Educational Administration: Theory and Practice
2024, 30(1), 7201-7212
ISSN: 2148-2403
https://kuey.net/

Research Article

Development And Optimization of SR Metformin Hcl Tablet Using Grafted Copolymers of Fenugreek Gum: Development, Optimization And In Vivo Study.

Dr. Asha Gandhi[1*], Nishant Singh[2] , Deepshikha[3], Krishma Kumari[4], Ms. Nishu[5], Hemlata[6]

[1*]Sri Sukhmani Institute of Pharmacy, Derabassi, Tel.:7986432097, E-mail:ashagandhi1991pharmacy@gmail.com
[2]Lala Birkha Ram College of Pharmacy, Panchkula
[3]Swami Devi Dyal Institute of Pharmacy, Panchkula
[4]Swami Devi Dyal Institute of Pharmacy, Panchkula
[5]R.K.S.D (P.G) College, Kaithal
[6]Swami Devi Dyal Institute of Pharmacy, Panchkula

Citation: Dr. Asha Gandhi, et.al (2024). Development And Optimization Of SR Metformin Hcl Tablet Using Grafted Copolymers Of Fenugreek Gum: Development, Optimization And In Vivo Study., *Educational Administration: Theory and Practice*, 30(1) 7201-7212
Doi: 10.53555/kuey.v30i1.10397

ARTICLE INFO

ABSTRACT

Background: SR formulation of grafted copolymers is preferable dosage form in exceptional cases where conventional dosage forms are unsuccessful for encounter the disease. Both fenugreek gum & metformin HCl shows better anti-diabetic effect as sustained manner.

Methods: This current study was to design and optimize the SR Metformin HCl tablets using graftedcopolymers of fenugreek gum. The gum was extracted from fenugreek seeds and modifiedinto grafted form by microwave-assisted method. Taguchi OA design was used to optimize the synthesis of graft copolymer that was characterized by Fourier transform in frared spectros copy(FTIR),X-ray diffraction (XRD), differential scanning calorimetry (DSC),Nuclear magnetic resonance (NMR) studies,surface morphology and swelling index (SI).Metformin HCl tablets were prepared using grafted copolymers and then optimized by CCDdesign.

Results: The formulated tablets were evaluated for physical characteristics like weights variations, hardness, friability, drug release and drug content that fulfilled all the officialrequirements of tablet dosage form. The release rate of SR tablets were carried out in HCl(pH1.2) followed by phosphate buffer (pH 6.8). In-vitro release study shows that the graftcopolymer-based SR formulations (TS9) exhibited the highest correlation value (r^2) for Higuchi kinetic model. The animal studies were conductedto accessed the potential of formulationfor antidiabetic action. The study was conducted for 21 days and biochemical parameters (Bodyweight, blood glucose level, triglycerides, HDL, LDL) and antioxidant parameters (CAT, MDA and GSH) were estimated.

Conclusion: In vivo studies indicated that the formula generated by CCD design showed better sustained release profile of the formulation.

Keywords: Taguchi OA design, Central composite design (CCD), Response surface methodology, Release kinetics, Pharmacodynamics study.

1. Introduction

Diabetes is third metabolic disease in the world in which body is unable to utilize the sugar. It characterized the higher or lower abnormalities in carbohydrates, lipids and proteins metabolism. In case of diabetic patient, the concentration of glucose in urine is 10% than healthy individual (1).It characterized by pancreatic beta-cell failure, declined insulin production and insensitivity. Hence decreased glucose transportation into liver, muscle and fat cell. It also increased fat disruption due to diabetes. Due to this defect, the glucagon level and glucose in liver cell increased during eating which does not change even after meal. Hence insulin resistanceincreased and due to insufficient levels of insulin, hyperglycemia is caused. Although in DM, abnormalities occur in activity of GIP, preserves the glucagon like peptide insulinotropic (GLP-1) effects.

Copyright © 2024 by Author/s and Licensed by Kuey. This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Thus GLP-1 shows probably beneficial therapeutic approach which is deactivated by Dipeptidyl peptidase. Due to central visceral adiposity, greater number of the peoples who are suffering from type DM are obese. Therefore, the adipose tissue also involved in the pathogenesis of DM (2,3,4).
Metformin HCl is an biguanide class anti-hyperglycemic agent that is widely used in themanagementof type-2diabetes.Itisahydrophilicdrug that'sslowly andincompletelyabsorbed from GI tract with its reported absolute bioavailability 50-60% (5).Due to shorter half-life (1.5to4.5hrs), larger doses are required for decreasing the patient compliance and required the need for administration of two or three times per day.Therefore these drawbacks can be overcome by designing SR formulation of Metformin HClusing grafted copolymers of fenugreek gum to maintain the drug plasma level for 8-12hrs which might to sufficient for one-time dosing formulation to prolong its action and toimprove patientcompliance (6,7,8).
Natural gums are the polysaccharide that shows various properties like easy availability, lowcost, bio availability and bio degrad ability with various pharmaceutical applications like adhesive, laxative, binder, disintegrants, lubricating, suspending and thickening agents. The limited accept ability of natural gum sisduet ocerta in short comings like microbial contamination, batch to batch variation, pH-dependent solubility, uncontrolled hydration andstability problem. For overcoming their drawbacks, modify the properties of polymer, graft polymerization is a quite promising technique that 's used nowadays(9).

2.Materials and methods

2.1 Materials
Fenugreek seeds were procured from local market, Panchkula, Haryana India. Metformin HCl standard was kindly supplied as a gift sample (park pharmaceuticals, Baddi). MCC, Magnesium stearate andPVPK-30 (Polyvinylpyrrolidone) was purchased from BASF Corporation, Ludwigshafen. STZ-NAD obtained from Sigma-Aldrich, Milwaukee, USA.

2.2 Preparation of grafted copolymers
Microwave-assisted graft copolymerization reaction was employed for the synthesis of copolymers of fenugreek gum which is isolated from fenugreek seed. Taguchi OA Design was usedfor optimization of grafting process. The characterization of grafted copolymers by FTIR Spectroscopy ,X-ray Powder diffraction, NMR Studies, Scanning Electron Microscopy (SEM), Swelling studies was done successfully (10,11).

2.3 Experimental design
Central composite design (CCD) was employed to optimized the tablet formulation. According to this design model, required amount of grafted fenugreek gum (X1) and PVP K30 (X2) were selected as the independent factors and studied at three level seach (12).

2.3.1 Formulation of SRM et form in HCl tablet
The SR tablet formulation of Metformin HCl was prepared bywet granulation method were shown in Table 1.Initially,the semisolid dough was prepared from the grafted fenugreek gum with the minimum amount of hot water (50°C), then Metformin HCl, MCC, and PVP K30 were mixedin timately with it. The formed mass was then sieved through #18 to obtain granules whichwere dried at60 °C for 20 min and further passed through # 18 mesh. These prepared granules were lubricated with purified talc and magnesium stearate and then compressed (13).

