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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF MUCOADHESIVE GASTRO RETENTIVE TABLET OF LEVAMISOLE BY USING PURIFIED POLYSACCHARIDE ISOLATED FROM *Terminalia bellerica* GUM

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ABSTRACT

The present investigation deals with the development of mucoadhesive gastro retensive tablets of levamisole using polysaccharide isolated from *Terminalia bellerica* gum (TBG). The TBG polysaccharide was tested for acute toxicity and drug–excipient compatibility study. The tablet formulations with TBG were evaluated for physical characteristics like hardness, % friability, % drug content and weight variations. Dissolution study was conducted to characterize release mechanism of the formulation and data are fitted to various kinetic models. The mucoadhesive abilities were studied by different *in vitro, Ex vivo* and *in vivo* models. The *in vitro* release study showed that the optimized formulation exhibited highest correlation (R) value in the case of Korsemeyer–Peppas model and the release mechanism study proved that the formulation showed a combination of diffusion and erosion processes. The mucoadhesive properties of polysaccharide are comparable to synthetic polymer in all *Ex vivo*, *in vitro* and *in vivo* models. There was a significant difference in the pharmacokinetic parameters (Tmax, Cmax, $AUC_{0-\infty}$, AUC_{0-t}, AUMC, MRT, T1/2, K_a and K_{el}) of the optimized formulation as compared to standard marketed formulation, which proves the controlled release and mucoadhesive property of the isolated polysaccharide.

Keywords- TBG polysaccharide, Scintigraphic, X-Ray study, Korsemeyer–Peppas model

1. INTRODUCTION

In recent years, in association with progress and innovation in the field of pharmaceutical technology, there has been an increasing effort to develop sustained release dosage forms for many drugs. Correspondingly, a growing number of new sustained release dosage forms have been submitted for regulatory approval. Prolonged release dosage forms have many advantages in safety and efficacy over immediate release drug products in that the frequency of dosing can be reduced, drug efficacy can be prolonged and the incidence and/or intensity of adverse effects can be decreased [1]. The main approaches to prolong the gastric residence of the pharmaceutical dosage forms include bioadhesive delivery system, which adheres to the mucosal surface; and release the active contents in a controlled manner [2].

Gums and mucilages of different sources and their derivatives represent a group of polymers widely used in pharmaceutical dosage forms. In recent years, a number of natural gums and mucilages have been evaluated for mucoadhesive drug delivery systems. This increased interest in natural polymer is due to their non-toxicity, cheap, easy availability, biodegradability and biocompatibility [3]. Apart from their safety, these natural polysaccharides are capable of providing the comparable drug release profiles with currently available sustained release products in markets.

Levamisole, marketed as the hydrochloride salt under the trade name Ergamisol (R12564), an anthelmintic and immunomodulator belonging to a class of synthetic imidazo-thiazole derivatives. The low bioavailability (47%) and short biological half-life (4.4 to 5.6 h) of levamisole following oral administration favors the development of a sustained release formulation. The objective of the present research includes *in vitro, Ex vivo* and *in vivo* evaluation of mucoadhesive properties of purified polysaccharides from *Terminalia bellerica* gum by using Levamisole hydrochloride as a model drug. The formulated tablets were characterized and optimized [4].

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MATERIALS AND METHODS

1.1. Materials

Levamisole was kindly gifted by Wockhardt Limited, Baddi, India. Polyvinyl Pyrrolidine K-30 (PVP K-30) was obtained from Alembic, Vadodra, India. Other chemicals were purchased from S.D Fine Chemicals Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

1.2. Extraction and purification of polysaccharide

Dried gums were collected from the trunk bark of *Terminalia belarica* plant and separated from fungal affected and other foreign object present during collection. They were converted into small pieces using a hammer. The pieces were then dried in oven dryer maintained between 40°C to 50°C for 72 h to obtain dry mass which were milled with a grinder to get the dry powder. The dried powder was passed through sieve number 40# and stored in an airtight container. The powder (1 kg) was extracted with boiling water three times. The aqueous extract was filtered through Whattman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and then centrifuged at 3000 rpm for 15 min. The supernatant was precipitated with three volumes of acetone, and stored overnight at 4°C. The precipitate was collected and washed again three times with acetone to get crude polysaccharide (60.4 g). The crude polysaccharide was subjected to DEAE- Sephadex A-50 column chromatography, washed with H_2O and eluted with 1.0 M NaCl solution. Most of the pigments were absorbed in the column. The Elutes collected were concentrated under reduced pressure to an appropriate volume, and then dialyzed against distilled water. The remaining portion was lyophilized to afford total purified polysaccharide [5].

1.3. Acute toxicity study of polysaccharide

Healthy male and female Swiss albino mice (8 weeks) were used for the acute oral toxicity study. They were breed and reared at the animal house of the institution. The animals were housed in polypropylene cages and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for one week prior to the experiment. The temperature in the animal house was maintained at 25 $\pm 2^0$C with a relative humidity of 30–70% and illumination cycle set to 12 h light and 12 h dark. The mice were fed with standard laboratory pelleted feed (M/s Gold Mohur Foods and Feeds Ltd. Bangalore, India). All the mice of both the sexes were fasted overnight before experimentation and were allowed to take food one hour after the experiment. Purified polysaccharide of TBG was administered orally at a dose of 2000 mg/kg body weight in distilled water. The animals were observed for any mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation) at an interval of 12 h for 14 days. This study was approved by the Animal Ethics Committee of Girijananda chowdhury institute of pharmaceutical sciences (CPCSEA Regn.No.1372/C/10/2013/CPCSEA, study approval No-GIPS/IAEC/10) [6]

1.4. Pre formulation study

1.4.1. Drug–excipient compatibility study by DSC

A differential scanning calorimetry (JADE DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug–excipient compatibility. Firstly, binary mixtures of levamisole and polysaccharide (in 1:1 mass/mass ratio) were prepared by using physical mixture technique. The drug–excipient mixture was scanned in the temperature range of 50–300°C under an atmosphere of nitrogen. The heating rate was 20 °C /min and the obtained thermo grams were observed for any type of interaction [7].

