PEG-Riz	Riz - solution	C-GP-Dox	C-GP-PEG-Dox	Dox - solution	Control
Glandular hyperplasia	+	+	++	+	+	+	-
Infiltration of the inflammatory cells	+	+	++	+	+	+	-
Epithelial hyperplasia	-	++	+++	+	++	+	-
Sluffing of mucosal epithelium	-	-	+	-	-	+	-
Congestion	-	+	-	-	-	-	-

+ : mild

++ : moderate

+++ : severe

- : no abnormality detected

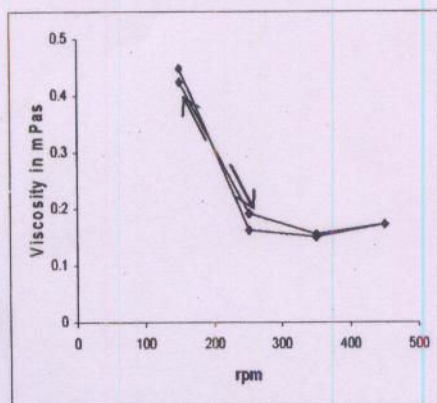
Table 4: Stability data of formulations at 5°C

Duration of month	Formulations at 5 °C ± 3 °C	Gelation temperature (°C)	Gelation time (min)	pH	Gel strength (sec)	Drug content
0	C-GP-Riz	37.4	7.32	6.77	9.48	99.05
1	C-GP-Riz	37.6	7.36	6.85	9.52	99.01
2	C-GP-Riz	37.4	7.38	6.72	9.50	99.89
3	C-GP-Riz	39	-	6.79	7	98.96
6	C-GP-Riz	41	-	6.80	6.36	98.92
0	C-GP-PEG-Riz	37	7.4	6.5	5.58	99.94
1	C-GP-PEG-Riz	37	7.45	6.53	5.46	99.89
2	C-GP-PEG-Riz	37.6	7.49	6.51	5.42	99.87
3	C-GP-PEG-Riz	38	-	6.48	5	99.85
6	C-GP-PEG-Riz	40	-	6.45	4	99.87
0	C-GP-Dox	37	7.34	6.72	9.48	99.96
1	C-GP-Dox	37.2	7.36	6.88	9.52	99.89
2	C-GP-Dox	37.2	7.36	6.89	9.50	99.15
3	C-GP-Dox	38.6	-	6.86	7	98.91
6	C-GP-Dox	39.4	-	6.85	6.36	98.92
0	C-GP-PEG-Dox	37	7.42	6.5	5.58	100.84
1	C-GP-PEG-Dox	37	7.45	6.53	5.46	99.89
2	C-GP-PEG-Dox	37.6	8.54	6.51	5.42	99.82
3	C-GP-PEG-Dox	38.2	-	6.40	5	99.79
6	C-GP-PEG-Dox	40	-	6.43	4	99.75

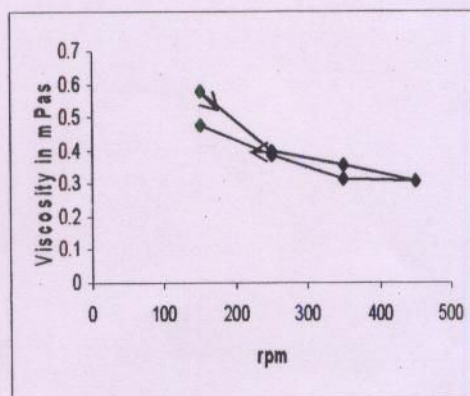


Table 5 Stability data of formulations at 25°C/60%RH

Duration of month	Formulations at 25°C/60%RH	Gelation temperature (°C)	Gelation time (min)	pH	Gel strength (sec)	Drug content
0	C-GP-Riz	37.4	7.32	6.93	9.56	99.5
1	C-GP-Riz	37	8.15	6.93	9.55	100.4
2	C-GP-Riz	37	7.40	6.95	8.30	99.89
3	C-GP-Riz	38.6	-	6.88	5.46	99.43
6	C-GP-Riz	41	-	6.93	5.11	99.5
0	C-GP-PEG-Riz	37	7.4	6.5	5.58	100.26
1	C-GP-PEG-Riz	37	7.45	6.53	5.46	99.89
2	C-GP-PEG-Riz	37.6	7.49	6.51	5.42	99.82
3	C-GP-PEG-Riz	38.8	-	6.48	5	99.79
6	C-GP-PEG-Riz	40	-	6.45	4	99.75
0	C-GP-Dox	37.4	7.32	6.93	9.56	99.5
1	C-GP-Dox	37	8.15	6.93	9.55	100.4
2	C-GP-Dox	37	7	6.9	9.38	99.89
3	C-GP-Dox	38.4	-	6.91	5.46	99.43
6	C-GP-Dox	42	-	6.94	4.39	99.5
0	C-GP-PEG-Dox	37	7.4	6.5	5.58	100.26
1	C-GP-PEG-Dox	37	7.45	6.53	5.46	99.89
2	C-GP-PEG-Dox	37.6	7.49	6.51	5.42	99.82
3	C-GP-PEG-Dox	38	-	6.48	5.14	99.79
6	C-GP-PEG-Dox	40	-	6.52	4.18	99.77