2.4 Statistical Analysis
On the basis of ANOVA provision, ten optimum check points formulation were selected to validate the chosen experimental design and polynomial equations. The resultantexperimental data of response properties were quantitatively compared with that of their predicted values. Obtained data were subjected to multiple regression analysis. The data were fitted ineq.(1,2,and3).

$$Y1 = \beta_o + \beta_1 A - \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2 \ldots\ldots\ldots (1)$$

$$Y2 = \beta_o - \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 - \beta_5 B^2 \ldots\ldots\ldots (2)$$

$$Y3 = \beta_o + \beta_1 A - \beta_2 B - \beta_3 AB - \beta_4 A^2 - \beta_5 B \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

2.5 Evaluation of granules
The prepared granules were evaluated for angle of repose, loose bulk density (LBD), tapped bulk density (TBD), compressibility index and drug content. The angle of repose was determined by funnel method. By using cylinder method, bulk density and tapped densitywere measured. Carr's index (CI) was used to evaluate the rate at which the powder was packeddown. Hausner's ratio was used to predict the flow properties of prepared granules. It ranges from 1.12 to 1.25, was thought to indicate good flow properties. The LOD of wet granules was evaluated using an electronic moisture balance and calculated the weight loss during drying (14, 15).

2.6 Evaluation of tablets
The formulated SR Metformin HCl tablets were evaluated for weight variation, hardness,thickness, friability

and drug content. The weight variation test was performed according tothe official method by comparing individual weights with that of their average weight. The hardness of the tablets was tested using a Monsanto hardness tester (Keshav Int.PvtLtd.India).The Friability of the tablets was determined in a Rochefriabilator (KeshavInt. Pvt. Ltd. India). The thickness of the tablets was measuredby avernier caliper. Drug content was determined using UV/ Vis spectrophotometer (ShimadzuIndia) (16, 17).

In vitro dissolution study

The dissolution study of Metformin HCl from the prepared batches of tablets was conducted in USP typeI dissolution apparatus in triplicate using three tablets from each batch. Dissolution media comprised of 900 ml 0.1N HCl and phosphate buffer (pH 6.8) for 24 hrs, maintained at 37.0 ± 0.5 °C for100 rpm. The release rate from the tablets were conducted indissolution medium of 0.1NHCl for 2hrs and thereafter in phosphate buffer pH 6.8 for 24hrs.An aliquot of 5 ml sample was withdrawn and replaced with another 5 ml of fresh dissolutionmedium at various time intervals. The % drug release in the sample was determined by measuring the absorbance at232nmusingUV–Visible Spectrophotometer (18, 19).

2.7 Invivo Pharmacodynamic studies

In vivo pharmacodynamic studies were carried out using six adult male albino wistar rats obtained from Niper,Mohali with its initial weight250-300g after approval from the institutional Animal Ethical Committee from Bilwar Med Chem and Research Laboratory pvt. ltd Rajasthan with Reg. No.- 2005/ PO/RCBT/S/18/CPCSEA. The animals were maintained inanimal colony of MMU University, district Ambala and further used for experiment. They were housed in standard environmental conditions under temperature 23 ± 2˚C with relative humidity 50-55% and photoperiod of 12hrs light and 12 hrs dark cycle prior to the study. All animals were provided proper animal feed, water and fasted over night priorto the study.

Hyperglycemia was induced experimentally in albino wistar rat by a single intra peritoneal administration of freshly prepared streptozotocin (STZ; 45mg/kg*i.p.*) prepared in freshcitrate buffer (pH 4.5), 15 minutes after nicotinamide (230 mg/kg, *i.p.*). After 72 h, STZ-NAD administration, blood glucose level was measured using a glucometer. Rats with blood glucose levels above 250mg /dlwere considered diabetic and used in this study (20, 21).

Theoptimized batch of tablets (TS9) of Metformin HCl was given in mini tablet form (lower 25mg/kg, medium 75mg/kg, higher 100mg/kg) orallyadministeredwithasmallamountof water. For the oral route of drug administration, the experimental rats were randomlydivided into seven groups of six animals each. Group I contained normal rats, who were orally administered with 1.5 mL aqua, Normal Control (NC); Group II comprises of rats who were given STZ –Nicotinamide to induce diabetes, further orally administered with1.5 mL aqua; Group III contain STZ induced diabetic rats, orally administered with marketed metformin HCl tablet in 1.5 mL aqua; Group IV comprised STZ effected diabetic rats, orally administered grafted fenugreek TS9 formulation (GFTS9 25 mg/kg, lower dose) in 1.5 mL aqua; Group V comprised of STZ induced diabetic rats, orally administered with grafted fenugreek TS9 formulation (GFTS9 75 mg/kg, medium dose) in 1.5 mL aqua; Group VI consisted STZ induced diabetic rats orally administered with grafted fenugreek TS9 formulation (GFTS9 100 mg/kg, higher dose) in 1.5 mL aqua; and Group VII include STZ induced diabetic rats orally administered with un-grafted fenugreek formulation 100 mg/kg in 1.5 mL aqua of pyrogallol red solution mixed in test tube. Absorbance taken at 232 nm for 30 sec at 15 sec interval (25, 26, 27).

At pre-determined time periods, 0.5 mLblood was collected from tail vein of ratsand put into heparinized plastic tubes.Collected blood sample were centrifuged for 10 min at 2000rpm and stored at -20°C till further use. The concentration of drug is estimated by HPLC method (22, 23, 24).

Antioxidant enzymes activity (Catalase, Malondialdehyde, Gluthione -S-transferase)

*Preparation of stock solution:*A standard of 1,1,3,3 tetra-ethoxy propane (TEP) was used.For preparation of 1M stock solution, 25µL TEP was dispersed in 100mL of de-ionized water. MDA was processed by hydrolysis of 1mL TEP stock solution in 50mL of 1% sulphuricacid and incubated for 2hrs at room temperature. Then the solution was reserved at 40°C and used after 4 weeks. The resulting solution of MDA standard (20n mol/mL) was further diluted with 1% sulphuric acid. Then obtained yield lies in concentration within1-20n mol/mL.

Procedure: Take aliquot 250 µL diluted plasma in the tube, 50 µL of 6M aquoues NaOH was put in it. Then incubate supernatant solution at 60°C on water bath for 30min. The protein bound MDA was precipitated by adding 125 µL of 35% (v/v) perchloric acid. Then centrifuged at 6000 rpm for 10min.The obtained supernatant put in to vial and mixed with 50 µL of 2,4 dinitrophenyl-Hydrazine. Then incubated for 30 min at room temperature.

Determination of GSH activity: Take 1mL of supernatant and 1mL of Trichloroacetate (10% w/v H_2O). Then centrifuge it at 10000 rpm for 10min. 0.5mL supernatant was further taken,in which 2ml of disodium Hydrogen phosphate was added andthen 0.25mL of DTNB (5,5 dithio-bis 2-nitro benzoic acid) (0.001M in 1.1%w/v sodium citrate) was added and mixed. Absorbances were checked at 412nm.

