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Research Article

A Novel Synergistic Galactomannan-Based Unit Dosage Form for Sustained Release of Acarbose

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Abstract. In the current study, the potential of a novel combination of a galactomannan with acarbose (100 mg) was evaluated for attaining a desired hypoglycaemic effect over a prolonged period of time. Three major antidiabetic galactomannans *viz*., fenugreek gum, boswellia gum, and locust bean gum were selected in order to achieve a synergistic effect in the treatment alongwith retardation in drug release. *In vitro* studies indicated that batches containing various proportions of fenugreek gum (AF40-60) were able to control drug release for a longer duration of approximately 10–12 h. In contrast, the matrices prepared using boswellia and locust bean gum were able to sustain the release for relatively shorter durations. Drug release mainly followed first-order release kinetics owing to the highly soluble nature of the drug. *In vivo* study depicted a significant reduction ($p<0.001$) in the postprandial blood glucose and triglyceride levels in the diabetic rats on treatment with formulation AF40. Thus, the developed system provides a better control of the postprandial glycaemic levels and it also obviates the need of conventional multiple dosing of acarbose. Furthermore, it also reduces the occurrence of side effects like diarrhea and loss of appetite.

KEY WORDS: acarbose; antidiabetic; boswellia gum; fenugreek gum; galactomannans; locust bean gum; sustained release.

INTRODUCTION

Nowadays, naturally occurring polysaccharides are being extensively exploited as release modifiers in drug delivery owing to their nontoxic, biodegradable nature, hydrophillicity, safety, easy availability, and low cost. The gums are being used both in controlling drug release as well as targeting it to specific sites, *e.g.*, colon (1–3). Thus, they are promising biomaterials in modifying drug release rate. In the current study, the potential of a novel synergistic combination of novel antidiabetic gums containing galactomannans with acarbose in achieving a desired controlled-release profile and improved glycaemic control was evaluated.

Galactomannans are heterogenous polysaccharides comprising mannose. In these molecules, the hydroxymethyl group at C-5 of certain D-mannose residues of a mannose polymer chain is substituted by D-galactose. They are mainly present as an extracellular deposit in the endosperm of seeds of plants of Fabales (Leguminosae). They have a mainstay in treatment of diabetes (1). Guar gum is the sole candidate of this group which is being extensively exploited commercially for its therapeutic efficacy in treatment of diabetes (2). Currently, many other gums containing galactomannans are also being evaluated for their hypoglycaemic activity.

Fenugreek gum (FG) is obtained from the seeds of an annual herb *Trigonella foenum graecum*. It is widely exploited in various regions of the world for its medicinal and flavoring properties. It contains an abundant quantity of soluble galactomannans (galactose/mannose ratio is 1.5:1; 4). An early study conducted in 1986, by Ribes *et al*., reported that testa and endosperm of the fenugreek seeds have significant antidiabetic potential. Many reports suggest the fact that the fiber fraction, extract, and gum obtained from fenugreek seeds have both antidiabetic and hypocholesterolemic effects in various animal and human models (5–9). Madar *et al.* have reported that a suspension of fenugreek powder lowers the postprandial sugar levels significantly in type 2 diabetic rats (10). It also aids in normalizing other clinical symptoms like as polyuria, polydypsia, weakness, and weight losses associated with diabetes (11). A majority of studies indicate that the gum fraction of the seeds is mainly responsible for lowering the plasma glucose levels and have a significantly beneficial effect on the serum lipid profiles. These activities are mainly attributed to the lowering in absorption of glucose, cholesterol, and bile acids from the intestinal segment of the gastrointestinal tract (12).

Locust bean gum (LBG) is a white to yellowish white powder obtained by crushing the endosperm of the seeds from the fruit pod of the carob tree (*Ceratonia siliqua* L.). It contains approximately 90–95% of pure D-galacto-D-mannan (4). It effectively modulates postprandial serum glucose concentration in rats similar to guar gum. This activity is mainly due to the reduction in the gastric motility on administration of the gum, which further reduces the absorption of the sugars from the food (13). Similar results have also been observed in case of human

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subjects (14,15). LBG has been successfully employed in the preparation of various controlled-release delivery systems like tablets, beads, microspheres, *etc.* (16–18).

Boswellia gum is the oleo gum resin obtained from *Boswellia serrata*. Traditionally, the gum possesses both antihyperglycemic and antihyperlipidemic potential. In 1986, Zutsi *et al.* reported that the gum has significant effect on the cholesterol biosynthesis in the rat liver (19). Hence, it has a good antihyperlipidemic and hypoglycaemic potential. Recently Ilango *et al.* studied the effect of the gum on the blood glucose and lipid profile of alloxan-induced diabetic albino rats. The study indicated that the diabetic animals treated with 400 mg/kg dose of methanolic extract of the oleogum resin exhibited significant hypoglycemic activity (20).

α-Glucosidase inhibitors are a more recently developed class of oral hypoglycaemics. This class of drugs reduces or delays carbohydrate digestion by competitive enzyme inhibition without causing any substantial effect on the serum insulin levels. Thus, they prevent postprandial increase in blood glucose without causing hypoglycemia (21). Acarbose is commonly used drug of this category. Other drugs belonging to this category are miglitol, voglibose, and emiglitate. Currently available regimens of acarbose include Precose® and Glucobay® (http://www.fda.gov/cder/ob/). Acarbose has a short half-life ($t_{1/2}$=2 h), which necessitates repetitive administration of the drug, *i.e.*, 25–100 mg, three to four times a day. Various clinical trials conducted in patients with non-insulin-dependent diabetes mellitus indicate that acarbose is a potential drug which reduces postprandial glucose levels significantly along with a marked effect on the hypertriglyceridaemia, which is closely linked to carbohydrate and insulin metabolism (22,23). However, during treatment with these systems, approximately 50% of the patients report gastrointestinal side effects of flatulence, abdominal distension, borborygmus, and diarrhea (24). Development of a modified-release system of acarbose (having a dose of 25–100 mg is to be taken at least thrice daily) will obviate the need of multiple dosing of the drug candidate and hence improve patient compliance with reduced gastrointestinal side effects by sustaining the release. Previous investigators have attempted to develop a simple sustained-release matrix system for sustained release of acarbose for stimulating weight loss (25) and for the treatment of diabetes or atherosclerosis (26) using conventional polymers.

The focus of the current study is to develop a synergistic combination of the drug with the natural-occurring polymer to obtain a better control of the blood glucose levels. The developed system is also aimed to obviate the need of repetitive administration of acarbose by sustaining the drug in the body over a longer period of time in turn reducing the gastrointestinal side effects commonly associated with the conventional repetitive administration of acarbose, *i.e.*, 25 mg *ter in die* (t.i.d).

MATERIAL AND METHODS

Materials

Acarbose (purity of 99.69%) was obtained as gift from Ranbaxy Research Laboratories Ltd. (Gurgaon, India). Fenugreek gum was a kind gift from Emerald Seed Products Ltd., Saskatchewan, S0H 0C0, Canada. Locust bean gum and boswellia gum were procured from Himedia, Mumbai (India) and a local retail shop in Dehradun (India). Microcrystalline cellulose (MCC, Avicel® PH 102), was a gift from the Ranbaxy Laboratories Ltd., Gurgoan (India). Talc and magnesium stearate (used as a lubricant in tableting) were obtained from S.D. Fine Chemicals Ltd., Mumbai (India). Fresh triple-distilled water (Sartorius, Göttingen, Germany) was used throughout this study. Blood glucose, cholesterol, and triglycerides kits were procured from Reckon Diagnostice Ltd., Baroda (India). All other chemicals and solvents were of analytical grade and used without further purification.