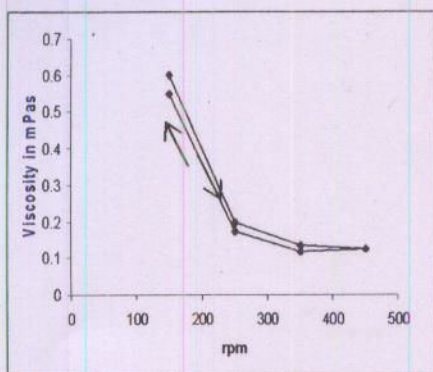
25°C



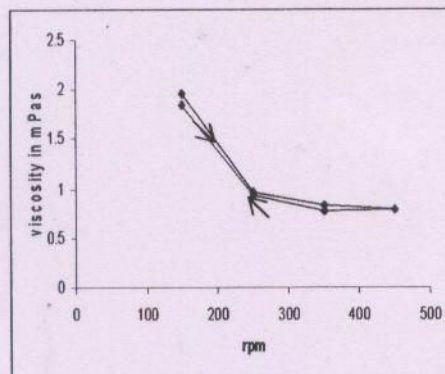
37°C

Fig 1.1: Rheological behavior of C-GP gels containing 10% GP and 2% chitosan





25°C



37°C

Fig 1.2: Rheological behaviour of C-GP-PEG gels containing 4.5% GP, 2% chitosan and 1% PEG

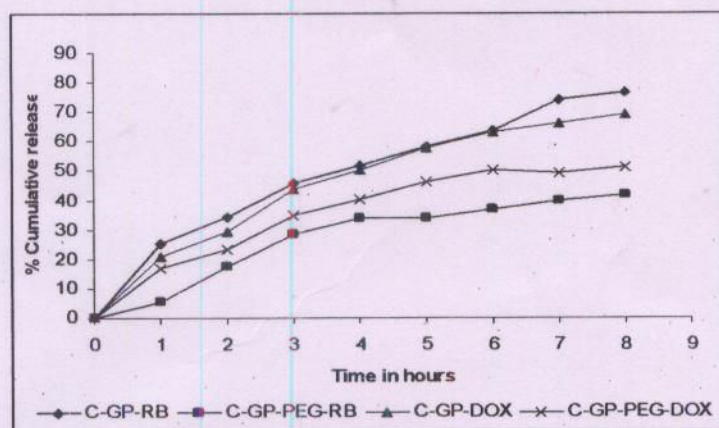
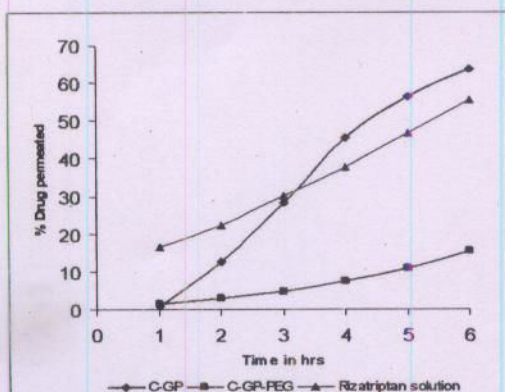
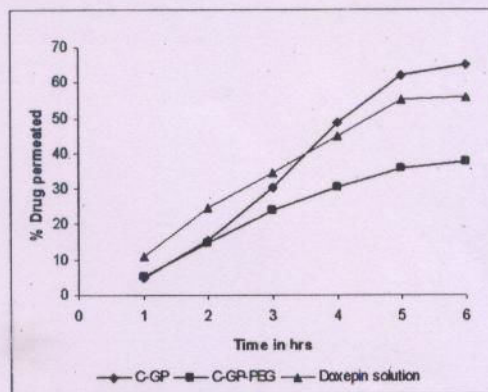