*Determination of CAT Activity***:** Take 0.05 ml of sample containing catalase enzyme and also added

Phosphate buffer (1.95 mL) and Hydrogen peroxide (1mL) in test and standard tube.Then test tube were vortexed and incubated at 37°C for two minutes. The aliquots from each test tube were removed and transferred to different tube containing ammonium molybdate. The test tubes were mixed well, 3mLpyrogallol red was added in each tube. The zero time valuation was noted at that point to which total amount

3. Results & Disscussion

FTIRanalysis

The FTIR spectroscopy of pure and physical mixture of Metformin HCl was shown in Fig.1A.The pure spectra of Metformin HCl indicated the peaks at respective wave numbers i.e. N-H stretching (3173.32cm−1), N-H stretching of the primary amine group –NH2 (1632.97), asymmetric NCN stretch (1567.51 cm−1),CH3 asymmetric and symmetricde formations (1471.41,1471.62cm−1),C-Nstretch (1061.34), CHrock (935.09cm−1). The FTIR of physical mixture of Metformin HCl, fenugreek gum, and acrylamide indicatedthe peaks at respective wave numbers i.e. N-H stretching (3168.59cm−1), N-H deformation(1614.31 cm−1), asymmetric NCN stretch (1553.36cm−1), CH3 asymmetric, and symmetricdeformations (1421.96cm−1), C-N stretch (1056.22 cm−1), CH rock (960.14cm−1) and CNstretch 1350.51. There were no interactions among all the excipient. So, the excipient were compatible with each other.

Effect of Formulation variables:

Summary of Formulation and response variables and their levels in CCD were shown in Table 2.

According to Design expert software, best-fitted model was quadratic for response Y1, Y2 &Y3 (% of drug released in 1 hr, % of drug released in 8 h, time to 50% drug release) and to establish full model (FM) poly nomial equation:% drug released in 1hr = 11.37 + 0.530990792 * A -0.555630555 * B + 0.351 * A * B +1.3026875*A^2+1.3534375*B^2%drugreleasedin8hr=54.8-3.255835387*A+1.374859415*B+2.25875*A*B+ 1.7889375*A^2+2.0304375*B^2Time of 50% drug release = 7.8 +0.905330086 * A-0.125 * B-0.25 * A* B-0.65 * A^2-0.4*B2The analysis of variance (ANOVA) was determined as per the provision of this software for Response1 (%drug released in 1hr), Response 2(%drug released in 8hr),Response 3(time to 50% drug release) to estimate the significance of the model. Using 5% significance level, amodel is considered significant and the p-value is less than 0.05. The model (formulation) parameters are affecting the response variables were described in Table3.

IncaseY1, factors X1,X2, X1^2,X2^2 were found and their effect was found to benegativei. e the amount of gum and PVP-K30 increases, the drug release from SR tablets decreased. Asimilar effect was also observed in the case of response Y2. In the case of Y3, all studiedvariables, quadratic effect and interaction term were found to be significant. A high levelof factor X1shows a high level of T50 % at all the levels. Thus X2indicates the increasingamount of grafted gum in SR Metformin HCl, increases the viscosity of the medium which inturn decreases the water diffusion into tablets and thereby decreases the release rate and inturn increases the T50%. The effect of independent factors on all of the observed responsesurfaces formedhillsides with large curvatures confirms that they were typically influenced by the interaction effect of concentrations on dependent factors that were shown in Fig.2.

Evaluation of granules

The prepared granules were evaluated for pre-compression parameters such as angle ofrepose, bulk density, tapped density, carr's index, hausner's ratio and LOD were shown in Table 4. The angle ofrepose was found to be in the range 20.57±0.42 to 35.33±0.39. Bulk density was found tobe between 0.33±0.004 to 0.44±0.003gm/cm^3 and tapped density between 0.36±0.03 to0.50±0.01gm/cm^3 for all formulations. Hausner's ratio was found in a range of 1.07±0.01to 1.21±0.02, Carr's index was found in a range of 7.20±1.15 to 17.66±1.32 and LODwas found in a range of 0.44±0.01 to 2.9±0.02. All the batches have shown good toexcellent flow properties. Hence, tablets were prepared with these granules in combination bythe wet granulation method.

Evaluation of tablet

The formulated tablets were evaluated by post-compression parameters such as weight variation, thickness, hardness, friability, swelling index and drug content that were shown inTable5. Weight variation was found to be in the range between 993.0±2.31 to 1002±5.72mg. The thickness of all the batches was found to be between 6.01±0.01mm to6.16±0.05mm. The hardness of the tablet was found to be in the range of 5.63±0.07to6.04±0.04.The % friability was found to be 0.26±0.11 to 0.87±0.41 that below 1%indicating the friability is within the prescribed limits. The equilibrium water uptake was observed from the ranges 92.60% to 144.54% in phosphate buffer, which was increased with an increasing amount of grafted fenugreek gum, it may be due to presence of differenthydrophilic groups. It shows that metformin HCl containing grafted fenugreek gum exhibitedthe maximum swelling ratio, indicating a high degree of swelling due to water uptake. Thepercent drug content in tablet batches was found to be within the range of 96.83±1.75to99.75±0.25 indicated uniformity of mixing.

3.7Invitro release study

In vitro drug release study of different tablet batches were shown in Table 6 (a) & 6(b).
T2 batch has shown maximum release up to96.37±0.46 % within 24 hrs as compared toother batches. T2 gives a prolonged release in a controlledfashion than in comparison toother batches. As soon as the graft copolymer tablet interacts with aqueous media, waterdiffuses into the tablet thereby causing the swelling of the matrix and this swelling continues.The hydrostatic pressure is developed within the tablets. With increased swelling, decreasedsolubility of the grafted polymeric matrix, which gets depleted slowly with slow diffusion ofthe drug from the matrix. The formulation batch T2 shows sustained release properties byprolongedreleasemore than24hrsuniformly.

Optimization

Response surface methodology gaveus ten solutions where the result soft he physical evaluation and tablet assay were found within limits. Table 7 lists the number of solutionswith their predicted and experimental values of all response variables with percentage error.The formulation number TS9 was chosen as the best-optimized formulation for MetforminHCl, as the error was minimum for the response of the dependent variables. The comparisonsof predicted and experimental results showed very close agreement, indicating the success of the design combined with a desirability function.

In vitro release comparison study of optimized grafted tablet of Metformin HCl (TS9), ungrafted fenugreek gum tablet and marketed formulation

The comparison of the dissolution profile of optimized grafted Met form in HCl tablet formulation (TS9), ungrafted Metformin HCl tablet formulation, and marketed formulationMetformin HCl [GLUCOMET SR 500mg] were shown in Fig.3.It can be observed thatthe Metformin HCl tablet (GLUCOMET -SR) released 79.27% of the drug in 24hrs, while the tablet formulated using ungrafted fenugreek gum released only 99.8% of the drug within 4hrs.Further, it can be observed that the release rate of the drug from the tablet formulated withgrafted fenugreek gum (TS9) was sustained (92.41%) for 24h than the tablet formulated with ungrafted fenugreek gum. TS9 gives a prolonged release in a sustained fashion than Other formulation batches. Because when grafted MetforminHCl tablet interacts with aqueous media, water diffusesinto the tablet thereby causing the swelling ofthe matrix and this swelling continues and a monotonically hydrostatic pressure is developedwithin the tablets.With increasein swelling, decreased solubility of the graftedpolymeric matrix, the polymeric matrix gets depleted slowly with slow diffusion of the drugfrom the matrix. The optimized batch TS9 showed that the sample has longer polymeric chains leading tomores welling and delayed drug release.