Preparation and Characterization of Matrix Tablets

Initially, different matrix tablets containing 100 mg of acarbose were prepared by direct compression of different homogenous blends containing fenugreek, locust bean, and boswellia gums in different ratios individually in order to achieve the desired drug release profile. MCC PH102 was used as the directly compressible vehicle, magnesium stearate (1% *w*/*w*) as the lubricant and talc (2% *w*/*w*) as the glidant. The ability of the various gums to retard the drug release for the desired duration of approximately 24 h and their release mechanisms were also evaluated. A detailed account of the matrix code and unit formula of all the matrices evaluated during the study is shown in Table I and divided in subgroups depending on the basis of the different gums used. Briefly, weighed quantity of drug was physically mixed with all the auxiliary excipients by geometric addition using a glass mortar and pestle for about 10 min. Then magnesium stearate and talc were added as the glidants/lubricant and thoroughly blended for 2 min. The homogeneous powder mixture thus obtained was weighed individually and manually fed into the

Table I. Composition of Various Batches

Batch No.	Acarbose (mg)	FG (mg)	LBG (mg)	BG (mg)	MCC PH101 (mg)
AF0	100	–	–	–	191
AF40	100	120	–	–	71
AF50	100	150	–	–	41
AF60	100	180	–	–	11
ALBG30	100	–	90	–	101
ALBG50	100	–	150	–	41
AB40	100	–	–	120	71
AB50	100	–	–	150	41

Values in table show weight of respective excipient in milligrams. The amount of talc and magnesium stearate was fixed at 6 and 3 mg, respectively, in all the batches (not shown in table).

die of a single-station tablet machine were prepared by direct compression using a single-punch tableting machine (Modern Engineering, New Delhi, India) equipped with biconcave die–punch set of 9.7 mm diameter and compressed to an average target weight of 300 mg per tablet. The prepared batches were evaluated for all the pharmacopoeial evaluation tests (weight variation, thickness, hardness, friability, drug content, and *in vitro* drug release) as per immunoprecipitation IP. For *in vivo* study, 50 matrix tablets weighing 50 mg (contains 10 mg acarbose per tablet) were prepared separately using 5.2 mm upper–lower punch set on the aforementioned tablet machine. The composition of the minitablet was kept proportionally similar to that of the optimized batch.

Content Determination

Ten tablets from each batch were weighed and powdered in a mortar and pestle. Powder equivalent to 100 mg of the drug was weighed and dissolved in 100 ml of water in order to obtain a solution of 1 mg/ml. The solution was sonicated for 5 min. It was further centrifuged for 10 min at 4,000 rpm. After centrifugation, the supernatant was taken and was subsequently diluted with water to 100 μg/ml. The drug content was calculated by analyzing the sample using a validated reversed-phase high-performance liquid chromatography (RP-HPLC) method supported with a UV spectrophotometer (λ_{max}=210 nm).

Particle Size Measurement

Particle size of the excipient also plays a critical role for optimum mixing of the tablet blends. Thus, the particle size of all the gums and the drug candidate was analyzed by the Malvern Mastersizer 2000 using the dry powder method. The instrument was operated at a pressure of 1 bar and the feed rate was set at 40% and the samples were analyzed using the airflow mode. Sufficient quantity of the sample was added in dry form to obtain the obscuration in the range of 0.5–6% and the particle size was measured in triplicate.

Fourier-Transform Infrared Spectroscopy)

Spectral analysis using Fourier-transform infrared spectroscopy (FTIR) is a useful technique to verify the formation of new complexes in the blends. FTIR studies were conducted on Perkin Elmer FTIR using KBr pellets to investigate possible interactions between acarbose and the respective gums, *i.e.*, FG, LBG, and boswellia gum (BG), respectively. Samples containing 1:1 physical mixtures of the drug with the gums were prepared. The weight ratio of a sample and potassium bromide was 1:100 mg. Background spectrum was collected before running each sample. The samples were compressed into pellets using a hydraulic press and the pellets thus obtained were analyzed between wave numbers 4,000 and 400 cm^{-1}.

Validated RP-HPLC Method for Analysis

The separations were carried out on a HPLC system (Shimadzu, Kyoto, Japan) consisting of a LC-10AT pump, a SPD-10A UV-visible detector and a DGU-14A degasser model. The separations were carried out on a reversed-phase column (Thermo Hypersil APS, 250×4.6 mm, S-5 μ). The column was operated at a temperature of 35° (±2°C). Sixty-five parts of ACN, 35 parts of phosphate buffer at a flow rate of 1.5 ml/min was used as the mobile phase. The wavelength of detection was set at 210 nm. The data were acquired and processed by the use of CLASS-VP software (Shimadzu, Kyoto, Japan).

Swelling Studies

Swelling studies were performed to study the rate of water uptake by the different matrices. It was determined equilibrium weight gain method similar to that reported by Sriamornsak *et al.* (27). This method is based on the fact that the matrices gain weight on swelling and therefore the percentage change in the tablet weight is proportional to the degree of swelling. However, as the matrix starts eroding with time, the value of this factor becomes less than zero which depicts matrix erosion. The study was performed in a closed shaker bath maintained at 37±2°C by placing the tablets in a 150 ml beaker containing 90 ml of water for 24 h. Initially, all the prepared matrix tablets were placed on a mesh and accurately weighed (w_0). The wire mesh was used to prevent the matrix from sticking to the glass walls and facilitate ease of weighing at various time points. Furthermore, it also aids in determining the radial and axial swelling at different time points. At the specified time points, the partially hydrated matrices were carefully removed along with the wire mesh and the tablets were lightly blotted with tissue paper to remove excess surface water and then reweighed (w_1) on an analytical balance (Sartorius LA120S). The percentage increase in weight due to absorbed liquid or water uptake was estimated at each time point from the following equation:

$$\% \text{ Weight change} = w_1 - w_0/w_0 \times 100 \quad (1)$$

Change in diameter of the tablets and the percent water uptake was recorded at regular time intervals.

Morphology of Swollen Tablets

Morphological examination of the swollen tablets was carried out using a digital camera (Sony Cybershot DSC-S750, USA) equipped with Sony lens (3.00× zoom (35–105 mm eq.)). Photoimaging was performed on each tablet formulation after hydrating in distilled water. The tablets were taken out from the medium and were photographed by a digital camera.

In Vitro Release Studies and Drug Release Kinetics

The dissolution of the developed matrix formulations (n=3) was carried out in United States Pharmacopeia (USP) type II (paddle) apparatus using USP apparatus type-II at 75 rpm in 900 ml of water and at a temperature of 37±0.5°C for 24 h. (Labindia, Mumbai, India). Five-milliliter dissolution medium was withdrawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h, filtered and analyzed using validated HPLC method. A mean dissolution time (MDT; 28) for release

of the drug from various batches was calculated using the following expression (Eq. 2):

$$MDT = \frac{\int_0^\infty t.W_d(t).dt}{\int_0^\infty W_d(t).dt} \quad (2)$$

where, W_d (t) is cumulative drug amount dissolved at any time, and the other parameters *viz*., $t_{50\%}$ and $t_{80\%}$ were calculated graphically.

In order to study the mechanism of drug release from the prepared matrix tablets, the release data obtained was evaluated using zero-order release kinetics, first-order kinetics, Higuchi's square root of time equation (29), Korsemeyer and Peppas equation (30), and Hixon–Crowells cube root of time equation (31). The goodness of fit was evaluated by comparing the correlation coefficient values for all the batches.

Stability Study

The final batch containing 40% fenugreek gum (AF40) was packed (HDPE bottles) and subjected to accelerated stability studies as per International Conference on Harmonisation (ICH) guidelines (40±2°C/75% RH±5% RH). The samples were withdrawn periodically (0, 30, 60, 90, 120, 150, and 180 days) and evaluated for the different physicochemical parameters *viz*. appearance, weight variation, thickness, hardness, and drug content. The *in vitro* dissolution studies were also performed.