Fig 2: Release profile of rizatriptan and doxepin from C-GP and C-GP-PEG gels



a) Rizatriptan containing formulation



b) Doxepin containing formulation

Fig 3: Ex-vivo permeation studies



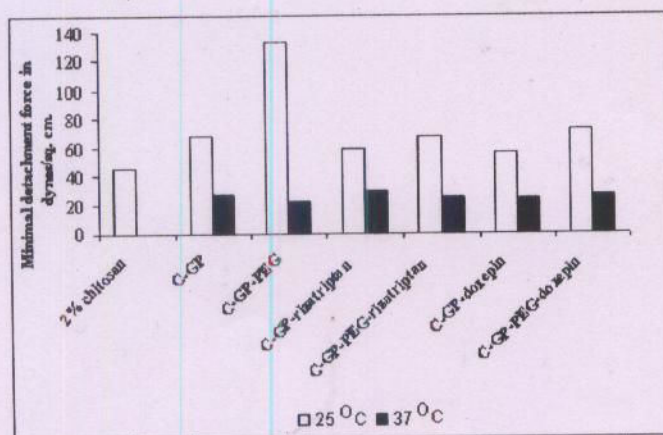


Fig 4: Mucoadhesion of formulations at 25 °C and 37 °C

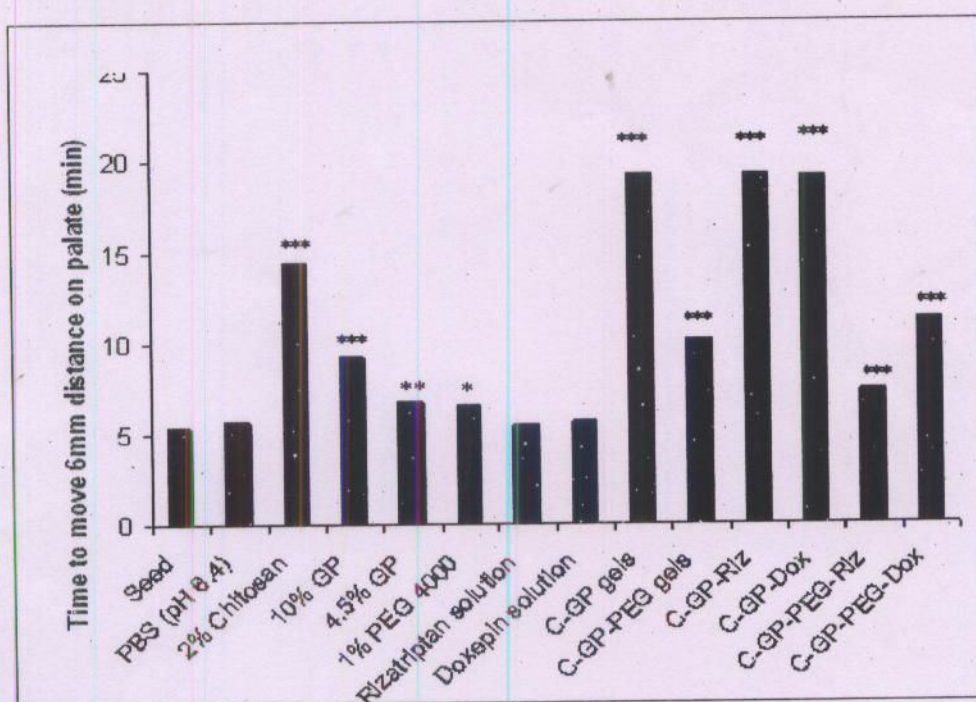
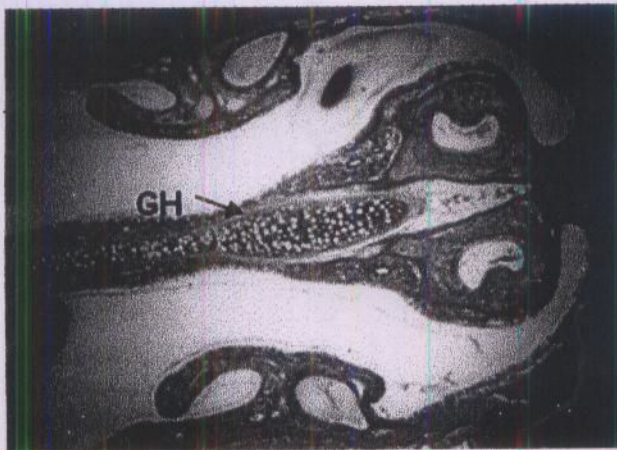