3.10 Estimation of Biochemical parameters

3.10.1 Body weight

In Body weight, all the treatment groups exhibited reduction in body weight which were shown in Fig.4. The groups treated with the fenugreek grafted formulation at a lower (50 mg/kg), medium (75 mg/kg) and higher dose (100 mg/kg) exhibited attenuation in body weight from day 14. This is very important to note that medium dose have provided the same level of attenuation in body weight as higher dose. Hence medium dose can be used to further research work due to equal level of body weight same as highest dose. Data are mentioned as mean±SEM (n = 6). $^{\alpha}$ $p < 0.05$, $^{\beta}$ $p < 0.01$, $^{\gamma}$ $p < 0.001$ compared to the normal group (NC). ^{a} $p < 0.05$, ^{b} $p < 0.01$, ^{c} $p < 0.001$ compared to the diabetic group [EC(D)] and $^{\varphi}$ $p < 0.05$, $^{\varphi\varphi}$ $p < 0.01$, $^{\varphi\varphi\varphi}p < 0.001$ compared to the Metformin-treated group [Metformin+(D)].

3.10.2 Blood glucose level

Fasting blood glucose level was increased in diabetic rats (423.33± 28.15) after interval of diabetic study. Oral intervention of different doses (lower dose 50 mg/kg, medium dose 75mg/kg and higher dose 100 mg/kg) reduced blood glucose level from 289.16± 43.13to 248.12 ± 7.00mg/ dL in lower dose group, from 279.16± 21.11to 203.16± 5.00 mg/ dL in medium dose group and from 267.13± 10.22to 173.12 ± 10.11mg/dL in higher dose group in the experimental animals groups that were shown in Fig.5. It note that medium dose have provided the same level of attenuation in blood glucose level as higher dose. It concludes the effect of diabetic activity of grafted formulation of metformin HCl at medium dose has shown better effect as compared to higher dose or other dose.

3.10.3Total Serum cholesterol level

Cholesterol level was found to be significantly increased in experimental animals after 21 days. When treated with different grafted doses, the level of cholesterol in rats reduced. Medium dose (75mg/kg) of GFTS9 formulation produced maximum attenuating effect same as that of higher dose of formulation on cholesterol were shown in Table 9.

3.10.4 Triglycerides level

Triglycerides level was increased in experimental animals after 21 days. when the treatment is done with different grafted doses, the level of triglycerides in rat groups decreased.The medium dose (75mg/kg)

produced same attenuating effect as to higher dose. Hence, it is observed that medium dose shows best action

3.10.5 Effect of HDL (High Density Lipoprotein) Level

HDL level was decreased significantly in experimental animals after 21 days of treatment with different doses (lower, medium and higher). It significantly attenuated the elevated level of HDL in rats. Medium dose (75 mg/kg) of GFTS9 formulation produced maximum attenuating effects same as higher dose. Hence, it is observed that medium dose shows best action.

3.10.6 Effect of LDL(Low Density Lipoprotein)

Iranian formula LDL= TC/1.19 +TG/1.9–HDL/1.1-38orLDL= TC-(HDL+TG/5) LDL level was decreased significantly in experimental animals after 21 days on treatment with different doses (lower, medium and higher). It significantly attenuated the elevated level of LDL in rats. 500 mg/kg of GFTS9 formulation produced maximum attenuating effect same as higher dose. Hence, it is observed that 500mg/kg dose shows best action.

3.11 Estimation of antioxidant parameters (CAT, MDA, GST)

The level of CAT and GSH were decreased and MDA level were increased which were shown in Table 10. After administration of different grafted tablets at lower, medium and higher dose (25mg/kg, 75mg/kg and 100mg/kg) produced maximum attenuating effect. Medium level dose shows the best effect same as higher dose.

CCD design was used as part of quality by design tool to get optimized formulation containing amounts of grafted fenugreek gum (X1) and PVP K30 (X2) as formulation parameters. The statistical parameters with polynomial equations were calculated that confirmed the designing check point analysis (TS9) to formulation, containing 347.98 mg of grafted gum and 74 mg of PVPK 30. The comparison study of TS9 formulation with dissolution profile showed that the drug shows delayed action, due to longer polymeric chains, showing greater extent of swelling. Beside, % drug content uniformity was also calculated within range 84.96 to 99.19%. Then selected formulation (TS9) indicates the success of the design which follows Higuchi model (R^2=0.994) with Fickian Diffusion (n = 0.994) to best fit. The consequence revealed the drug was released via grafted tablet by sustain manner. The results of animal studies were conducted for 21 days, in which the Biochemical parameters (reduced body weight, glucose level, Cholesterol, Triglycerides, LDL, VLDL and increased HDL level)and antioxidant parameters (decreased the level of CAT and GSH and increased MDA) were observed.

4. Conclusion

In the present study, the graft copolymers of fenugreek gum were synthesized. After optimization of the grafting batch, designed the SR Metformin HCl tablet using these grafted copolymers. The selected tablet formulation (TS9) were indicating the success of the designcombined with a desirability function exhibited a better-sustained release action with graftedfenugreek gum as compare with un-grafted and marketed formulation by applying the animal design.

Declaration of Competing Interest

The author is responsible for the content and writing of this article.

Acknowledgements

The author is thankful to Sri Sukhmani Institute of Pharmacy, Derabassi for providing the support for this research work.

References

1. Pala S,GhorajaMK, Ghoshb S, Udayabhanua G. Flocculation properties of polyacrylamide graft e dcarboxy methyl guargum(CMG-g-PAM) synthesized by conventional and microwave assisted method. Journal of Hazardous materials 2011; 1580–1588. doi:10.1016/j.jhazmat.2011.06.083.
2. Berg M, Jeremy TL. The integration of metabolism. In T. L. Berg M. Jeremy, Biochemistry 2008; 5: 858-859.
3. Fujioka K. Pathophysiology of type 2 diabetes and the role of incretin hormones and beta-cell dysfunction. JAAPA. 2007; 3-8. doi: 10.1097/01720610-200712000-00001.
4. Abdulfatai B, Olokoba A, Olusegun Obateru, Lateefat B Olokoba. Type 2 Diabetes Mellitus: A Review of Current Trends. Oman Medical Journal. 2007; 27(4): 269-273. doi: 10. 5001/omj.2012.68.
5. Chandra Basak S, Senthil Kesevan S, Ramalingam M. Design and release characteristics ofsustainedrelease tablet containing met for min HCl.Brazilian Journal of Pharmaceutical Sciences. 2008; 477-483. doi:10.1590/S1516-93322008000300018.