1.4.2. Drug–excipient compatibility study by FT-IR spectroscopy

FT-IR spectra were recorded on a Bruker spectrophotometer (Model-220, Germany) using KBr discs in the range of 4000–4500 cm^{-1}. FT-IR analysis has been performed using sample of levamisole with polysaccharide at 1:1 mass/mass ratio [8]

1.5. Formulation of tablet

Terminalia bellerica gum polysaccharide based mucoadhesive tablets of levamisole were prepared by wet granulation method using different compositions as per Table 1 All the ingredients were screened through sieve no. 60 and then blended (except magnesium stearate) for 15 min. A blend of all ingredients was granulated with 95% isopropyl alcohol. The wet masses were passed through sieve no. 12 and the resulting granules were dried at 40°C. The dried granules were again passed through sieve no. 22. Finally magnesium stearate was added and mixed for 5min. The micro-meritic studies were carried out for all the granules. The results of angle of repose, Carr's Index and Hausner ratio indicated that the granules possess good flow property and good packing ability. Tablets were compressed on a 8-station Mini Press-I rotary tablet compression machine (Shakti Pvt Ltd) fitted with 8-mm flat-shaped punches using sufficient compression force to obtain a hardness of 4 to 5 Kg/cm^2 containing 50 mg of levamisole per Tablet. Manufacturing defects were observed in tablets like capping, lamination and chipping [9].

Table 1: Formulae code of levamisole mucoadhesive tablet

Ingredients	Weight of Ingredients mg/tablet								
	F10	**F15**	**F20**	**F25**	**F30**	**F35**	**F40**	**F45**	**F50**
Levamisole	50	50	50	50	50	50	50	50	50
TBP	16	24	32	40	48	56	64	72	80
Polyvinyl pyrollidone (K 30)	5	5	5	5	5	5	5	5	5
Microcrystalline cellulose	84.2	76.2	68.2	60.2	52.2	44.2	36.2	28.2	20.2
Magnesium stearate	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
Talc	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Total Tablet weight	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**

1.6. Evaluation of tablets

Thickness of each tablet was measured in mm using a digital Vernier Caliper (Mitutoyo Dial Thickness Gauge, Mitutoyo, Japan). The mean and standard deviation values were calculated and reported. Hardness was determined by using a Monsanto tablet hardness tester (n = 6). The friability of the tablets was tested by using Roche friabilator. From each batch 20 tablets were taken, weighed and finely triturated. An adequate amount of this powder equivalent to 100 mg of the drug was accurately weighed and shaken with 150 ml of 0.1N HCl for 10 minutes. The mixture was diluted with 0.1N HCl to produce 200ml and filtered. 10 ml of the filtrate was diluted to 100 ml with distilled water and the absorbance was measured at 265 nm. The drug content in the formulation was calculated using the standard curve. From each batch 20 tablets were taken, weighed together and individually for the determination of uniformity of weight of tablets. The mean and percent deviations were determined [10, 11].

1.7. *In vitro* dissolution rate study

Drug release was assessed by dissolution test under the following conditions: n = 6 (in triplicate), USP type II dissolution apparatus (Lab India, DISSO 2000) at 50 rpm in 900 ml of 0.1 N HCL maintained at 37 ± 0.5˚C. The tablet was allowed to sink to the bottom of the flask before stirring. Special precaution was taken not to form air pockets on the surface of the tablet. Five milliliters of the sample was withdrawn by using a syringe filter at regular intervals and replaced with the same volume of pre warmed (37 ± 0.5°C) fresh dissolution medium. The drug content in each sample was analyzed after suitable dilution using UV spectrophotometer method at 215 nm [12, 13].

1.8. *In-vitro* release kinetics

In the model-dependent approaches, the order of drug release from matrix systems was described by using zero order or first order kinetics. The mechanism of drug release from matrix systems was studied by using Higuchi diffusion model and Hixon-Crowell erosion model. Korsemeyer-Peppas support the drug release mechanism for further judgment. The respective equations for these models according to Korsmeyer-Peppas equation, the release exponent 'n' value is used to characterize different release mechanisms for a dosage form with cylindrical shape and summarized [14, 15].

1.9. Swelling Studies of the tablet

Swelling study of individual batch was carried out using USP type II dissolution apparatus (Basket type, Lab India, DISSO 2000). For each formulation batch, one tablet was weighed and placed in the stainless steel basket of the dissolution apparatus and weighed. The basket was then placed in the dissolution beaker containing 900 ml of 0.1 N HCl media and rotating speed of the basket is 100rpm. The tablets were allowed to swell at 37 ±0.5˚C; the basket was periodically weighed up to 12hr after removing the excess water on the surface with a filter paper. The swelling index was calculated using following formula. % Swelling Index = $\{(W_t) - (W_0)/ (W_0)\}$ x 100**.** Where, W_0 was the weight of the tablet before placing into the dissolution basket. W_t the difference of the {(basket and tablet weight after time t) - tablet weight at the initial time (W_0)} [16]

1.10. *In vitro* evaluation of mucoadhesion

1.10.1. Shear stress method

The shear stress measure the force that cause a mucoadhesive to polysaccharide with respect to the mucus layer in directional parallel to their place of contact of adhesion. The study was carried out on 3% w/v aqueous solutions of isolated polysaccharide. Two smooth polished glass blocks of 10×10×0.5 cm were selected. One glass plate was fixed on a leveled table with an adhesive. To the upper block a thread was tied and passed through a pulley at a length of 12 cm. At the end of the thread a pan of 3 g weight was attached for the addition of weights. A drop of polysaccharide solution at a temperature of 25^0C was kept at the center of the fixed block with a pipette, and the movable block was placed on it. A load of 100 g was applied such that the drop of polymer spread as a uniform film in between the two glass blocks. After keeping it for fixed time intervals of 5, 10, and 15 min, the applied load was removed and the weights were added into the pan just sufficient to pull the upper movable block or to make it slide down from the fixed base block. The sheer stress was expressed in gm/cm^2. The result was compared with the result of the synthetic polymer as reported in literature [17].