In Vivo Bioadhesion Testing of the Tablets to the Rat Gastrointestinal Tract

A novel method was developed inhouse to evaluate the bioadhesive potential of the optimized tablet formulation. Male Wistar rats weighing approximately 250 g were fasted 18 h before experimentation and during this period, they received only portable water. They were divided into two groups; one group was kept on fasting and the other on fed state during the study. Tablets (50 mg) containing 40% of fenugreek gum and 33.33% of charcoal instead of acarbose were formulated and administered to all the animals to trace the path of the dosage form during the course of the day. The tablets containing charcoal had similar properties as those prepared with the drug. Early morning, these tablets were administered orally to all the animals with the aid of a canula. Subsequently, studies were carried out with the fasted group receiving no food and animals of the other group were supplied with 40 g of normal rat chow thrice a day. The animals were sacrificed at defined time intervals and dissected to isolate the gastrointestinal tract to locate the position of the dosage form in order to estimate the transit time and the bioadhesive strength of the designed formulation in the presence and absence of food.

In Vivo Pharmacodynamic Studies

Acarbose acts locally in the small intestine, and only 0.5–1.7% of the drug is absorbed. Due to the negligible oral absorption of acarbose, measuring the drug concentration in plasma *via* pharmacokinetic studies is almost impractical. Hence, a suitable pharmacodynamic protocol was established to evaluate the efficacy of the synergistic effect of the active pharmaceutical ingredient with the natural gum in the final formulation AF40.

Animal Housing and Handling

Six male Wistar rats per groups, weighing 150 g, were used in the study in accordance with a protocol approved by the Institutional Ethical Committee (letter approval number: (1-12/IAEC 03/09/2009)), at the central animal house facilities of Panjab University, Chandigarh, India. The experiments were conducted as per Committee for Prevention, Control and Supervision of Experimental Animals guidelines. All rats were housed properly in cages under environmentally controlled conditions (23±2°C; 55±5% relative humidity, 12 h light/dark cycle). All rats were housed with free access to food and water, except for the final 24 h before experimentation. After 1 week, blood was withdrawn from the optical vein through standard heparinized capillary in Eppendorf's tube. After collection, blood was centrifuged at 3,000 rpm for 20 min in order to separate the serum. This fraction was then used for estimation of normal blood glucose, cholesterol, and triglycerides. All the animals were found healthy and non diabetic (fasting blood sugar below 60 mg/dl). Type II diabetes was induced in the rats with the aid of high fructose diet. All the animals except those of the control group were fed on high fructose formulated inhouse for approximately within 40 days after which the treatment protocol was started.

Control and Fructose Diet

The control diet for the rats contained 50.0% nitrogen-free extract, 20% protein, 8% fat, 4% cellulose, 1–1.5% of each mineral, and vitamin mix. The control diet was obtained from Ashirwad Industries (Chandigarh. India) and the high-fructose diet (HFD) were prepared in-house as per the formula given by Maurya *et al*. (32). It contained fructose 50%, casein 19%, Dalda (Vanaspati Ghee) 11%, wheat flour 15%, and vitamin and mineral mixture approximately 1%.

Induction of Diabetes

Group A received normal diet and served as control while the rats of the other groups received HFD pellets for 45 days. On 45th day, blood samples were withdrawn from the retro-orbital plexus after an overnight fast. Serum was analyzed for blood glucose, cholesterol, and triglycerides using commercially available kits from Reckon Diagnostics Pvt. Ltd. (Baroda, India) to ensure the induction of type II diabetes in all the groups.

Experimental Design

All the animals were 4 weeks of age, weighing around 150 g at the time of dietary manipulation. Animals were

randomly assigned into five groups of six animals each as given below:

Group A Nondiabetic, normal control rats, received tap water and control diet (normal rat chow)
Group B Diabetic, fructose-fed rats, received tap water and fructose diet
Group C Insulin resistance-treated, fructose-fed rats, treated with immediate release formulation (2.5 mg acar bose per tablet thrice a day at 0, 5, and 10 h, respectively)
Group D Controlled Release-treated, fructose-fed rats, treated with sustained-release formulation (10 mg acarbose per tablet once a day)
Group E Placebo-treated, fructose-fed rats, treated with placebo sustained-release formulation.

Tablets were administered orally with the aid of forceps and canula to administer 2–4 ml water. The animals were maintained in their respective groups for 45 days and treatment with the dosage form was started on the 46th day after confirmation of induction of type II diabetes in all the fructose-fed groups further the dosing schedule was carried out for 72 h and the body weight, plasma glucose, cholesterol, and triglycerides of all animals were measured at defined time intervals.

Estimation of Blood Glucose

Blood glucose of the experimental animals was estimated by glucose oxidase/Peroxidase method as described by Trinder (33). In this method, glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. The Merck Auto Analyzer was used to analyze the prepared samples.

Estimation of Triglycerides and Cholesterol Levels

Triglycerides were estimated by the glycerol-3-phosphate oxidase-phenol+4-aminoantipyrine (PAP) method using the Agappe diagnostic kit (Kerala, India). Enzymatic determination of triglycerides was based on treatment with glycerol kinase followed by glycerol-3-phosphate oxidase and peroxidase, respectively, which resulted in the formation of a red quinoneimine the intensity of which was measured at 505 nm. The Merck auto analyzer was used to analyze all the prepared samples. Cholesterol was measured by cholesterol oxidase–PAP method. In this method, the cholesterol esters are hydrolysed to free cholesterol by cholesterol esterase. The free cholesterol is then oxidized by cholesterol oxidase to cholesten 4-en-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenolic compound in the presence of peroxidase to yield a colored complex which is read at 505 nm. The intensity of color produced is directly proportional to the concentration of total cholesterol in the sample.

Estimation of Body Weight Loss and Frequency of Diarrhea

Changes in the body weights of all the rats in the various treated and untreated groups were recorded at regular time intervals to initially evaluate the induction of the metabolic disorder which is accompanied by significant weight gain and latter to check the efficacy of the designed formulation. Occurrence of diarrhea was measured every 24 h for 72 h. The cages used in the study consisted of a grid, and all feces expelled from the rats fell through this grid onto a plate and the number of intact feces and the halos formed due to diarrhea were calculated. Diarrhea was scored cumulatively, according to an adaptation of the method of Hedge and coworkers as follows: 0 for normal feces or no feces, 1 for well-shaped wet feces, 2 for shapeless feces, and 3 for unshaped feces with large amounts of liquid (34,35).

Estimation of Food Intake

Food intake was measured periodically manually for 72 h. A known amount of normal rat chow (*i.e.*, 40 g per six animals per diet) was given to all the groups of animals during the study. The food was administered after a defined period of time to simulate the normal feeding cycle. Food was given at 0, 5, and 10 h after administration of the formulation every day, i.e., 120 g per day. The food was reweighed before the following set of administration and the difference was used to measure the amount of food consumed.

RESULTS AND DISCUSSION

Physical Evaluation and Content Determination of the Prepared Matrices

The results indicated that all the tablets prepared in this study meet the IP requirements for weight variation tolerance. Drug content of all tablet formulations were found in the range of 99.01–100.70%. The thickness, diameter, and hardness variation of all the prepared batches are indicated in Table II.

Table II. Physical Evaluation Parameters for All the Batches

Formulation code	Weight (mg) (mean±SD)	Thickness (mm)	Hardness (kg cm^2) (mean±SD)	Friability (% weight loss)
AF0	299.32±1.14	3.98	4.87±0.09	0.55
AF40	301.11±0.98	4.20	4.10±0.11	0.80
AF50	300.28±0.17	4.12	4.39±0.21	0.75
AF60	299.78±0.04	4.00	4.59±0.11	0.63
ALBG30	301.2±0.48	4.76	3.22±0.79	0.84
ALBG50	299.69±0.44	4.62	3.39±0.16	0.72
AB40	298.78±1.12	4.12	3.23±0.32	0.71
AB50	299.04±0.99	4.12	3.45±0.20	0.72

Particle Size Measurement

The mean particle size of acarbose was found to be 134.617 μm. During direct compression for effective compaction, the blend should be uniform without any significant segregation. Therefore, the particle sizes of the gums used in the study were also evaluated. The mean particle size of fenugreek, locust bean, and boswellia gum was 110.86, 188.87, and 102.49 μm, respectively. Thus, the mean particle sizes of all the selected components of the tablet blend were comparable to that of the major active ingredient. As a result, it will assist in uniform mixing of all the components in the blend avoiding the segregation of the particles of different excipients during the compression process resulting in a better formulation.