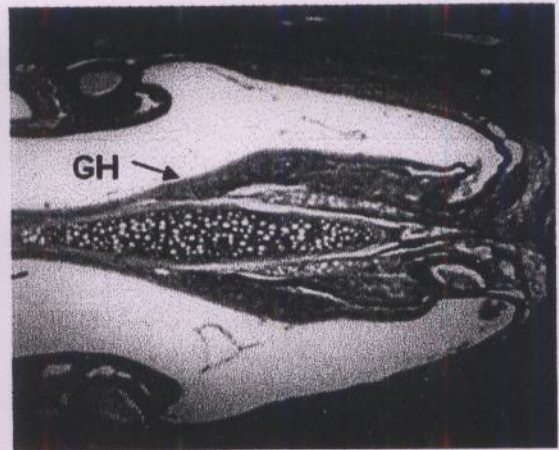
Fig5: Mucociliary trans- port rate

Data was analyzed by one way ANOVA followed by Bonferroni's test; (n =6), * P<0.05
 P<0.01 *P< 0.001

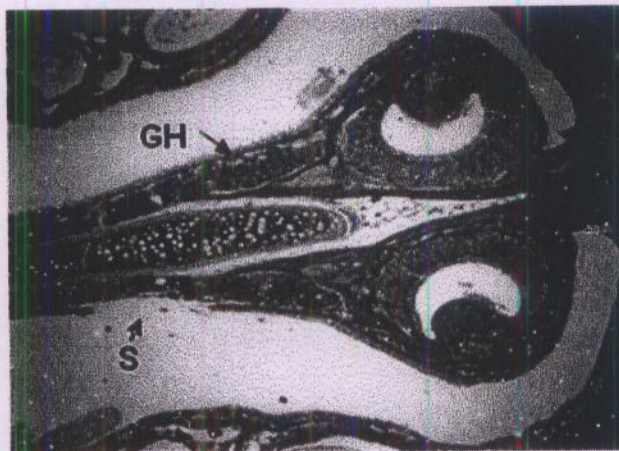




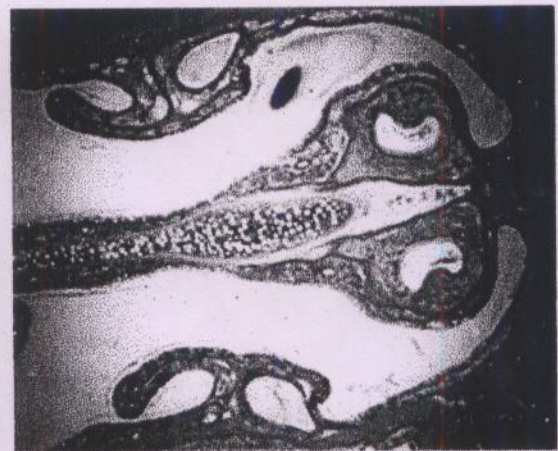
C-GP-Riz



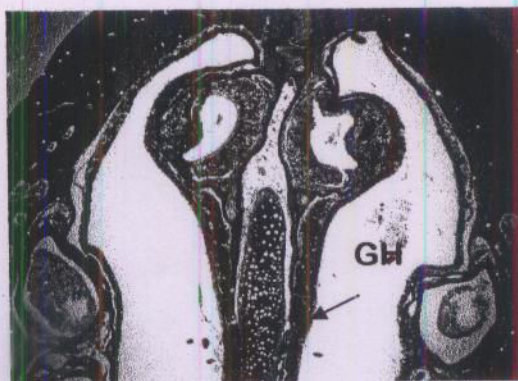
C-GP-PEG-Riz



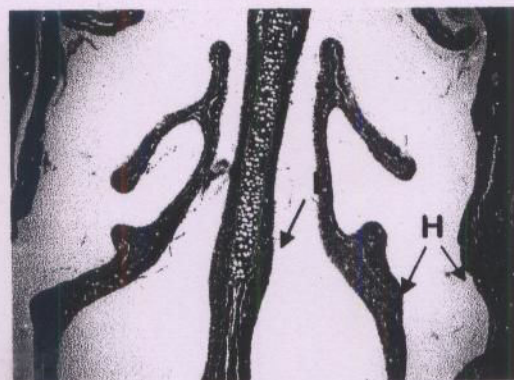
Rizatriptan solution



Control



C-GP-Dox