1.10.2. Falling sphere method

To characterize the mucoadhesive strength, the falling sphere method was used .For this purpose a clean burette was taken and filled with 10% mucus solution and fixed in a stainless steel tube. Mustard grain which retained on sieve size # 12 were taken and dipped in polysaccharide solution of various concentrations (1 % - 5 % w/v) and then each grain were slowly placed at the

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top of the mucus layer. Time taken by the grain to fall 50 divisions in the burette was noted and values were tabulated. The result was compared with the result of the synthetic polymer in place of isolated polysaccharide as reported in literature [18].

1.11. *Ex vivo* evaluation of mucoadhesion

1.11.1. Mucoadhesive strength

Ex-vivo mucoadhesive strength of levamisole tablets was measured by using texture profile analyzer (TAXT Plus, Stable Micro Systems, and UK). Fresh goat gastric mucosa membrane was obtained from the local slaughterhouse and stored in phosphate buffer pH 7.4 and the experiment was performed within 3 hr of procurement of goat mucosa. The mucosal membrane was excised by removing the underlying connective tissue and was placed on the base of Texture Analyzer. A tablet was attached to the stainless steel probe to the mobile arm of the texture analyzer with the help of a both sided tape. The area of contact of mucosa was moistened with 50 µl of simulated gastric fluid. The mobile arm was lowered at a rate of 0.5 mm/s until a contact with the membrane was made. A contact force of 1 N was maintained for 60 s, after which the probe was withdrawn from the membrane at a 0.5 mm/s to the distance of 15 mm. the peak detachment force was recorded as mucoadhesive force [19].

1.11.2. Mucoadhesive time

The fresh goat stomach mucosa was tied on the glass slide with the help of double sided tape and the tablet was wetted with 1 drop of 0.1 M HCl (pH 1.2) and pasted to the goat stomach mucosa by applying a light force with a fingertip for 30 s. The glass slide was placed at the bottom of vessel paddle type USP Type-II (Lab India, DS 8000) apparatus. The test was performed with 900 ml of the 0.1 N HCl at 37 ± 10^0C. After 2 min, a 50 rpm stirring rate was applied to simulate the stomach environment, and tablet adhesion was monitored for 24 h. The time for the tablet to detach from the goat stomach mucosa was recorded as the mucoadhesion time [20]

1.12. *In vivo* evaluation of mucoadhesion

1.12.1. Scintigraphic study

The *in vivo* mucoadhesive ability was studied by gamma scintigraphy in a healthy rabbit of 2.2 kg throughout the study. The total study protocol was approved by animal ethics committee GIPS (CPCSEA Regn. No.1372/C/10/2013/CPCSEA. Study approval No-GIPS/IAEC/10). Tc-99 m is most widely used radionuclide in nuclear medicine. It has a very short half-life of 6 h and emits photons but not particulate radiation (β rays harmful to tissues). A dose of 6 MBq sodium pntatectate(Tc-99m) was incorporated into the tablet blend during manufacture to facilitate scintigraphic imaging at radioactivity was counted in a well-type counter (gamma ray spectrometer: GRS 23C, ECIL) at IICB, Kolkata. Then put into the led shell. Optimized Levamisole tablet was prepared with polysaccharide and radionuclide by melt granulation method in the similar way as described in their preparation method. The prepared formulation (4mm tablets, weight 80mg) was administered to the rabbit by oral feeding tube (tracheal tube no 4) with mild local anaesthetic (xylocaine gel 2%). During the study the rabbit was not allowed to eat any food, but water was given *ad libitum.* Static Imaging studies were performed and dosage form was visualized using a gamma camera (low energy high resolution colorimeter integrated with an ENTEGRA work station) by moving the table under the camera at Thakurpukur Cancer Research centre (Regional Radiation Monitoring Centre, Kolkata) under dual-head gamma camera (GE Hawkeyes).The Images were recorded at intervals of 1, 2, 3, 4, 5, 6, 7 and 8 h. [20]

1.13. Pharmacokinetics study

In vivo study of TBG polysaccharide matrix tablets was performed in healthy rabbits (New Zealand, white) of either sex weighing 2.8-3.2 kg. They were given access to normal standard diet and tap water *ad libitium*, during the experiment. The animals were housed, two per cage in standard rabbit cages maintained at 22 ± 3^0C under 12 h light and 12 h dark cycle. The animals were acclimatized at least one week prior to the start of the experiment. All rabbits had free access to water throughout the study. The animals were divided into two groups each consisting of three animals. The first group received 25 mg dose of optimized formulation of levamisole TBG polysaccharide matrix tablet (F45) by oral administration. The second group received the same dose of standard levamisole in distilled water. The tablets were put behind the tongue to avoid their destruction due to biting. The Institutional Animal Ethical Committee approved the protocol for this study (CPCSEA Regn. No.1372/C/10/2013/CPCSEA. Study approval No-GIPS/IAEC/10). Blood samples (1.5 ml) were withdrawn from a heparinized catheter placed in the marginal vein of the ear before administration and at predetermined times, using EDTA as anticoagulant. Plasma samples were immediately separated by centrifugation at 4000 rpm for 30 min using Remi centrifuge and stored at -20^0C until analysis. Baseline plasma samples obtained prior to the tablet administration at time 0 served as the blank control for each animal. Various pharmacokinetic parameters such as peak plasma concentration (Cmax), time at which Cmax occurred (Tmax), area under the curve (AUC), elimination rate constant (Kel), biological half life (t½), absorption rate constant (Ka) and mean residence time (MRT), Area under the moment time curve (AUMC) were calculated in each case from the plasma drug concentration data by using PK solver pharmacokinetic software [21].