Fourier-Transform Infrared Spectroscopy

FTIR study was performed to establish the compatibility of the drug and the polymers used. FTIR spectra for various blends are presented in Fig. 1. The FTIR spectra A refers to pure acarbose while spectra B, C, and D represent its blend with fenugreek, locust bean, and boswellia gums, respectively. In case of pure drug, a broad band appearing at 3,371 cm^{-1} denotes O–H stretching, 1,652 cm^{-1} depicts the stretching zone of C=C, 1,418 cm^{-1} depicts the bending zone of O–H group, and 1,152 cm^{-1} depicts the C–O stretching vibration which is in concordance with the literature values (36). All these bands were also identified in spectra B, C, and D, *i.e.*, the physical mixtures of acarbose with fenugreek, locust bean, and boswellia gum, respectively, with minor differences in frequencies. Thus, it is suggested that there was no interaction between the drug and the selected polymers.

Method Validation

A validated RP-HPLC method was developed for analysis of acarbose in all the batches developed in the study. The calibration curve for acarbose was linear (r^2=0.9998;

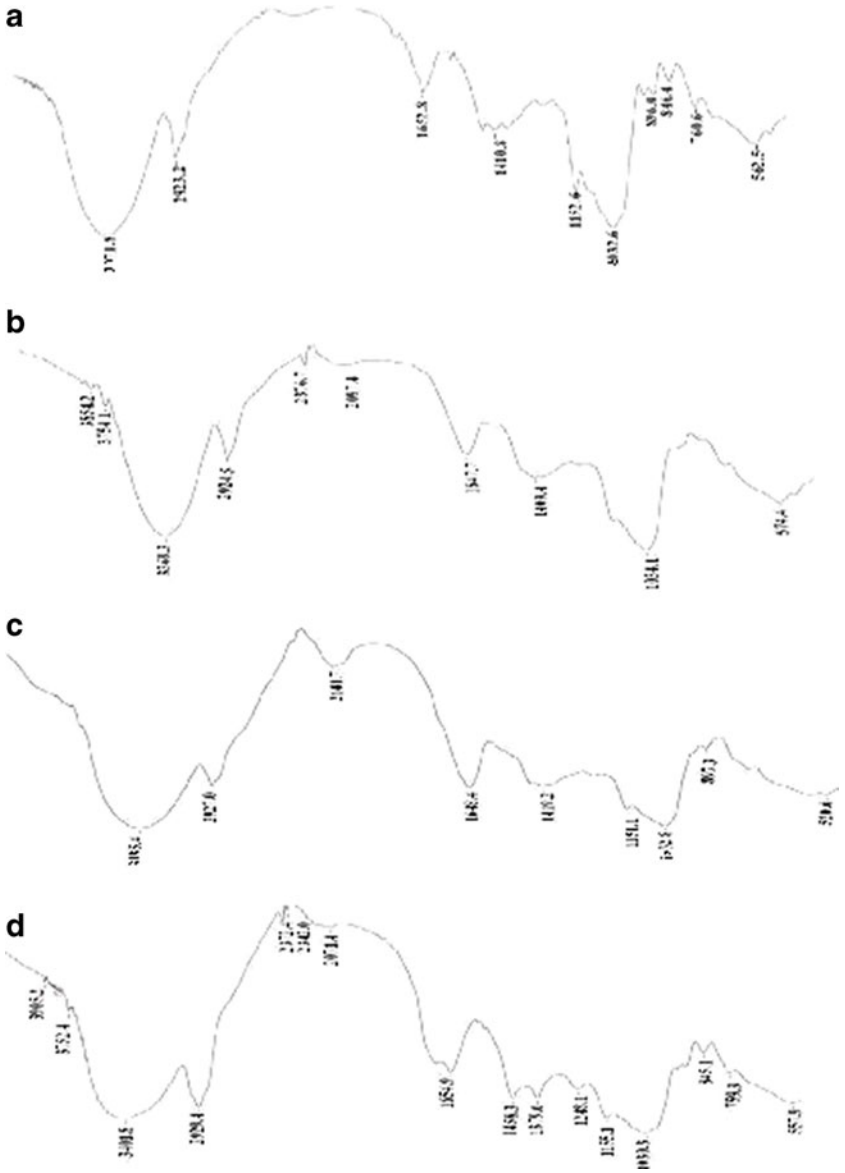


Fig. 1. FTIR spectra of (**a**) pure acarbose (**b**) physical mixture of acarbose with and FG (1:1) (**c**) physical mixture of acarbose with LBG (1:1) (**d**) physical mixture of acarbose with BG (1:1)

(±0.0001)) in the range of 5–200 μg ml^{-1}. The limit of detection and limit of quantification of the method were 0.899 and 2.72 μg/ml, respectively. Relative standard deviation values for all the key parameters like interday and intraday precision was less than 5.0%. The recovery of the drug was found to be 99.42–103.61%. The method was found to be robust and reproducible. Hence, the developed method was successfully employed for the estimation of the drug content and the drug release in the *in vitro* samples.

Swelling Studies

Swelling studies aid in studying the degree of hydration of the polymer matrices which in turn modify the rate and kinetics of drug release from the delivery systems. The swelling studies were performed on all the prepared batches. The water sorption behavior of all the aforementioned hydrophillic gum matrices are shown in Fig. 2(a–c). Batches (AF40-60) containing various proportion of fenugreek gum showed an increase in swelling with a corresponding increase in the polymer concentration. It is well documented in literature that fenugreek gum consists of a highly substituted mannose backbone (37). In a recent study, Doyle *et al.* have reported that there is a significant degree of hyperentanglement in the galactose side chains of the mannan backbone of the fenugreek gum in the presence of water (38). Thus, the swelling of the matrices is attributed to the hyperentanglement of the gum particles in the dissolution media. The swelling of the matrices started initially within approximately 30 min and increased linearly till 12 h followed by a constant phase after that. As a result, a continuous gain in the weight of the matrices was recorded up to 12 h. This behavior was attributed to the corresponding increase in the strength of the diffusional path length to be traveled by the dissolution media with the increase in the gum concentration in the matrix core.

In contrast, the matrices containing locust bean and boswellia gum showed an initial increase in water uptake in first 2 h followed by erosion. These results were supported by the fact that locust bean gum is known to have a lower degree of solubility and water-binding capacity at ambient temperature (39,40). It has been reported previously that locust bean gum matrices tend to show a relatively lower degree of swelling compared to the other galactomannans (41). Locust bean gum matrices (ALBG30-60) showed a slightly higher degree of axial swelling relative to radial swelling. They showed an initial rapid axial swelling in the first 30 min followed by gradually decline in the swelling rates. This behavior is mainly attributed to the poor swellability of the gum. Similarly, the percent weight loss from the boswellia gum-based tablets (AB40-50) increased progressively with the swelling time. These matrices showed complete erosion within 4 h. The extent of erosion in water increased progressively, as the percentage remaining of the tablet mass decreased, with the increased swelling time. Boswelia gum displayed the highest erosion rate and lowest swelling index and hence these matrices resulted in lack of control of drug release.