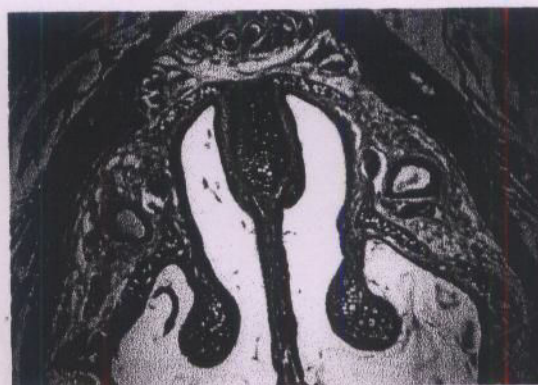


C-GP-PEG-Dox





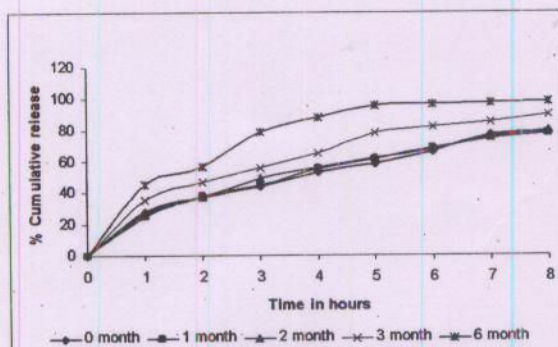
Doxepin solution



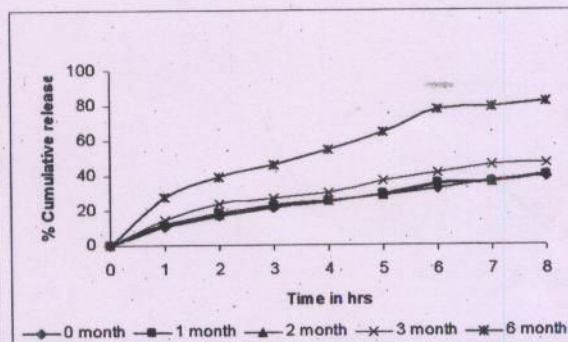
Control

Fig 6: Morphological study of nasal tissue in mice for group treated with drug containing formulations

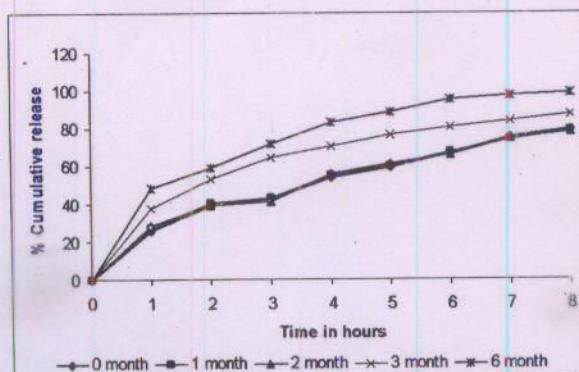
GH: Glandular hyperplasia; I: Infiltration of inflammatory cells; H: Epithelial hyperplasia
S: Sluffing of nasal mucosa