1.14. Extended stability studies

To assess the formulation and drug stability, stability studies were done according to ICH guidelines. The optimized formulation of Levamisole (45% TBG polysaccharide) was subjected to stability study at 40 ± 2ºC and 75 ± 5% RH for 30, 60, 90 days. The samples were evaluated for physical changes, hardness, friability, drug content, mucoadhesive properties and percentage drug release during the stability studies [22, 23].

2. RESULT AND DISCUSSION

3.1. Acute toxicity study

The purpose of this study was to evaluate toxicity profile of the *Terminalia bellerica* gum polysaccharide. 14 day acute oral toxicity study was performed in Swiss albino mice. The LD50 of *Terminalia bellerica* gum polysaccharide were not further studied as it was found to be safe up to 2000 mg/kg on 24 h study basis. It was observed that the animals fed with the *Terminalia bellerica* gum polysaccharide were found to be healthy. The animals were observed for any mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation) at an interval of 12 h for 14 days. No unusual changes in behavior or in locomotors activity, ataxia, and signs of toxicity were observed during the 14 days period. No differences were found in growth behavior between the control and treatment group in 14 days of study

3.2. Pre formulation study

3.2.1. Drug–excipient compatibility study by DSC

The DSC curves of levamisole, TBG polysaccharide, levamisole-TBG mixtures and combination of three are presented in Fig.1 Levamisole exhibited a single melting endothermic peak corresponding to the melting of the drug. Onset of melting of Levamisole was observed at 235.23˚C. Any sign of interaction would be reflected by a change in melting endothermic of levamisole. The results from DSC study confirmed that there was no significant change in the melting endotherm of levamisole in the mixture with TBG polysaccharide as it moves to 203.79˚C which was not significant for considering as prominent drug excipient interaction. Hence, there was no well defined chemical interaction between drug and polysaccharide [7]

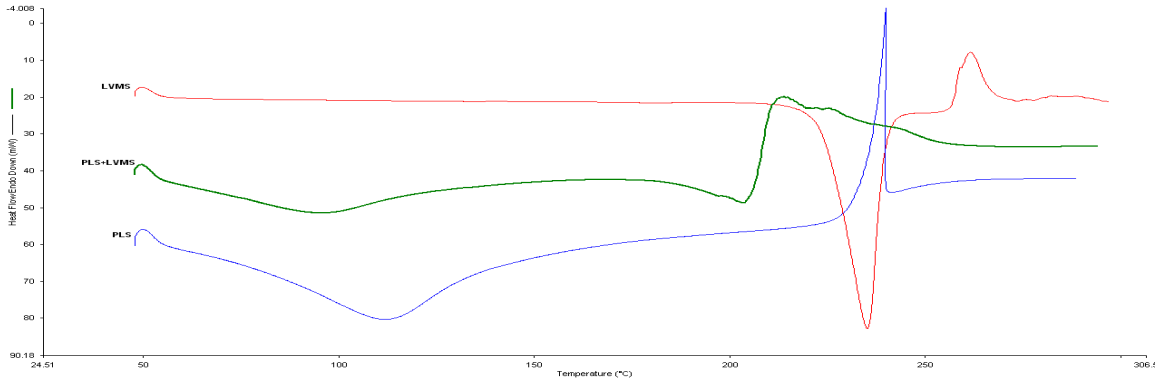

Figure 1: Combine DSC thermogram of Levamisole, Terminalia bellerica Polysaccharide & polysaccharide & Levamisole mixture

3.2.2. Drug–excipient compatibility study by FT-IR spectroscopy

The FTIR spectra of pure drug levamisole, TBG Polysaccharide and levamisole-TBG mixture are shown in Fig.2. The FTIR spectrum of levamisole showed peak at 2631.69cm^{-1} due to C=N stretching, peaks at 1572.48, 1519.44 & 1435.14cm^{-1} due to C=C (aromatic) stretching, 1201.33 cm^{-1} due to C-N stretching, 734.99 cm^{-1} due to C-S, 1964.92 cm^{-1} due to S (=O) $_2$ asymmetric stretching and 838.17 cm^{-1} due to C-Cl symmetric stretching confirming the drug structure.