Morphology of Swollen Tablets

Visual inspection indicated that the matrices containing fenugreek gum showed the maximum degree of radial swelling and formed a viscous gel mass around the matrix core (Fig. 3). This gelatinous layer was tough, pulpable, and exhibited a good adhesive tendency. All the matrix cores were intact and swelled completely by the end of 24 h. On the other hand, the locust bean gum matrices showed an initial swelling followed by erosion and complete dissolution of the matrix within 12 h. However, boswellia gum matrices eroded much faster than the other two gums and complete erosion of the matrix was observed within 6 h. These systems showed a

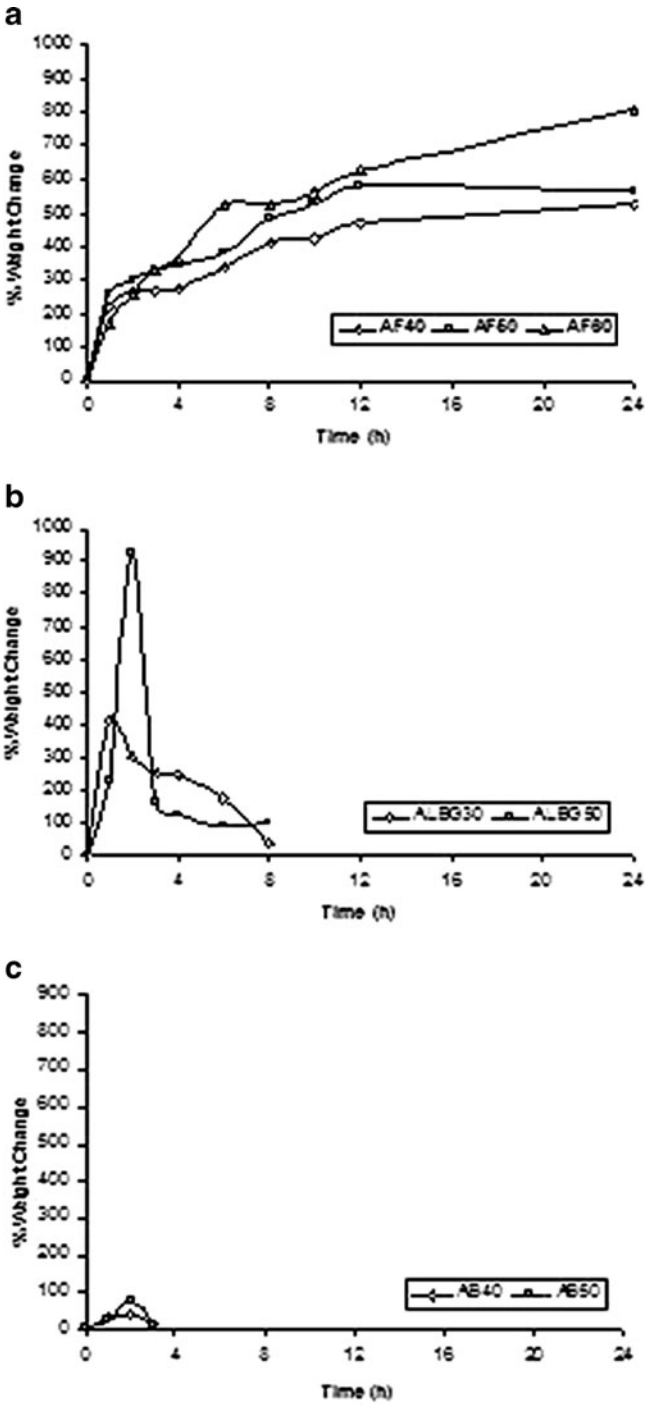


Fig. 2. Percent weight change of different formulations containing various proportions of (**a**) fenugreek gum (AF40, AF50 and AF60) (**b**) locust bean gum (ALBG30 and ALBG50) (**c**) boswellia gum (AB40 and AB50) at different time points

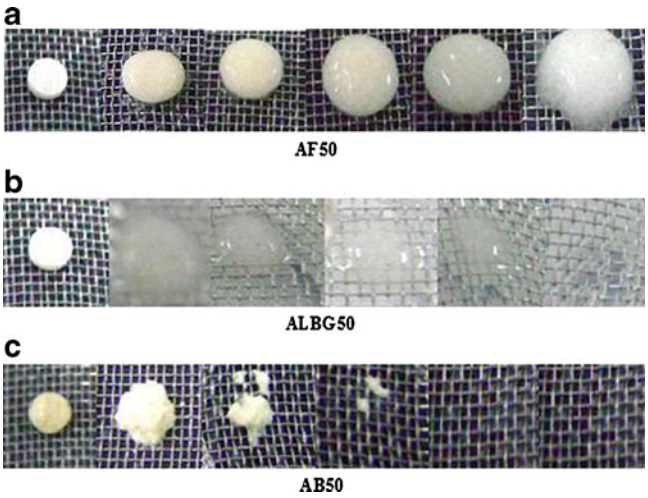


Fig. 3. Photographs of different ACR tablets (**a**) Fenugreek gum matrices (**b**) Locust bean gum matrices (**c**) Boswellia gum matrices hydrated in distilled water (Dissolution Media) at 0, 2, 4, 6, 10 and 24 h at same drug to polymer ratio (1:1.5)

peculiar behavior as these matrix fragmented into flakes which deposited at the bottom of the vessel. Visual inspection indicated that the matrices containing fenugreek gum showed the maximum degree of radial swelling and formed a viscous gel mass around the matrix core. These results were in good concordance with the dissolution results.

In Vitro Release Results

The results for dissolution studies carried out on various formulations are shown in Fig. 4a–c. It was observed that the immediate release formulation (batch AF0) containing drug and MCC only showed almost complete drug release of 99.67±0.73% in 1 h. It was observed that after 2 h, 50.15±3.03%, 48.08±0.23%, and 40.73±3.49% cumulative amounts of acarbose were released from batches AF40, AF50, and AF60, respectively. At the end of 8 h, AF40 and AF50 formulations released 87.66±0.18% and 84.36±1.40% of acarbose, respectively. A relatively faster rate of drug release was recorded in all the formulated batches in the initial 3 h followed by a constant drug release. These results were in good agreement with the observations of the swelling studies. According to the swelling studies, the fenugreek gum-based matrices showed swelling proportional to the amount of gum in the system. As a result, the drug release was correspondingly controlled on increasing the fraction of gum in the tablet. Almost 90% of the drug was released from both the formulations within 12 h followed by complete release in 24 h owing to the high solubility of the drug candidate. Furthermore, batch AF60 containing 60% fenugreek gum depicted a slightly slower rate of drug release relative to batches AF40 and AF50.

Figure 4b, c depicts the *in vitro* release pattern of locust bean gum batches (ALBG30 and ALBG50) and boswellia gum batches (AB40 and AB50). Matrices containing locust bean gum (ALBG30 and ALBG50) and boswellia gum (AB40 and AB50) showed variable degrees of controlled release. Batches containing 30% (ALBG30) and 50%