6.Badsnta RK, Satyanarayan NV. Preparation and in vitro & in vivo evaluation of cephalexin matrix tablets. Brazilian Journal of Pharmaceutical Sciences. 2018;1-12.doi:http://dx.doi.org/10.1590/s2175-9790 2018000317277.
7. AnibandeepB, WuiTin S, Navjot. Formulationdevelopmentandoptimizationofsustained release matrix tablet of Itopride HCl by response surface methodology and itsevaluation of release kinetics. Saudi Pharmaceutical Journal. 2013; 201–213.doi:10.1016/j.jsps.2012.03.006.
8. Kenneth N, Parthasarathy N, Narendra C, Kalyani P. Development and evaluation of oralcontrolled release matrix tablets of lamivudine: optimization and in vitro-in vivo studies. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 95-101.https://www.researchgate.net/publication/339876280.
9. Cooper JM. Mechanisms of diabetic complications, Physiol Rev. 2013;137-188.
10. Malviya R, Sharma PK, Dubey SK.Microwave Controlled the green synthesis ofacrylamide graft copolymers of Azadirachita indica gum for the waste water management. Current Applied Polymer Science 2018;130-149.
11. Bal T & Swain S. Microwave assisted synthesis of polyacrylmide grafted polymeric blendof fenugreek seed mucilage-polyvinyl alcohol (FSM-PVA-g-PAM) and its characterization sastissue engineered scaff old and asa drug delivery device.DARUJournalofPharmaceuticalSciences.2019 ; https://doi.org/10.1007/s40199-019-00237-8
12. Sarkar D, Nandi G, Changder A, Hudati P, Sarkar S, Ghosh LK. Sustained release gastroretentive tablet of metformin hydrochloride based on poly (acrylic acid)-grafted-gellan. International Journal of Biological Macromolecules. 2017;137-148.
13. USP29-NF24. 2017; 28 (2)
14. Lachmman L, Liberman HA, Konig JL. The Theory and practice of industrial pharmacy. Vargheese publishing house, Bombay. 1991; 297-301.
15. Otsuka M, Gao J, Matsuda Y. Effect of Amount of Added Water During Extrusion-Spheronization Process on Pharmaceutical Properties of Granules. Drug Development and Industrial Pharmacy.1994; 2977-2992.
16. De Leersnyder F, Vanhoorne V, Bekaert H, Vercruysse J, Ghijs M, Bostijn N, De Beer T. Breakage and drying behaviour of granules in a continuous fluid bed dryer: Influence of process parameters and wet granule transfer. European Journal of Pharmaceutical Sciences. 2018; 223-232.
17. Kumar A, Singh K, Ahuja M. Xanthan-g-poly (acrylamide): Microwave-assisted synthesis, characterization and in vitro release behavior. Carbohydrate Polymers.2009; 76: 261-267.
18. Mandal S, Kumar Basu S, Biswanath Sa. Ca2+ ion cross-linked interpenetrating network matrix tablets of polyacrylamide-grafted-sodium alginate and sodium alginate for sustained release of diltiazem hydrochloride. Carbohydrate Polymers. 2010; 867-873.
19. Bhagwat Durgacharan A, Kolekar Vandana A, Nadaf Sameer J, Choudhari Prafulla B, More Harinath N, Killedar Suresh G. Acrylamide grafted neem (Azadirachta indica) gum polymer: Screening and exploration as a drug release retardant for tablet formulation. Carbohydrate Polymers. 2020; 229
20. Kaur N, Kishore L, Singh N. Attenuation of STZ- induced diabetic nephropathy by cucurbita Peo L. Seed extract chacterized by GCMS. Wiley Journal of Food Biochemistry. 2017; https://doi.org/1 0.1111/jfbc.12420.
21. Garud N, Garud A. Acute toxicity studies of Metformin Microsphere prepared by two different methods. Pelagia Research Library.2012; 604-609
22. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974; 20(4):470-5.
23. Dietz AA, Rubinstein HM. Laboratory note--standardization of the Ellman reaction. Clinical Biochem. 1972; 136-138.
24. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem. 1977; 882-4.
25. Gupta M, Bajaj KV, Katriya P, Yadav S, Kamal R, Gupta RS. Evaluation of antidiabetic and antioxidant activity of Moringa oleifera in experimental diabetes. Journal of Diabetes. 2012; 164–171
26. Rasha A, Al-Eisa, Hend M, Tag Mohamed Salah El 3Naggar, Heba MA, Abdelrazek 4 and Nahla S, El-Shenawy. Evaluation of Tribulus terrestris extracts relative to Metformin on oxidative stress and histopathology of the liver for diabetic male rats. Diabetology. 2022; 46–55. https://doi.org/10.3390 /diabetology3010004.
27. Asagba SO, Kadiri HE, Ezedom T. Biochemical changes in diabetic rats treated with ethanolic extract of Chysophyllum albidum fruit-skin. The Journal of Basic and Applied Zoology. 2019; https://doi.org/10.1186/s41936-019-0118-y 1-10.

Figure Legends:

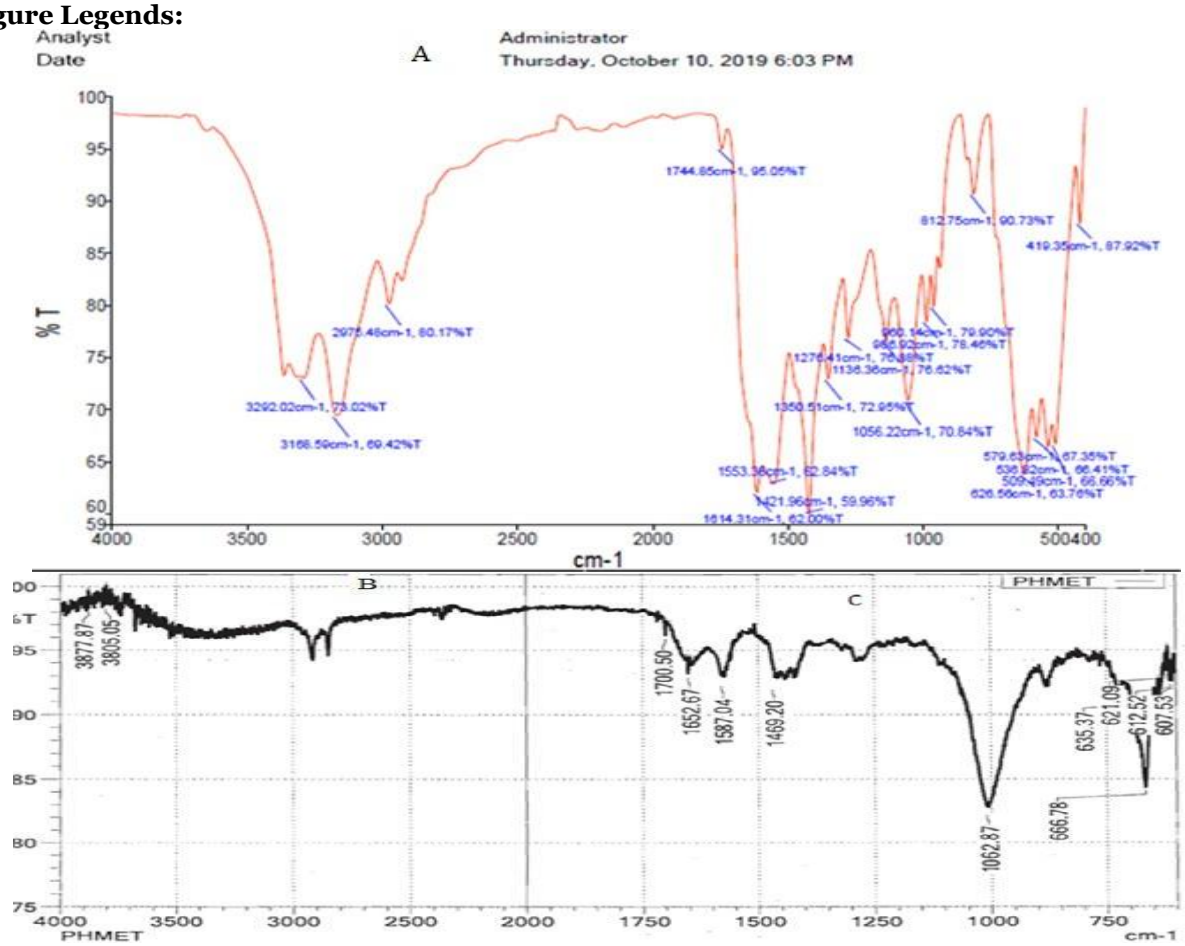

Fig.1(A) FTIR spectrum of Metformin HCl (B) FTIR spectrum of physical mixture (Metformin HCl, grafted fenugreek gum,PVPK30, MCC, Magnesium stearate andtalc)