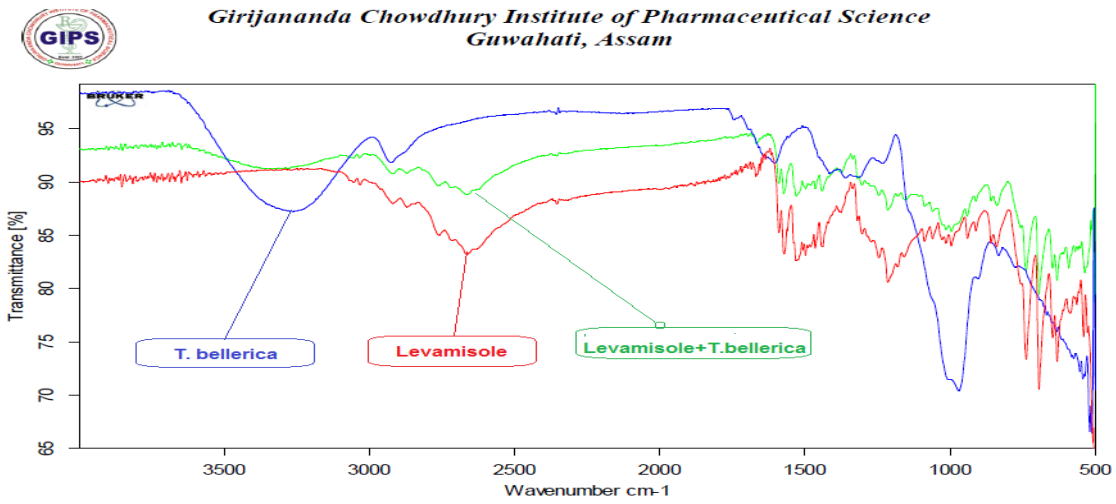

Figure 2: FTIR Spectrum of Levamisole, Polysaccharide and Levamisole-polysaccharide mixture

The FTIR spectrum of TBG polysaccharide shows Peaks at 969.40 and 831.75 are due to finger print regions for carbohydrates, the absorption peaks at 1604.43 cm^{-1} and 1412.15 cm^{-1} are indicative of acetyl group. The absence of significant aromatic stretches in the 1600-1690 cm^{-1} region and the weakness of the stretches imply that there is modest amount of cross linking by peptides. The FTIR spectrum of mixture of the tablet showed all the characteristic peaks of levamisole indicating the undisturbed drug in the formulation. All the peaks of TBG Polysaccharide remain unchanged in the physical mixture. The results from FTIR spectroscopy showed that there was no significant change in the FTIR spectrum of levamisole in mixture with TBG Polysaccharide (Fig.2).

3.3. Evaluation of physical parameters of tablets

The tablet thickness of the various formulations was found to be in the range of 2.62 ± 0.076 to 2.92 ± 0.067. The hardness of all tablet were in the range of 3.5 ± 0.3 to 7.2 ± 0.4 kg/cm^2. Hardness increased as the amount of concentration of TBG Polysaccharide increased. This indicates the binding potentiality of the polysaccharide. The tablets prepared in each batch were found to have within the limit of weight variation and content uniformity. The content of each individual preparations were found to be within the limits of 85-115%.

3.4. *In vitro* drug release study of all batches

The *in vitro* drug release profiles are shown in Fig.3. The results indicated slow and controlled release of levamisole from the matrix tablet. In the formulations, release of levamisole in the first hour varied between 15.13±0.51 in F10 and 5.06±0.47 in F45. The release of drug extended from 10hr in F25 to more than 12hr in F40, F45 and F50 batches. The total amount of drug is released within 6-7hr from the batches F10 to F20. The drug release decreases when the TBG concentrations have increased. Batch F25 also had a controlled pattern of drug release but not so significant as compared to the other five bathes. Among the five batches (F30-F50) the F45 showed mean release of 11.96% drug in first 2hr, while within the first 6hr and 8hr mean release were 69.79% and 74.44%, remaining portion of drug was released during last 4 hr of observation and yet 4.69% of drug remain in the formulation. The dissolution for the first 12 hr period of F45 batch is steady till the polymer relaxation gets predominant. A controlled release pattern of drug was observed from the tablets of batch F45 throughout the 12 hr of dissolution study. Hence, F45 batch was selected as the optimized batch for further stability and pharmacokinetic study.

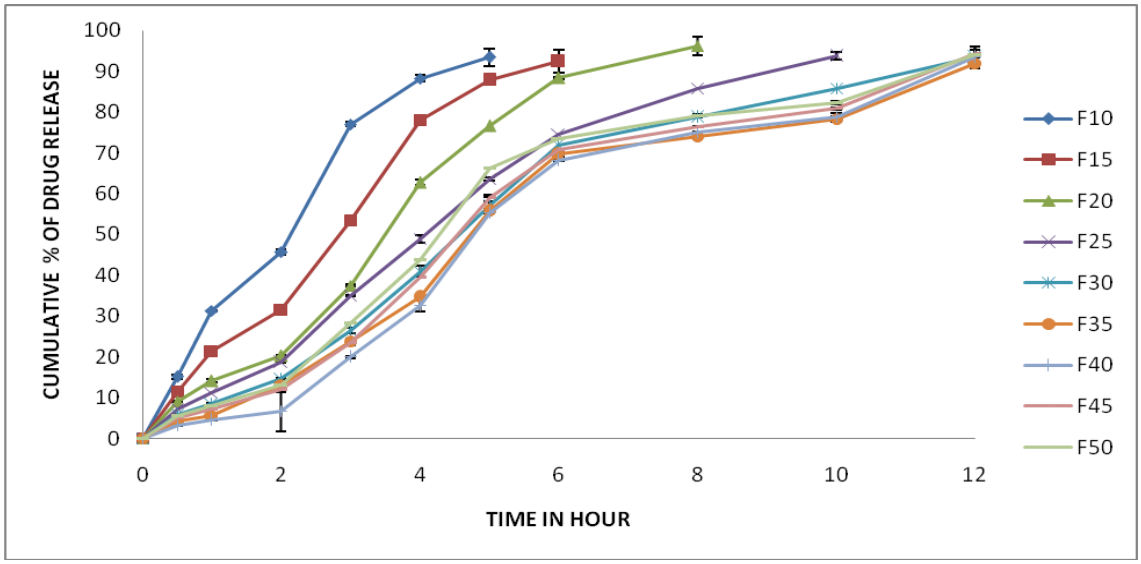

Figure 3: Comparative dissolution drug profile of all batches (Mean value ± SD)