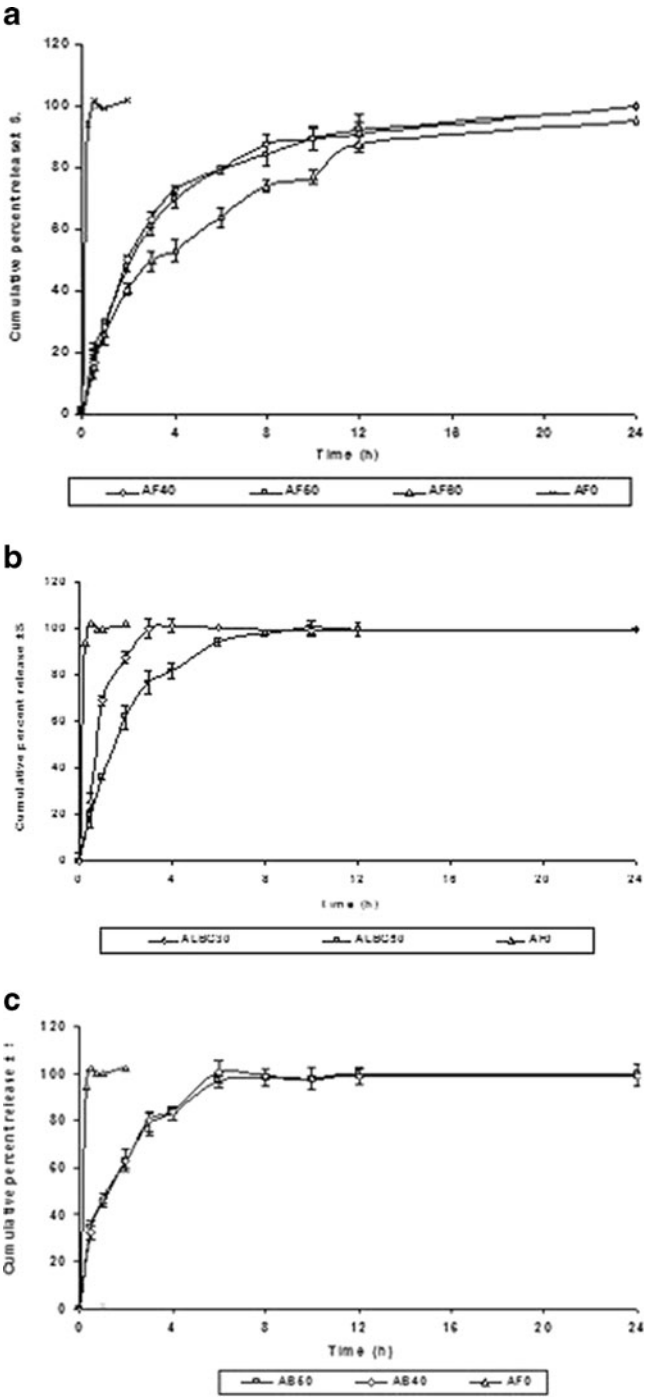


Fig. 4. Mean cumulative percentage release of acarbose from matrix tablets containing various proportions of (**a**) fenugreek gum (**b**) locust bean gum (**c**) boswellia gum plotted as a function of time. Each point depicts the mean value of three samples (n=3)

(ALBG50) of locust bean gum depicted complete drug release of 100.05±2.43% and 100.69±1.33% in 3 and 10 h, respectively. Moreover, batches containing boswellia gum also depicted a relatively slower rate of controlled release of the drug with complete drug release in 6 and 8 h in batches AB40 and AB50, respectively. Both these polymers showed initial burst release within the initial 4 h, *i.e.*, 81.82%±4.8% and 82.84%±3.13% at a polymer concentration of 50% in batches ALBG50 and AB50, respectively. A considerable

lack of drug release control obtained in these batches was attributed to the lesser gel strength of the developed matrices.

The release behavior can be explained on the basis of swelling studies. Thus, erosion dominated over swelling in the case of locust bean gum and boswellia gum matrices. These matrices eroded within 10 and 6 h for locust bean gum (ALBG30, ALBG50) and boswellia gum (AB40, AF50), respectively. As a result, almost-complete drug release was obtained from all the batches containing either of these polymers within approximately 8 h. As both locust bean and boswellia gum provided very poor control in drug release even at higher concentrations of 50%, therefore no further batches were formulated with them.

MDT is used to characterize drug release rate from a dosage form and indicates the drug release retarding efficiency of polymer. Tablets prepared with fenugreek gum (AF40-60) showed highest MDT value (3.88–7.50 h) in comparison to tablets prepared with locust bean (ALBG30 and ALBG50) or boswellia gum (AB40 and AB50; 3.02–2.20 and 2.02–2.22 h, respectively) indicating sustained release of the drug.

The $t_{50\%}$ and $t_{80\%}$ values were also calculated graphically as shown in Table III. Generally, values of both $t_{50\%}$ and $t_{80\%}$ increased on increasing the proportion of the polymer in the matrix system. In the present study, fenugreek gum batches showed the highest $t_{50\%}$ and $t_{80\%}$ in the range of 2–3.2 and 6–12 h, respectively, indicating good control of drug release from the developed matrices. However, batches containing various proportions of locust bean and boswellia gum showed relatively faster release rate and hence lower $t_{50\%}$ and $t_{80\%}$ compared with those of fenugreek gum. This finding can be attributed to the lower swelling and higher erosion property of these polymers, which retarded drug release from the matrix.

Drug Release Kinetics

Drug release from the controlled-release dosage forms is controlled by key parameters like nature and proportion of the polymer used, solubility of the active pharmaceutical ingredient, and the swelling and erosion behavior of the polymers used (42). Values of drug release exponents and coefficient of correlation for all the release models were calculated for all the prepared batches. The release data was found to be in good agreement with the first-order release model, with coefficient of regression values between 0.902 and 0.997. Water-soluble drugs are predominantly released by diffusion resulting from the relaxation of the macromolecular polymer chains followed by matrix erosion. Many reports have shown that the release of water soluble moieties typically follow first-order release kinetics (43–46). Hence, the results obtained were in good concordance with previous literature. Therefore, it was concluded that the drug release from the galactomannan-based matrices is concentration dependent.

Table III. $t_{50\%}$ and $t_{80\%}$ Values of Acarbose Release from the Matrix Tablets

Batches	$t_{50\%}$ (h)	$t_{80\%}$ (h)
AF40	2	6
AF50	2.12	6
AF60	3.2	12
ALBG30	0.8	1.6
ALBG50	2.24	4
AB40	1.25	3.0
AB50	1.25	3.2

Stability Studies

The accelerated stability data for the selected formulation showed no significant change in parameters like weight, thickness, hardness, and drug content. However, the tablets were observed to be pale yellow in appearance after 4 months which is attributed to the change in color of the drug on storage. No significant change in the release profiles was observed (similarity factor f_2 was found to be 83 after storing for 6 months, which is >50). As there was no significant change in the drug content of the designed batch, the formulation was found to be stable. The shelf-life of the optimized formulation was evaluated as per the ICH Q1E (evaluation of stability data) guidelines for drug substances and drug products intended for storage at room temperature (www.ich.org). Shelf-life of batch AF40 was found to be 1.47 years. *In vitro* release studies were also performed on the stability samples.

In Vivo Studies on Adhesion of the Tablet in the Gastrointestinal Tract

Mucilage derived from seeds of *Trigoenella foenum* possesses significant mucoadhesion, relatively higher than many established synthetic polymers *viz.*, carbopol, HPMC (47). The optimized formulation containing 40% of fenugreek gum (AF40) swells on imbibition of the dissolution media and forms a gelatinous layer around the matrix. This layer assists in controlling the rate of release acarbose form the matrix system. The mucoadhesive stregth of this layer was studied by comparing the residence time of tablets of AF40 in the various segments of the gastrointestinal tract of the rat in fasted and fed state. Rats were selected for the study because of the ease of use, lower cost, and better identification of the dosage forms in each compartment of the gastrointestinal tract after animal sacrifice. Charcoal was used as a marker for the tablets in this case. Matrix tablets of batch AF40 containing charcoal instead of the drug were administered orally to collect data for the transit time of the delivery system in fasted and fed conditions. This method is better than radiography or gammascintigraphy as these techniques are more suitable in case of large animals (48). Furthermore, it also obviates the use of a radioactive material.

The controlled-release formulation (AF40) was administered in both fast and fed conditions to evaluate the effect of food on the residence time of the formulation. Due to higher degree of gut motility in case of fasted state, the chyme leaves the stomach anytime between few minutes to approximately 3 h (49). Therefore in the fasted rats, the tablet was located in the lower segment of the large intestine approximately within

4 h of administration. However, the controlled-release tablets were located in the upper segment of the gastrointestinal tract till approximately 6 h.