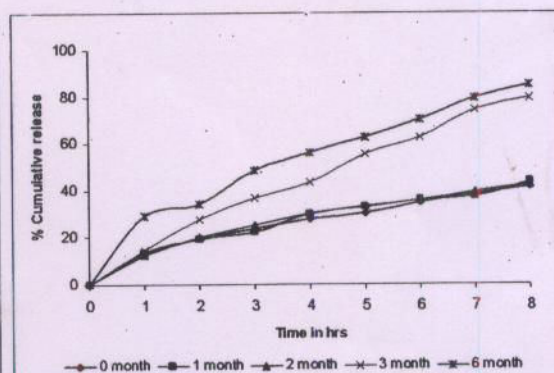
C-GP-Riz gels at 5°C ± 3°C



C-GP-PEG-Riz gels at 5°C ± 3°C

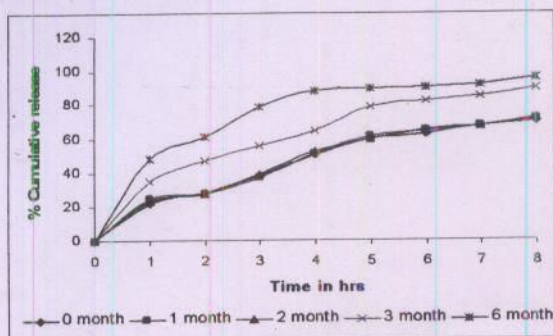


C-GP-Riz gels at 25°C/60%

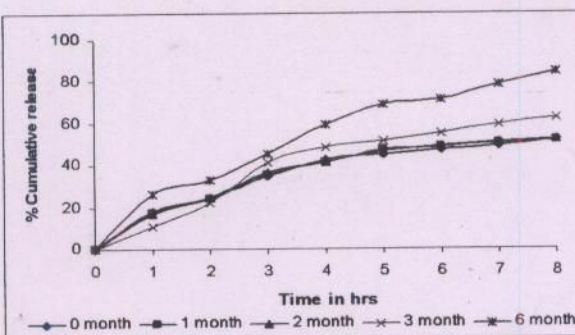


C-GP-PEG-Riz gels at 25°C/60%

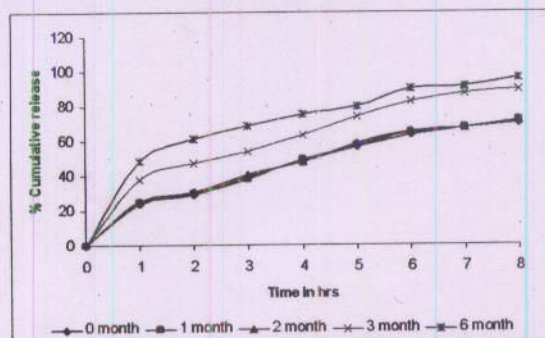




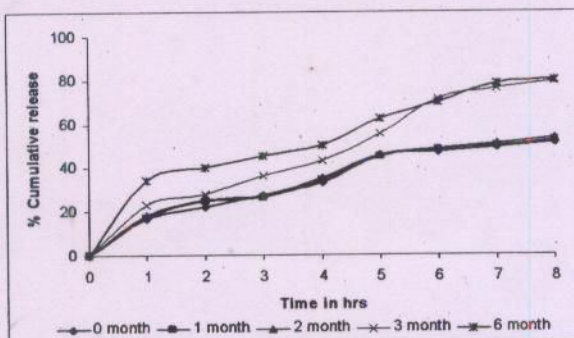
C-GP-Dox gels at 5°C ± 3°C



C-GP-PEG-Dox gels at 5°C ± 3°C



C-GP-Dox gels at 25°C/60% RH



C-GP-PEG-Dox gels at 25°C/60% RH

Fig 7 Percent cumulative release of drugs from gels at 5°C and 25°C/60%

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Publications and presentations

1. Research article entitled "**Thermoreversible biogels for intranasal delivery of rizatriptan benzoate**" in Indian Journal of Pharmaceutical Sciences, 2009, 71(6), 723-724
2. Poster on **Thermoreversible Biogels For Intranasal Delivery of Rizatriptan Benzoate**, Chand R., Naik A.A., Nair H.A. at IPA symposium on Nasal and pulmonary drug delivery, 2009
3. Poster on **Thermosensitive Biogels For Intranasal Delivery of Doxepin**, Rajawat G. S., Naik A. A., Nair H. A, at 10th International symposium on advances in Technology & business potential of New drug delivery systems, 2010

Thermoreversible Biogels for Intranasal Delivery of Rizatriptan Benzoate

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Chand *et al.*: Thermoreversible Biogels of Rizatriptan Benzoate

The objective of the present study was to formulate and evaluate a thermoreversible formulation containing rizatriptan benzoate for intranasal administration. Chitosan and aqueous β -glycerolphosphate were mixed in cold condition to obtain chitosan- β -glycerolphosphate mixtures, which served as the thermoreversible systems. Rizatriptan benzoate was incorporated at a final strength of 25 mg/ml. Both *in vitro* release and *ex vivo* permeation of rizatriptan from gels were measured at 37° using Franz diffusion cells. Formulations were tested *in vivo* in mice for reduction in locomotor activity using digital actophotometer and nasal mucosal tissues were examined histopathologically.

Key words: Thermoreversible gels, rizatriptan benzoate, chitosan

The nasal route has been successfully exploited for systemic delivery of drugs and vaccines. This route also offers the possibility of preferential targeting of drugs to CNS via olfactory pathway, bypassing the blood brain barrier^[1]. The objective of the present study was to formulate and evaluate a thermoreversible formulation containing the antimigraine drug rizatriptan benzoate (RB) for intranasal (IN) administration. The gels are based on the mucoadhesive biopolymer chitosan and utilize β -glycerolphosphate (GP) (C-GP-PEG) and without PEG (polyethylene glycol) (C-GP).