3.5. Kinetics and mechanism of drug release

The release rate constant was calculated from the slope of the appropriate equations and the correlation coefficient (R) was determined for all formulations (Table-2). In most of the formulated tablets the R values were higher in first order model than in zero order models indicating that the drug release from most of the tablets followed first order kinetics. Determination of correlation coefficient from various formulations, containing different proportions of TBG Polysaccharide indicates that first-order and Korsemeyer–Peppas equations seemed to be better fit than other equations. The R values of 0.980, 0.981, 0.965, 0.975, 0.959, 0.950, 0.981, 0.998, 0.937 for the batches of tablets F10, F15, F20, F25, F30, F35, F40, F45 and F50 respectively which showed good linearity between log cumulative amount of drug release versus log time. The n values were found to be 0.728, 0.861, 0.908, 0.824, 0.802, 0.842, 0.917, 0.828, and 0.763 for the tablets F10, F15, F20, F25, F30, F35, F40, F45 and F50 respectively. Hence, the mechanism of drug release from the tablets was predicted from Korsemeyer–Peppas equations and from the obtained n values was a coupling of both the process of diffusion and erosion.

Table 2: Release Kinetics study of formulated baches of TBGP mucoadhesive matrix tablets

Model	Parameters	Formulation Code								
		F10	F15	F20	F25	F30	F35	F40	F45	F50
Zero Order	R^2	0.941	0.973	0.961	0.957	0.935	0.936	0.938	0.932	0.901
	K_0	0.356	0.284	0.226	0.179	0.151	0.143	0.142	0.148	0.152
First Order	R^2	0.968	0.946	0.919	0.955	0.948	0.938	0.913	0.937	0.941
	K_1	0.007	0.005	0.004	0.003	0.003	0.002	0.002	0.003	0.004
Higuchi	R^2	0.937	0.897	0.856	0.890	0.879	0.856	0.820	0.860	0.872
	KH	5.21	4.47	3.93	3.48	3.21	3.02	2.98	3.13	3.27
Hixon-Crowel	R^2	0.985	0.970	0.949	0.977	0.969	0.957	0.937	0.957	0.958
	KHC	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Korsemayer – Peppas	R^2	0.980	0.981	0.965	0.975	0.959	0.950	0.981	0.998	0.937
	KKP	1.563	0.621	0.387	0.521	0.517	0.382	0.239	0.432	0.668
	Release Exponent (n)	0.728	0.861	0.908	0.824	0.802	0.842	0.917	0.828	0.763

3.6. Swelling study

Appropriate swelling behaviour of mucoadhesive tablet is very much essential for uniform prolong release of the drug and effective mucoadhesion .The percentage of swelling ranging from 15.2±4.6% to 20.1±3.8 in first hour but increases over 200% in 10-12 hr. Some tablet shows decrease amount of swelling between 10 -12 hour because of erosion and breaking occurred in 10-12 hour. When the concentration of the polymer increases, swelling increases with time which has been showed in (Fig 4).

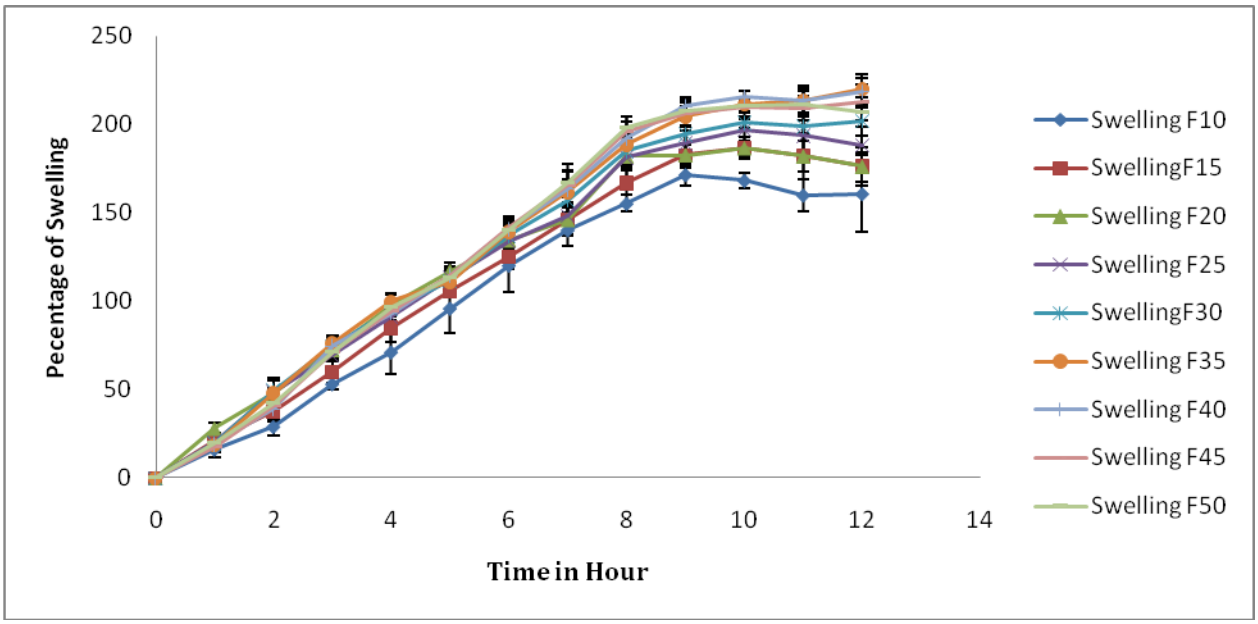

Figure 4: Swelling index of the tablet

3.7. *In vitro* mucoadhesive property

3.7.1. Shear stress method

The bioadhesion of the formulations was measured *in vitro* as a detachment force measurement, or the force required detaching the formulation from the mucosal tissue. After each measurement, the tissue was replaced by fresh piece and finally detachment force was measured. The amount of shear in gram increases as the holding time increases. After 5, 10 and 15 minute the detachment weight requirement was 11.78 ± 0.48gm, 32.90±0.32 and 84.73±0.26 respectively. This result is comparable to the result reported in literature for synthetic polymer [23]. This indicates that higher holding time requires the most maximum force in grams to break the bond between the polysaccharide and the mucosal surface. Further, the wetting time is more as the holding time increases and the carboxylic acid structure in polysaccharide gradually undergo hydrogen bonding.