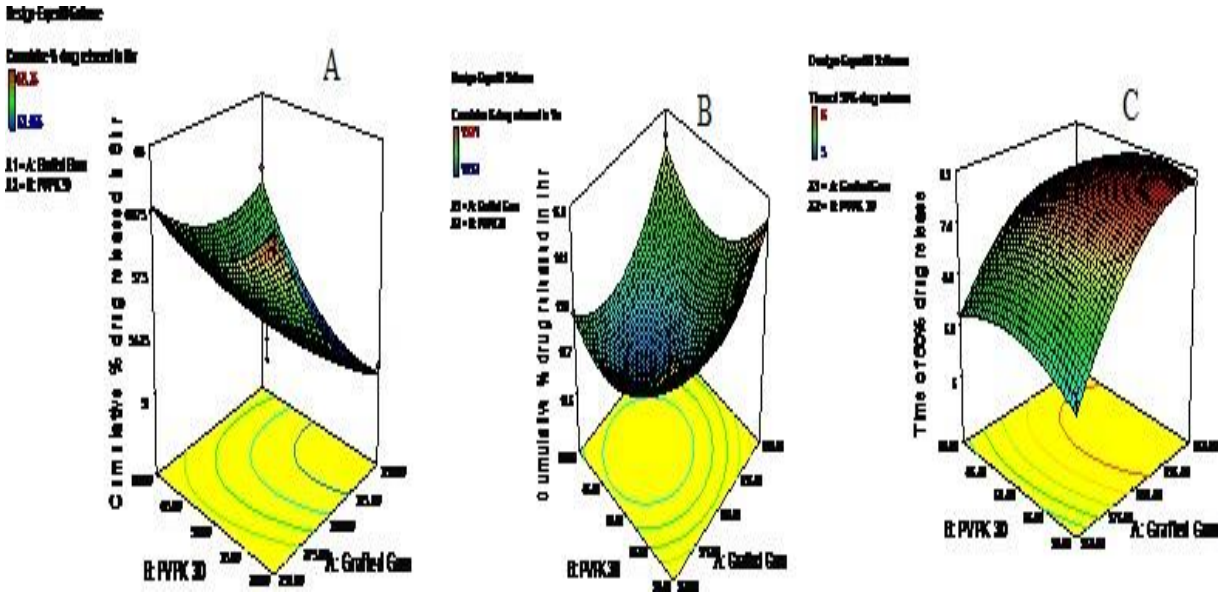

Fig.2. (A) 3D Response surface plot of% drug released in 1hr(B) % drug released in 8hrs (C) Time of 50% drug release

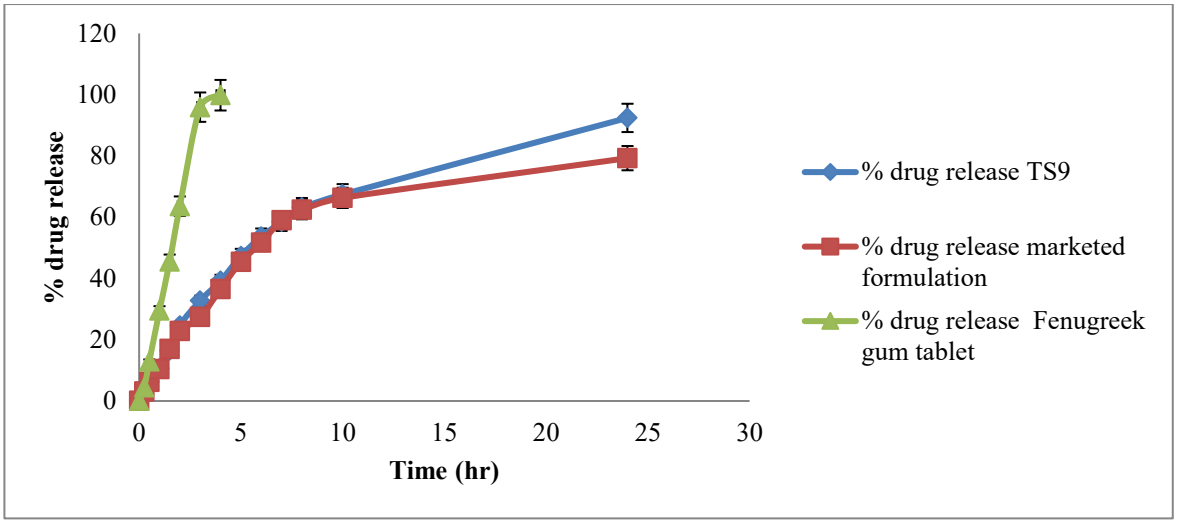

Fig.3.Percentage drug release of optimized fenugreek grafted tablet (TS9), marketed formulation and fenugreek gum tablets

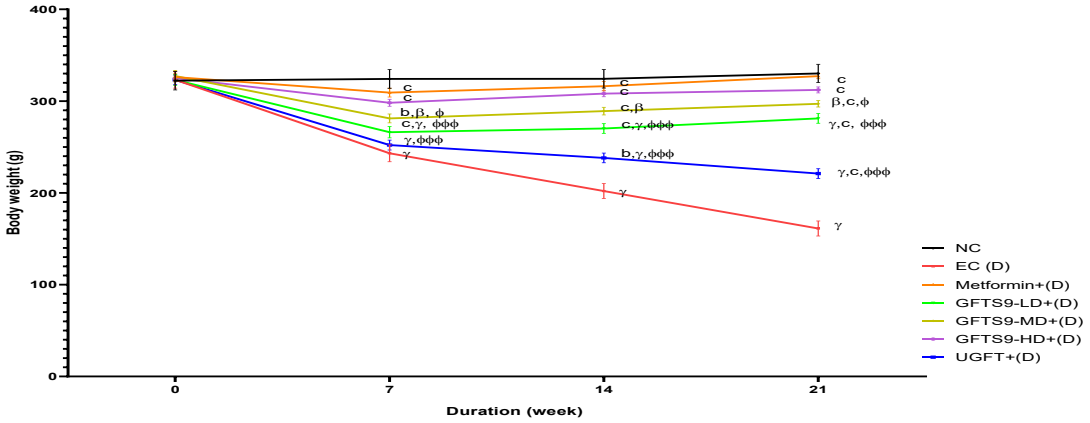

Fig.4. Effect on body weight
Fig.5. Effect on blood glucose level

Table 1:

Table1. Composition of SRM et formin HCl tablet formulation

Formulationcode	Drug(mg)	Graftedgum(mg)	PVPK30 (mg)	MagnesiumStearate (mg)	Talc(mg)	MCC (mg)	Total (mg)
TD1	500	229.29	50.00	10	10	200.00	1000
TD2	500	300.00	50.00	10	10	130.00	1000
TD3	500	300.00	50.00	10	10	130.00	1000
TD4	500	250.00	20.00	10	10	210.00	1000
TD5	500	370.71	50.00	10	10	59.29	1000
TD6	500	250.00	80.00	10	10	150.00	1000
TD7	500	350.00	80.00	10	10	50.00	1000
TD8	500	300.00	7.57	10	10	172.43	1000
TD9	500	300.00	92.43	10	10	87.57	1000
TD10	500	350.00	20.00	10	10	110.00	1000
TD11	500	300.00	50.00	10	10	130.00	1000
TD12	500	300.00	50.00	10	10	130.00	1000
TD13	500	300.00	50.00	10	10	130.00	1000