MATERIALS AND METHODS

Chitosan (degree of deacetylation~89%) and RB were gifted by CIFT and Cipla Pvt. Ltd. respectively. GP was purchased from CDH and PEG 4000 from S. D. Fine Chem, Mumbai, India. All other reagents used in the study were of analytical grade.

Preparation of gels:

Chitosan dissolved in 0.1N HCl and aqueous GP were mixed in cold condition to obtain chitosan-GP (C-GP) mixtures, which served as the thermoreversible systems. Formulations containing PEG 1% w/v (C-GP-PEG) with lower GP content were also prepared. RB was incorporated at a final strength of 25 mg/ml.

Evaluation of Gels:

The gelling temperature and time were measured by gradually warming the sols until movement of the meniscus was arrested. Gel strength was measured at 37° in terms of time taken for a 7 g stainless steel ball to fall through 4 cm height of gel. Both *in vitro* release and *ex vivo* permeation of RB from gels into PBS (pH 6.4) were measured at 37° using Franz diffusion cells across parchment paper and sheep nasal mucosa, respectively followed by UV spectrophotometric analysis^[2]. Mucoadhesive strength of the formulations in both sol and gel states were determined using a modified two-pan balance as the force required to separate two porcine mucin coated surfaces with gel between them. Formulations were tested *in vivo* in mice for reduction in locomotor activity using digital actophotometer and nasal mucosal tissues were examined histopathologically. Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test whenever applicable (P value<0.001).

RESULTS AND DISCUSSION

The formulations were fluid at room temperature and were rapidly transformed to viscous gels at 37°. Both gels showed initial burst followed by gradual release and permeation and release from C-GP gels were more rapid than from the gels with PEG (figs. 1 and 2). The gels had mucoadhesion comparable to or greater than chitosan at 25°, but the adhesiveness was significantly reduced on gelation at 37° (fig. 3). *In vivo* results revealed significant and sustained reduction in

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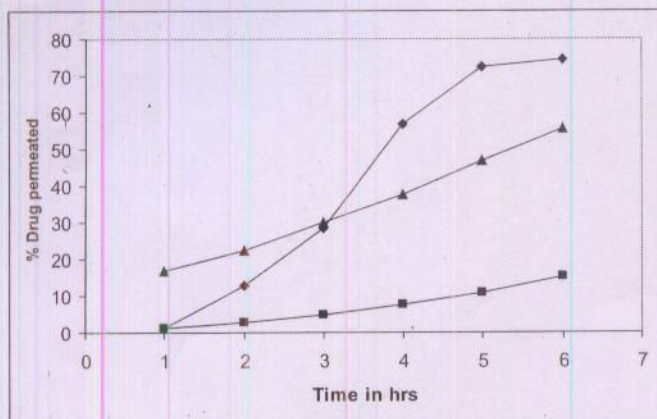


Fig. 1: *Ex vivo* permeation studies

Ex vivo permeation studies of RB from solution and from thermoreversible formulations, (—♦—) C-GP, (—■—) C-GP-PEG and (—▲—) drug solution

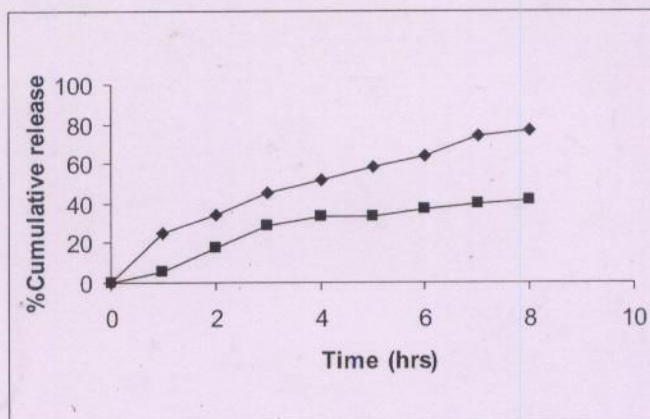


Fig. 2: *In vitro* drug release studies

In vitro drug release of RB from thermoreversible formulations. (—♦—) C-GP and (—■—) C-GP-PEG