3.7.2. Falling sphere Method

In vitro mucoadhesive property of the TBG polysaccharide was evaluated by falling sphere method. It was observed that as concentration of the polysaccharide was increased, resistance in terms of time to move of the mustard towards bottom side was also

increased. Time in seconds required to move the mustard grain from top to bottom in 5 % w/v solution was found to be 67.353 ± 0.34 Seconds. This indicates that higher the concentration of the polysaccharide increases the adhesiveness of the polysaccharide containing mustard and decreases the downward movement. This result is comparable to the result reported in literature for synthetic polymer [18].

3.8. *Ex-vivo* evaluation studies

3.8.1. Mucoadhesive strength studies

The result depicts an increase trend in mucoadhesive strength, with an increase in the TBG Polysaccharide concentration. After each measurement the tissue was replaced by fresh tissue, the tissue was replaced by fresh piece and finally detachment force was measured. The mucoadhesive strength was increased from 19.4 ± 0.51N to 73.6 ± 1.07 N at low and high level of isolated polysaccharide. Increasing the polymer amount provides more adhesive sites and polymer chains for interpenetration with mucin, resulting in augmentation of bioadhesive strength [24].

3.8.2. Mucoadhesive time studies

The residence time of the formulations ranged between 106 min and 490 min. The results showed that the mucoadhesive time is concentration dependent. Increasing contact time may provide inter diffusion and chain entanglement between the polysaccharide and mucin chain in mucus membrane. This is in agreement with Leung and Robinson, who demonstrated that mucoadhesion of carbomer was a time dependent process supporting the proposed interpenetration as being a time dependent process [25]. An increase in contact resulted in an increase in formation of secondary bonds and diffusional path or depth of interpenetration between two macromolecules. Increasing contact time between the mucoadhesive polysaccharide and the mucus layer could, therefore, increase the mucoadhesive strength.

3.8.3. Scintigraphic study

The scintigraphic method was adopted to monitor the system in the gastric region of rabbit in different time interval. The selected scintigraphic images of the formulation at different time intervals are presented in Fig. 5. From the scintigraphic images, it can be observed that stagnation of the formulation took place in between stomach and upper portion of the small intestine. The scintigraph clearly explained that the liberated radioactivity was distributed across the whole stomach of the rabbit indicating the mucoadhesive action of the polysaccharide. The scintigraphic image taken after 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 7 hr and 8 hr revealed that the formulation remains intact in the stomach at the end of 8 hr by adhesion in the mucus membrane. The result is comparable with the results of studies *in vivo* in animals relating to mucoadhesion of polymers [26].

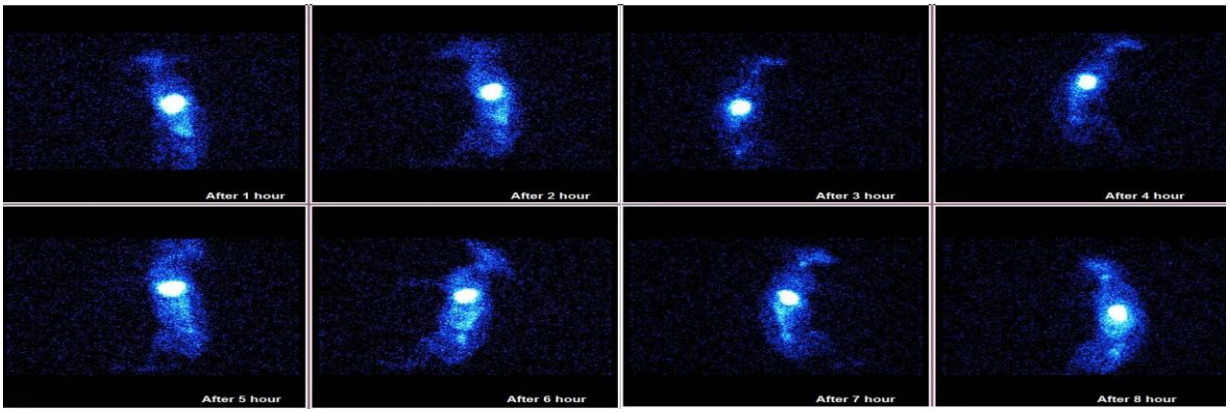

Figure 5: Scintigraphic image of rabbit showing mucoadhesion of Terminalia bellerica matrix tablet

3.9. *In vivo* plasma concentration-time data

The mean pharmacokinetic parameters of the optimized formulation and standard levamisole formulation (Levasol-50), (C_{max}, T_{max}, K_a, K_{el}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ and $MRT_{0-t,}$ and AUMC) calculated from the individual plasma concentrations of the *in vivo* experiments are represented in Table 3. The mean plasma concentration-time curves of optimized formulation with compare to standard formulation following oral administration are given in Fig. 6. The absorption of levamisole is rapid in standard formulation than the test; the mean T_{max} was 3.17 hr, while in the optimized tablet containing TBG polysaccharide, the mean T_{max} was delayed by more than 6.19 hr. This shows that the mucoadhesive TBG Polysaccharide tablet was effective in delaying the peak plasma concentration of levamisole. The peak plasma concentration (C_{max}) of levamisole standard formulation and optimized formulation were found to be 5.33 and 5.63 µg/ml respectively. The K_{el} values were found to be 0.15 and 0.30 hr^{-1} calculated from the slope of the terminal phase of the plasma concentration time data. A decrease in elimination rate constant of optimized formulation indicated the slow release rate of the drug in rabbit [27].