Table2:

Table2. Summary of Formulation and response variables and their level sin CCD

Formulation variables	Unit	Low	High	.-alpha	.+alpha
X1=Grafted Gum	Mg	250	350	229.29	370.71
X2=PVPK 30	Mg	20	80	7.57	92.43
Response variables					

Response variables	Unit
Y1= % drug released in 1hr	%
Y2= % drug released in 8hr	%
Y3=Time of 50% drug release	hrs

Table3:

Table3. Summary of ANOVA table for formulation variable from CCD % Drug released in 1hr

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	26.93	5	5.38	15.98	0.0010	significant
A-Grafted gum	2.25	1	2.25	6.69	0.0361	
B-PVPK 30	2.47	1	2.47	7.33	0.0303	
AB	0.49	1	0.493	1.46	0.2658	
A^2	11.80	1	11.80	35.03	0.0006	
B^2	12.74	1	12.74	37.82	0.0005	
Residual	2.35	7	0.33			
Lack of Fit	0.44	3	0.149	0.311	0.8178	not significant

% drug released in 8hrs

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	165.45	5	33.09	17.562	0.0008	Significant
A-Grafted gum	84.80	1	84.80	45.007	0.0003	
B-PVPK 30	15.12	1	15.12	8.026	0.0253	
AB	20.40	1	20.40	10.831	0.0133	
A^2	22.26	1	22.26	11.815	0.0109	
B^2	28.67	1	28.67	15.221	0.0059	
Residual	13.19	7	1.88			
Lack of Fit	2.64	3	0.88	0.334	0.8029	not significant
Pure Error	10.54	4	2.637			
Cor Total	178.64	12				

Time to 50% drug release

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	10.574	5	2.115	13.241	0.0019	significant
A-Grafted Gum	6.557	1	6.557	41.054	0.0004	
B-PVPK 30	0.125	1	0.125	0.783	0.4057	
AB	0.250	1	0.250	1.565	0.2511	
A^2	2.939	1	2.939	18.402	0.0036	
B^2	1.113	1	1.113	6.969	0.0334	
Residual	1.118	7	0.160			
Lack of Fit	0.318	3	0.106	0.530	0.6855	not significant
Pure Error	0.800	4	0.200			
Cor Total	11.692	12				

Table4:

Table 4. Characterization of different composition of grafted tablet granules(Mean±SD)

Formulation Code	Angleofrepose (θ)	Bulkdensity(g/cm3)	Tapdensity(g/cm3)	Hausner'sRatio (Mean±SD)	Carr'sindex(%) (Mean±SD)	LOD
TD1	26.49±0.56	0.44±0.03	0.50±0.01	1.12±0.01	11.28±1.40	2.12±0.02
TD2	24.02±0.31	0.34±0.05	0.38±0.07	1.09±0.02	8.92±2.22	1.03±0.01
TD3	23.18±0.21	0.37±0.07	0.40±0.01	1.08±0.01	7.77±1.18	1.05±0.01
TD4	33.18±0.15	0.38±0.06	0.44±0.02	1.14±0.02	12.44±1.67	0.86±0.03
TD5	30.20±0.56	0.33±0.04	0.37±0.03	1.12±0.01	11.05±0.95	0.44±0.01
TD6	33.20±0.24	0.37±0.05	0.45±0.17	1.19±0.03	15.95±2.63	1.72±0.07
TD7	25.24±0.20	0.35±0.05	0.38±0.01	1.08±0.01	7.83±1.19	2.39±0.04
TD8	25.95±0.40	0.33±0.06	0.40±0.01	1.21±0.02	17.66±1.32	0.64±0.02
TD9	35.33±0.39	0.41±0.05	0.46±0.07	1.12±0.02	11.04±2.29	2.55±0.01
TD10	30.22±0.23	0.35±0.07	0.41±0.06	1.17±0.03	14.47±2.78	2.90±0.02
TD11	21.47±0.19	0.35±0.05	0.39±0.01	1.10±0.02	9.66±1.60	1.07±0.02
TD12	20.57±0.42	0.35±0.06	0.38±0.07	1.07±0.01	7.20±1.15	1.12±0.02

TD13	23.03±0.12	0.33±0.03	0.36±0.03	1.11±0.06	10.31±0.44	0.95±0.01

Table5:

Table5.Characterizationofpostcompressionparametersoftablets

Sr. no.	Formulation Code	Weight variation (mg)	Thickness(mm)	Hardness(kg/cm²)	%Friability	SwellingIndex 0.1NHCl Content (pH1.2)	Phosphatebuffer (pH6.8)24h4h	%Drug
1	TD1	1000.6±4.9	6.06±0.03	5.63±0.07	0.53±0.305	19.47±0.03	92.60±0.05	98.00±0.901
2	TD2	995.6±3.44	6.10±0.02	5.76±0.13	0.65±0.13	26.89±0.02	129.63±0.01	99.75±0.25
3	TD3	993.5±2.07	6.06±0.03	5.82±0.06	0.51±0.118	29.45±0.04	127.33±0.09	98.33±0.87
4	TD4	1000.4±4.25	6.12±0.01	5.79±0.09	0.33±0.115	32.29±0.04	98.88±0.02	97.91±0.80
5	TD5	1002±5.72	6.16±0.05	6.00±0.02	0.51±0.102	30.29±0.06	129.23±0.01	99.66±0.52
6	TD6	993.5±3.31	6.05±0.02	5.85±0.04	0.87±0.418	23.01±0.04	86.57±0.01	98.75±0.50
7	TD7	999.8±4.37	6.04±0.03	6.04±0.04	0.63±0.058	21.20±0.05	144.54±0.07	96.83±1.75
8	TD8	1001.8±5.29	6.07±0.02	5.93±0.07	0.31±0.102	18.36±0.06	139.15±0.03	99.66±0.52
9	TD9	992.8±2.04	6.01±0.01	5.94±0.05	0.53±0.233	24.55±0.06	137.79±0.06	97.41±0.52
10	TD10	998.1±3.6	6.02±0.02	6.03±0.02	0.30±0.173	26.89±0.02	140.14±0.08	97.83±0.57
11	TD11	998.7±4.95	6.14±0.03	5.82±0.04	0.26±0.115	29.49±0.01	127.41±0.05	99.25±0.43
12	TD12	993.0±2.31	6.06±0.02	5.76±0.12	0.53±0.233	22.78±0.02	117.92±0.04	98.58±0.38
13	TD13	997.1±1.37	6.09±0.02	5.71±0.03	0.40±0.200	28.71±0.05	121.83±0.01	98.08±0.76

Table 6(a):