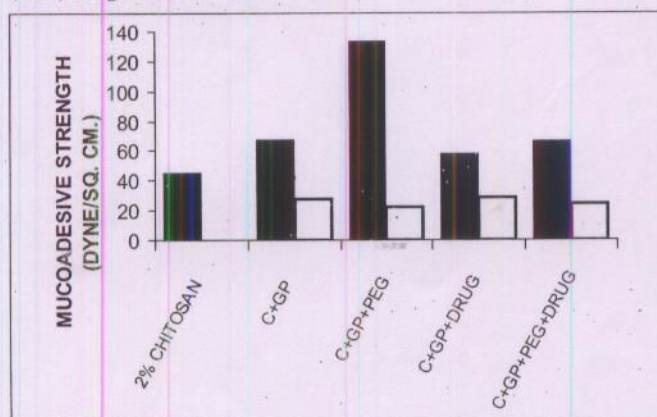


Fig. 3: Mucoadhesive strength

Mucoadhesive strength of formulations at (■) 25° and (□) 37°

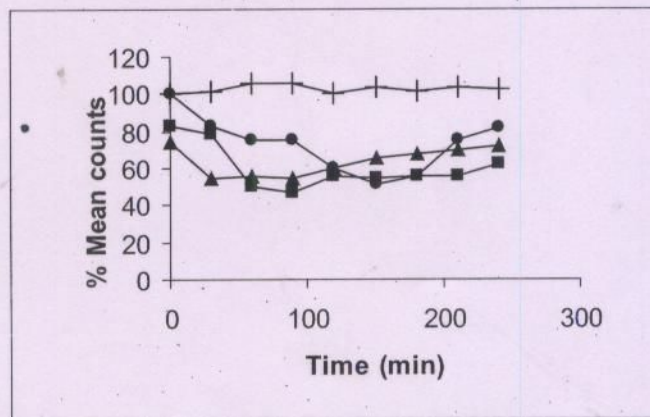


Fig. 4: Locomotor activity of mice after intranasal administration

Locomotor activity of mice after intranasal administration of rizatriptan benzoate. (—+—) control, (—■—) C-GP, (—●—) C-GP-PEG and (—▲—) drug solution

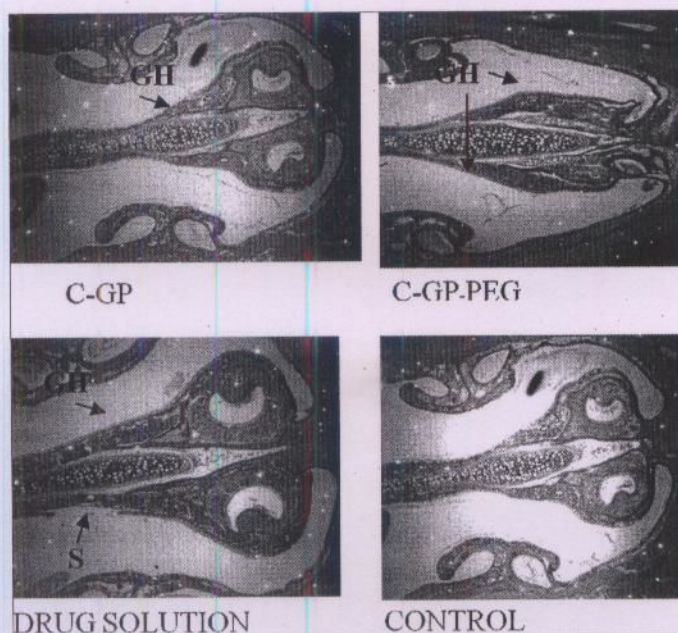


Fig. 5: Histopathology of nasal mucosa after 5 days of exposure
S: Sluffing of nasal mucosa, GH: Glandular hyperplasia. The tissues were stained with haematoxylin-eosin stain

locomotor activity of mice on IN administration of both formulations in comparison to drug solution (fig. 4). Histopathology revealed minor damage to nasal tissues after 5 days of exposure (fig. 5).

The weakly basic GP prevents precipitation of chitosan on increase in pH and facilitates hydrophobic interactions on slight elevation of temperature resulting in thermoreversible systems. The sols showed good mucoadhesion but gelling reduced this effect due to stronger bonding within polymeric chains rather than with mucin. The pronounced and prolonged depression in locomotor activity of mice strengthens the hypothesis of direct delivery to brain. Preliminary studies also indicate a good safety profile. In conclusion, the developed biogel formulations

could prove to be promising alternative therapy with RB. The formulations offer convenience of administration and prolong nasal residence time and thereby nasal absorption of RB.

ACKNOWLEDGEMENTS

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