The plasma $t_{1/2}$ values of levamisole matrix tablet of optimized formulation and standard levamisole tablet were found to be 4.63 and 2.31 hr respectively. The controlled release characteristics of the matrix tablet were reflected in the MRT. The MRT was noticeably increased following oral administration of the levamisole matrix tablet of optimized formulation (12.63 hr) as compared to levamisole standard tablet (6.35 hr). The

AUC_{0-t} of levamisole standard tablet was found to be 54.20 µg.hr/ml, whereas increase in AUC_{0-t} was observed in optimized formulation (86.42 µg.hr/ml), which indicated the increased bioavailability of the optimized formulation. The other parameters viz $AUC_{0-\infty}$, AUMC were also found to be much higher in the levamisole matrix tablet of optimized formulation. Significant difference was observed in all the pharmacokinetic parameters between both the formulations. Thus, the results of the present study clearly indicated the applicability of polysaccharide for the *in vivo* sustained release of the levamisole.

Table 3 - Pharmacokinetic parameters Optimized Mucoadhesive levamisole tablet of test TBGP 45%and standard levamisole formulation in healthy animals

Parameter	Unit	TBGP 45%	Standard formulation
T_{max}	H	6.19±0.14	3.17±0.03
C_{max}	µg/ml	5.63±0.23	5.33±0.07
K_{el}	hr^{-1}	0.15±0.01	0.30±0.03
K_a	hr^{-1}	0.16±0.006	0.32±0.002
$t_{1/2}$	hr	4.63±0.31	2.31±0.06
AUC_{0-t}	µg.hr/ml	86.42±2.60	54.20±0.82
$AUC_{0-\infty}$	µg.hr /ml	96.88±4.09	54.45±0.84
AUMC	µg /ml.hr^2	1225.63±99.26	346.13±7.99
MRT	Hr	12.63±0.46	6.35±0.07

The results are mean±s.d

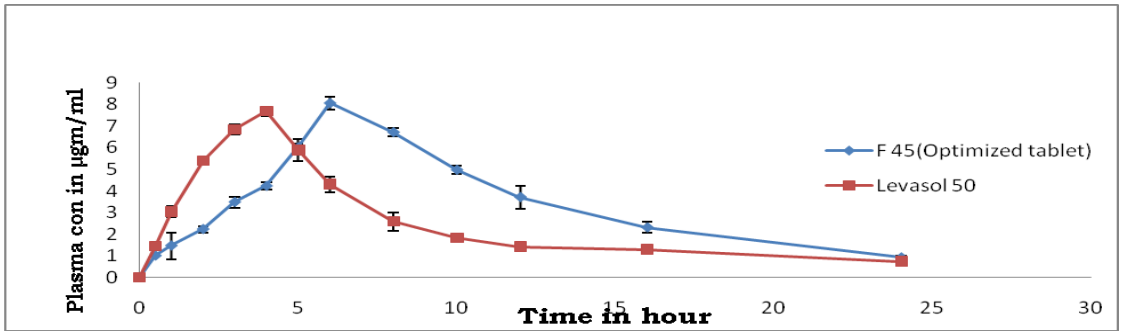

Figure 6: Mean plasma concentration vs. time profile of Test (F45) and standard (levasol-50)

3.10. Extended stability study

Extended stability study of the optimized mucoadhesive tablets levamisole (F45) were carried out under proper condition for 90 days. There was no significant change in the percentage of hardness, friability and of the amount of drug content as well as in mucoadhesive strength after 30, 60 & 90 days mentioned in Table 4. Based on the result it was opined that the tablets of batch F45 were stable after 3 months of storage at accelerated stability conditions.

Table 4: Extended stability study of Optimized Levamisole Tablet (45% TBG polysaccharide).

	Optimized formulation (F45)		
Parameters	**After30 Days**	**After 60 Days**	**After 90 Days**
Hardness (Kg/cm^2) [a]	3.2±0.35	3.8±0.2	3.85±0.15
Friability (% w/w) [b]	1.55±0.12	1.40±0.15	1.95±0.15
Uniformity of Weight (mg) [b]	157.87±1.01	161.20±2.99	163.57±1.20
Uniformity of Content (% w/w) [b]	97.16±0.12	95.15±0.61	94.15±0.25
Mucoadhesive Strength(N)	34.19±1.91	29.95±0.92	20.33±0.93

4. CONCLUSION

From the present study, conclusion was focused on the formulation of 12 hr mucoadhesive controlled release tablets of levamisole using *Terminalia bellerica* gum polysaccharide as matrix forming hydrophillic polymer. It was summarized from the dissolution studies that the tablets containing less concentration of *Terminalia bellerica* gum polysaccharide were disintegrated readily but at higher concentration of *Terminalia bellerica* gum polysaccharide the tablets released the drug in a controlled manner over 12 h. The study of release mechanism exhibited anomalous non-fickian diffusion that involved both diffusion and erosion mechanisms. Among all the formulations, the F45 batch exhibited optimum drug release profile and significant

mucoadhesive properties. The pharmacokinetic study of the optimized batch (F45) in rabbits was carried out in triplicate. The plasma drug concentration versus time interval was estimated by using in house developed HPLC method procedure. The optimized controlled release tablets exhibited first order absorption rate kinetics in the release of drug from the tablets. The increase in Tmax value and decrease in Cmax value in case of the optimized formulation F45 from the values of the conventional tablet of levimasole revealed that the TBG polysaccharide can be used as carrier for developing mucoadhesive formulations.

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