After 8 h of administration, the tablet was located in the latter segment of the ileum. The location of the charcoal labeled tablets after 2 and 6 h of administration is fed state are shown in Fig. 5a, b. On dissection after 24 h, no intact formulation was present in the gastrointestinal tract of the rats, only a few blackish fragments of charcoal were observed in the latter part of the gastrointestinal tract in the animals kept in the fed state. The results obtained indicated that the incorporation of fenugreek gum in the matrices (batch AF40) provided adequate bioadhesive strength increased the residence time of the delivery system in the body and aided in achieving a desired controlled-release profile for acarbose.

In Vivo Pharmacodynamic Studies

Based on the *in vitro* study results, batch AF40 was selected for the animal studies as it provides the desired controlled-release profile and contains an antidiabetic polymer which is predicted to provide a synergistic antidiabetic effect on the antidiabetic properties of the formulation along with the drug. Another aim of the study was to decrease the incidence of side-effects associated with repetitive administration of the drug. Thus, various hematological parameters *viz.*, blood glucose, triglycerides, cholesterol were studied along with the effect of the designed formulation on the frequency of occurrence of various side effects like diarrhea, food intake, and body weight gain.

Postprandial Glucose Level Measurements Post-Administration of Different Formulations

Initially, in the study, type II diabetes was induced in male Wistar rats. A number of earlier reports have shown that type II diabetes can be successfully be induced within 20–50 days of feeding the animals on a diet containing approximately 60% fructose (32,50). Therefore, the animals were fed on a high-fructose diet to induce diabetes. Fasting blood glucose, cholesterol, and triglycerides levels were periodically determined (0, 15, 20, 30, and 45 days). A significant elevation ($p<0.001$) in the blood glucose levels among all the groups being fed on a high fructose diet relative to the control group within 45 days confirming the induction of diabetes.

Acarbose prevents increase in postprandial blood glucose which is responsible for various complications in type II diabetics (51). After the induction of diabetes in groups B–E, both IR (AF0) and CR (AF40) formulations of acarbose were administered in groups C and D, respectively. Group A and B were kept as the control and the positive control groups in the study comprising of nondiabetic and diabetic rats, respectively. Furthermore, group E was treated with a placebo batch containing only fenugreek gum (40%) to evaluate the contribution of this fraction in decreasing the glycaemic levels. The tablets were administered to the treatment groups as per the standard dosing regimen, *i.e.*, 30 min before the administration of food. Normal rat chow was administered to all the groups thrice a day at 0, 5, and 10 h each day for 5 days along with portable water.

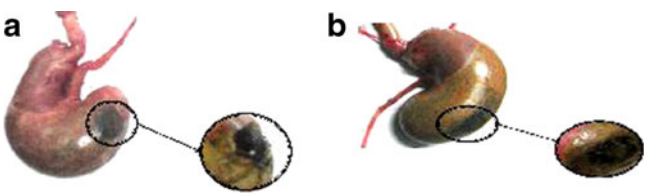


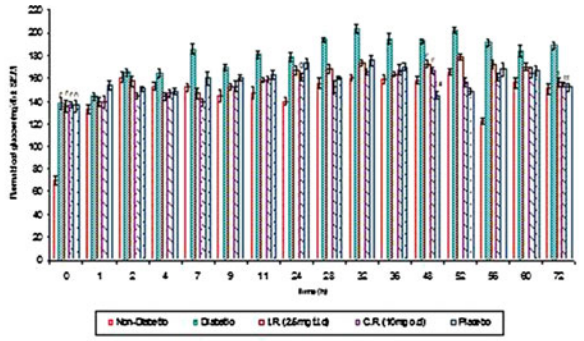
Fig. 5. Location of the tablet (AF40) in the stomach after (**a**) 2 h (**b**) 6 h of administration in fed state

Postprandial blood glucose levels were determined for all the groups at regular time intervals (0, 1, 2, 4, 7, 9, 11, 24, 28, 32, 36, 48, 52, 56, 60, and 72 h) in the following study and the results are compiled in Fig. 6. Results obtained in the initial hours following the administration of both the dosage forms (AF0 and AF40), indicated no significant difference in the postprandial glucose levels among the diabetic groups (groups C, D, and E). Glucose levels were almost similar to those of the control animals (group A). This peculiar behavior is attributed to the lag time taken by the animals to acclimatize to the change in nature of diet (from high fructose diet to normal rat chow). After 7 h of drug administration, significant difference in the blood glucose levels were initialized and maintained further throughout the study. The postprandial blood glucose levels followed the following order group B>group E>group A>group C >group D at 7 h. Furthermore, a steady decrease in the blood glucose levels were constantly recorded in group D being fed on AF40 (10 mg o.d.) with respect to group C treated with AF0 (2.5 mg, t.i.d) at 7, 9, 11, 24, 28, 32, 36, 48, 52, 56, and 72 h, respectively, indicating the increased effectiveness of the developed system in lowering blood sugar on continuous treatment. No statistically significant differences were recorded in the glucose levels of the animals belonging to groups C and D with respect to the glucose levels of the animals of control nondiabetic group A at the aforementioned time points, indicating the maintenance of near to normoglycaemic levels after the administration of food.

Significant lowering in the glucose levels of group E (*i.e.* the group being treated with the placebo tablets containing only fenugreek gum) were also recorded subsequently on days 2 and 3 of the study. Therefore, another significant finding of this study was that the fenugreek gum used in the formulation of the tablets is antidiabetic in nature as it significantly lowered ($p<0.001$) the glucose levels of the diabetic group even in absence of the drug. Thus, the polymer contributed dually, both therapeutically as well as a release retardant. Moreover, the treatment improved progressively on the subsequent days of the study indicating the beneficial effects of the developed system on prolonged therapy.

Effect on Lipid Profile

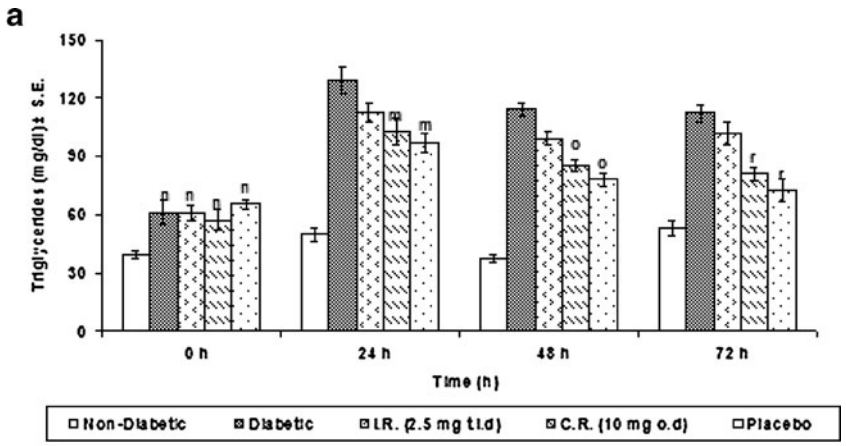
Acarbose reduces serum triglycerides significantly in both rats and humans (24,52,53). This effect is mainly associated with a decreased hepatic very low-density



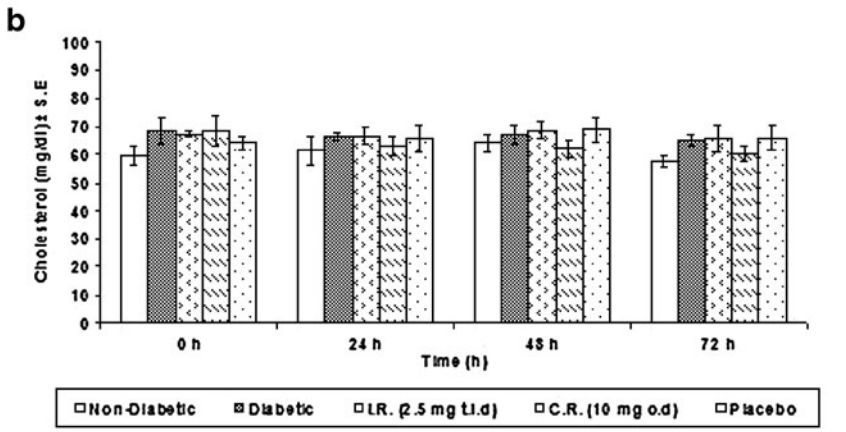
* All data are expressed as Mean±S.E.M. *p<0.05 by one-way ANOVA followed by Tukey test when values of group A were compared with groups B, C, D and E at 0 h, establishing the induction of diabetes. #p<0.05 by one-way ANOVA followed by Tukey test when values of group D were compared with group B at 24 h. *p<0.001 by one-way ANOVA followed by Tukey test when values of group B were compared with groups C, D and E and $^{a}p<0.001$ by one-way ANOVA followed by Tukey test when values of group E were compared with groups A, B, C and D. $^{b}p<0.001$ by one-way ANOVA followed by Tukey test when values of group B were compared with groups C, D and E at 72 h.