Table 6(a). Invitrodrugreleasestudyofdifferenttablet batches

Dissolution Media	Time(h)	%drug release TD1	%drug releaseTD2	%drug release TD3	%drug releaseTD4	%drug releaseTD5	%drugrelease TD6	%drugrelease TD7
0.1NHCl	0.25	0.82±0.10	1.87±0.05	1.23±0.07	1.63±0.07	3.73±0.03	5.88±0.07	3.14±0.01
	0.5	5.14±0.05	8.73±0.01	5.73±0.03	6.35±0.12	9.76±0.04	10.93±0.01	7.83±0.01
	1	13.42±0.06	11.59±0.01	10.52±0.01	14.04±0.06	14.64±0.05	12.64±0.07	14.60±0.02
	1.5	21.32±0.02	14.94±0.06	15.94±0.07	21.77±0.05	18.77±0.04	18.94±0.06	18.26±0.06
	2	32.50±0.34	20.46±0.06	21.46±0.03	27.34±1.08	25.34±0.35	24.47±0.46	23.61±0.23
pH6.8 PhosphateBuffer	3	40.53±0.20	25.75±0.53	26.75±0.41	33.73±0.18	34.37±0.24	29.75±0.41	32.84±0.49
	4	45.11±0.30	36.97±0.34	32.97±0.60	39.51±0.57	37.27±0.24	36.97±0.34	39.48±0.12
	5	50.94±0.11	45.65±0.26	40.65±0.26	45.03±0.77	40.07±0.45	45.65±0.26	43.11±0.68
	6	55.24±0.28	49.15±0.11	45.15±0.26	52.76±0.68	43.51±0.34	53.15±0.65	48.53±0.31
	8	63.75±0.68	56.65±0.30	52.65±0.06	62.01±0.36	53.02±0.58	60.65±0.70	59.73±0.41
	10	69.75±0.33	60.72±0.41	59.72±0.71	66.63±0.27	57.43±0.19	64.73±0.44	64.21±0.67
	24	82.51±0.48	96.37±0.46	94.37±0.69	84.12±0.88	89.58±0.41	97.38±0.35	96.98±0.21

Table 6(b):

Table 6(b). Invitrodrugreleasestudyofdifferenttablet batches

DissolutionMedia	Time(hr)	%drugrelease TD8	%drug release TD9	%drugrelease TD10	%drugrelease TD11	%drugrelease TD12	%drugrelease TD13
0.1NHCl	0.25	0.53±0.08	0.73±0.12	7.73±0.088	2.06±0.07	1.32±0.09	1.03±0.10
	0.5	6.74±0.04	6.76±0.03	10.76±0.07	9.35±0.07	6.54±0.11	4.01±0.07
	1	15.21±0.08	13.05±0.05	14.6±0.058	10.76±0.09	12.01±0.07	11.97±0.02
	1.5	21.73±0.06	16.21±0.06	22.77±0.43	17.26±0.07	16.42±0.07	15.43±0.05
	2	26.52±0.36	22.34±0.37	29.34±0.43	22.72±0.41	21.73±0.08	20.75±0.22
	3	35.63±0.64	31.37±0.21	38.37±0.28	30.32±0.68	29.05±0.56	24.98±0.57

Table 7:

Table 7.Comparison data of optimized formulation, predicted and experimental data

Number ofsolutions	Graftedgum	PVPK30	Predictedresponse %drugreleasedin1hr	Predictedresponse %drugreleasedin8hrs	Predictedresponse Time to 50%drugrelease	Observedresponse %drugreleasedin1hr	Observedresponse %drugreleasedin8hrs	Observedresponse Timeto 50% drugrelease	Percentageerror %drugreleasedin1hr	Percentageerror %drugreleasedin8hrs	Timeto50% drug release
TS1	273.97	40	11.82	57.12	7	10.56	63.12	6	0.89	4.23	0.78
TS2	264.09	33	12.53	58.83	7	10.78	66.07	5	1.24	5.11	1.17
TS3	271.69	55	11.39	57.29	7	13.76	58.81	6	1.67	1.07	0.75
TS4	302.92	21	13.21	55.07	8	14.32	59.54	7	0.79	3.15	0.42

TS5	283.06	56	11.26	56.29	7	10.02	51.98	8	0.87	3.04	0.42
TS6	299.19	77	11.93	57.64	7	15.56	53.32	8	2.56	3.05	0.45
TS7	251.86	42	12.42	59.98	6	16.89	57.11	7	3.15	2.02	0.52
TS8	307.02	78	12.19	57.76	7	14.92	55.78	7	1.93	1.40	0.29
TS9	**347.98**	**74**	**13.74**	**57.35**	**8**	**13.61**	**56.01**	**8**	**0.09**	**0.94**	**0.32**
TS10	262.49	59	11.58	58.32	7	12.96	56.02	6	0.97	1.62	0.52

PhosphateBuffer pH6.8	4	39.42±0.04	38.27±1.05	42.27±0.48	34.12±0.50	34.11±0.56	30.52±0.58
	5	43.82±0.31	43.07±0.49	45.07±0.12	40.18±0.40	43.85±0.50	42.53±0.54
	6	49.25±0.56	48.51±0.36	47.51±0.45	45.53±0.77	48.74±0.74	47.35±0.49
	8	57.21±0.36	60.52±0.59	55.43±0.47	53.75±0.53	55.97±0.71	54.98±0.75
	10	62.65±0.42	65.43±0.16	55.43±0.34	59.63±0.69	61.97±0.36	61.97±0.53
	24	82.62±0.51	80.58±0.42	92.58±0.30	95.12±0.68	96.01±0.28	95.84±0.69

Table9:

Table 9.EffectofformulationsonTotalCholesterolinstreptozotocininduceddiabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
Total cholesterol	112.12 6.66 6	261.15 6.19 6	148.1 5.62 6	194.16 6.53 6	179.17 6.18 6	166.12 6.08 6	238.14 6.72 6

Effectof formulationsonTriglycerideslevelinstreptozotocindiabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
Serum Triglyceride	76.12 5.22 6	171.22 4.27 6	85.26 4.16 6	134.18 4.11 6	111.22 6.11 6	97.23 6.96 6	150.19 7.12 6

Effectof formulationsonHDLinstreptozotocininduceddiabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
HDL (mg/dL)	46.13 2.11 6	23.16 3.56 6	41.16 2.16	32.18 1.17 6	36.18 2.43 6	39.14 2.26	28.17 2.26

EffectofformulationsonLDLinstreptozotocininduceddiabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
LDL cholesterol (mg/dL)	54.13 0.82 6	164.12 2.81 6	66.11 1.93 6	98.12 2.19 6	81.12 2.57 6	71.03 2.13 6	154.23 3.06 6

Table 10: Antioxidant parameters

Table 10. Effect of formulation son CAT instreptozotocin induced diabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
CAT	87.06 2.81 6	29.12 2.81 6	81.14 1.93 6	42.19 2.11 6	70.14 2.61 6	74.03 2.66 6	30.11 2.03 6

Effect of formulation son GSH instreptozotoc in induced diabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
GSH (/mg of protein)	54.22 1.78 6	19.06 1.32 6	49.23 1.19 6	32.16 1.41 6	43.21 1.43 6	47.11 1.1 6	24.23 1.06 6

Effect of formulation son MDA instreptozotoc in induced diabeticrat

	NC	EC (D)	Metformin+(D)	GFTS9-LD+(D)	GFTS9-MD+(D)	GFTS9-HD+(D)	UGFT+(D)
MDA (nmol/mg of protein)	54.22 1.78 6	19.06 1.32 6	49.23 1.19 6	32.16 1.41 6	43.21 1.43 6	47.11 1.1 6	24.23 1.06 6