Fig. 6. A comparative profile indicating postprandial plasma glucose levels (mg/dl) in all the experimental groups at different time points

lipoprotein triglyceride secretion rate which is also known to lead to a slight decrease in serum cholesterol concentrations. In the present study, a notable effect of the high-fructose diet on the lipid profiles of the animals was recorded on the induction of diabetes. On the 45th day, groups B, C, D, and E being fed on the high-fructose diet showed a significant difference ($p<0.001$) from the control animals (group A) in the fasting serum triglycerides and relatively lesser changes in the serum cholesterol levels. The lipid profile was studied every 24 h for 72 h (*i.e.*, 0, 24, 48, and 72 h). After treatment with AF0 and AF40 in group C and D, respectively, in the initial 24 h, a significant lowering ($p<0.05$) in the triglyceride levels of the animals belonging to group D and E was recorded in comparison to the diabetic animals (group B). The results are depicted in Fig. 7. A statistically significant difference ($p<0.001$) in the triglyceride levels in groups D and E with respect to group B were recorded in 48 and 72 h. As this decrease progressed significantly with the



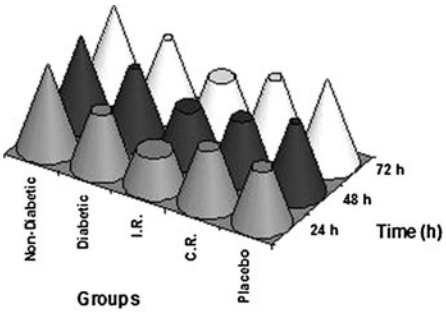
* All data are expressed as Mean±S.E.M. *p<0.05 by one-way ANOVA followed by Tukey test when values of group A are compared with groups B, C, D and E at 0 h. #p<0.05 by one-way ANOVA followed by Tukey test when values of group B are compared with groups D and E at 24 h. *p<0.001 by one-way ANOVA followed by Tukey test when values of group B are compared with groups D and E at 48 h. #p<0.001 by one-way ANOVA followed by Tukey test when values of group B are compared with groups D and E at 72 h.



* All data are expressed as Mean±S.E.M. No statistically significant difference was recorded amongst the groups at all the time points.

Fig. 7. A comparative profile indicating (**a**) triglyceride and (**b**) cholesterol levels (mg/dl) in all the experimental groups before and after treatment with the immediate release (2.5 mg t.i.d) and controlled release (10 mg o.d) and placebo formulations (n=6)

passage of treatment, it was concluded that both the controlled-release (AF40) and placebo formulation can control the triglyceride levels effectively in diabetics. After the induction of diabetes, the rats were moderately hypercholesterolemic as compared to the normal rats. It is well documented in literature that the administration of acarbose does not lower the total cholesterol levels significantly (21,54,55). Similar observations were made in the present study also, as treatment with acarbose in both immediate and controlled-release formulations (AF0 and AF40) resulted in the maintenance of cholesterol levels near to those of the nondiabetic animals without any significant reduction in their levels.



* depicts complete food intake of 120 g. A relative consumption of food in different treatment groups has been depicted at different time intervals with the aid of different colors. A significant improvement in food consumption was recorded in the C.R treated group D with the progression of treatment

Fig. 8. Extent of food intake in various treated groups (n=6)

Body Weight Loss

Acarbose has the therapeutic potential to act as an adjunct agent in the treatment of obesity and/or other disorders of carbohydrate intolerance (56). It lowers the body weight gain significantly at various dose levels both in the rats and in humans (57–59). An initial significant increase ($p<0.001$) in weights of the animals belonging to the nondiabetic and diabetic groups (fructose fed) was observed. Subsequently, in sustained-release formulation fed rats, a small decrease in body weight was observed within 5 days of acarbose administration. The body weight gain reduced from 84.33±3.35 to 66.78±5.78 g in 5 days. Similar changes in body weight were observed in animals treated with the placebo formulation, *i.e.*, body weight gain decreased from 94.0±5.46 to 82.78±7.45 g. This indicated that slow release of acarbose from the dosage form affected the body weight and assisted in its subsequent reduction.

Estimation of Food Intake

The most frequent side-effects of acarbose are fullness of stomach, flatulence, and diarrhea (23). It has a negative effect on the food consumption by the diabetic individual due to slower rate of digestion of the administered diet. Therefore, food intake in various treatment groups was measured and compared to establish the efficacy of the developed once-a-day formulation AF40 over the conventional available immediate release formulation (AF0) administered t.i.d. Food intake was measured periodically manually for 72 h. A known amount of normal rat chow (*i.e.*, 40 g per six animals per diet) was given three times a day to all the groups of animals during the study. The food was administered after a defined period of time to simulate the normal feeding cycle. Food was given at 0, 5, and 10 h after administration of the formulation every day, *i.e.*, 120 g per day. The food was reweighed before the following set of administration of food (40 g in each lot) and the difference was used to measure the amount of food consumed. Figure 8 depicts the extent of food intake in various treated groups. Almost-complete consumption of food was observed in groups A, B, and E due to the absence of acarbose in the treatment regime. Furthermore, a significant increase in the food consumption was observed constantly over the period of 72 h in the group D (fed on AF40 o.d.), *i.e.*, 80, 80, and 95 g after 24, 48, and 72 h, respectively. In comparison to that the animals of group C, showed a lower rate of food consumption with a maximum consumption of 70 g after 72 h of treatment.

Estimation of Frequency of Diarrhea

Single doses of the optimized controlled-release formulation (AF40) when given orally 30 min before the administration of food resulted in reduction of occurrence of diarrhea. Normal feces were recorded in the case of control group A and group E consisting of nondiabetic rats and rats treated with placebo formulation, respectively, after the ingestion of normal meals (*i.e.*, score 0). However, slight extent of watery stools (semisolid stools with a slight halo, score 2) were observed in case of animals being fed on the IR tablets (t.i.d) in group C on the second and third day. However, AF40-treated group D produced normal feces (score 0). Thus, the results indicate the alleviation of a major side effect associated with the repetitive administration of the conventional immediate release formulation of acarbose on treatment with a suitable controlled-release formulation.

CONCLUSIONS

Sustained-release formulations of acarbose were designed in the current study to inhibit α-glucosidase enzyme present in the lumen of the small intestine, for a longer duration of time and thus obviate the need of repetitive administration of the drug. The study results clearly indicate the presence of synergism between the antidiabetic drug and the galactomannan in controlling the postprandial glucose, triglycerides, and cholesterol levels. The optimized formulation also resulted in a marked reduction in the incidence of gastrointestinal side-effects like diarrhea, fullness of stomach, and flatulence along with a noticeable increase in the food intake. Thus, it is concluded that such novel synergistic approaches can be beneficially exploited for the designing of an efficacious delivery system for the sustained release of the drug along with the therapeutic benefits of release rate controlling polymer.

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