ORIGINAL ARTICLE

Formulation of Glipizide Tablets using Fenugreek Seed Mucilage: Optimization by Factorial Design

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Abstract

Introduction: Fenugreek seed has antidiabetic activity. It also contains a major proportion of mucilage. This research work aims to develop glipizide tablets using fenugreek seed mucilage (FSM). **Materials and Methods:** The mucilage was extracted by double distilled water and precipitated with ethanol. The tablet utilizing the mucilage was prepared by wet granulation method. Full 3^2 factorial design was applied to study the effect of amount of FSM (X_1) and amount of microcrystalline cellulose (X_2) on release of drug at 1 h, Q_1 (Y_1), at the end of 12 h, Q_{12} (Y_2), and time taken to release 50% of drug, $t_{50\%}$ (Y_3). Diabetes was induced in Wistar albino rats using streptozotocin, and effect of formulation on blood glucose level was determined. **Results and Discussions:** All evaluated parameters for tablet were found within the limits of the United States Pharmacopeia NF 24/19. Fourier transform infrared spectrum reveals that there is no incompatibility between the ingredients. It was observed that the selected independent factors significantly affect the dependent responses. Independent factors, X_1 and X_2, have a negative and positive effect on the dependent responses, respectively. Formulation brought blood glucose level to normal at the end of the antidiabetic study, however, group treated with glipizide alone was still showing elevated blood glucose level. **Conclusion:** It can be concluded that FSM can be used to formulate glipizide tablets and formulation shows better hypoglycemic activity than glipizide alone. This may be attributed to the antidiabetic activity of fenugreek seeds itself.

Key words: Diabetes, factorial design, fenugreek seed, glipizide, mucilage

INTRODUCTION

Polymers used in the manufacturing of any formulation affect the stability of formulation and therapeutic efficacy of the active pharmaceutical ingredient.[1] Polymers can be obtained from synthetic, semi-synthetic, and natural sources. Nowadays, natural gums and mucilages have gained popularity over synthetic and semi-synthetic polymers due to their low cost, safety, and availability. Natural gums and mucilages have been used for the preparation of tablets. Fenugreek seeds, methi in Hindi, are obtained from *Trigonella foenum graecum L.* belonging to Leguminosae or Fabaceae family. These seeds commonly available and are used as spice and preservatives. Activities such as immunostimulatory, antidiabetic, antihypertensive, and cholesterol-lowering ability are reported in literature. Glipizide is extensively and widely used for management of glucose level in Type 2 diabetic patients. Glipizide is sulfonyl urea antidiabetic agents, having pKa 5.9, partially soluble in water and falls in class II category of drugs according to the Biopharmaceutical Classification System.[2]

Designs of traditional pharmaceutical formulations are based on changing one variable at a time approach. It consumes time and does not consider the interaction between the variables. Factorial design and analysis of the response are considered as powerful, efficient, and systematic tool in optimization. It develops dosage forms in lesser time and improves research and development work.[3]. Many literatures reveal the application

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of factorial design by researchers for development of dosage forms.[4,5] In the current study, we aim to develop glipizide tablets utilizing FSM by applying 3^2 full factorial designs. The effect of amount of FSM (X_1) and microcrystalline cellulose (MCC) (X_2) was selected as independent variables while the percentage of drug released in 1 h, Q_1 (Y_1), percentage of drug released in 12 h, Q_{12} (Y_2), and time required for 50% of drug release, $t_{50\%}$ (Y_3) were selected as dependent variables.

MATERIALS AND METHODS

Chemical and reagent

Glipizide was a gift from Dishman Pharmaceuticals and Chemicals Ltd, Ahmedabad. MCC, Talc, and Magnesium Stearate were procured from SD Fine Chemicals Ltd., India. All the other chemicals used were of analytical grades.

Extraction of mucilage

The collected fenugreek seed was washed with distilled water to remove any adherents. The seed was grinded with small amount of water using mixer grinder to form slurry. The slurry thus prepared was precipitated with ethanol with continuous stirring with mechanical stirrer. The precipitate was then washed with ethanol for three times and dried at 401°C ± 1°C. The dried material was grinded using a mechanical grinder and passed through # 60 mesh sieve. The powdered mucilage was stored in a desiccator till further use.

Full factorial design

Full factorial design taking 2 factors at 3 levels was used for development of tablet. According to the model, 9 experiments were conducted varying both the factors at same time. This factorial design involves independent variables X and dependent variables Y. The two independent variables selected for this study were amount of FSM (X_1) and amount of MCC (X_2). Each independent variable was varied at 3 levels. The levels of independent variables are shown in Table 1. The dependent variables selected were the amount of drug released in 1 h, Q_1 (Y_1), amount of drug released in 12 h, Q_{12} (Y_2), and the time required to release 50% of drug, $t_{50\%}$ (Y_3). The result of factorial design can be expressed either as:

Simple linear equation:

$$Y_i=b_0+b_1X_1+b_2X_2+b_3X_3 \quad (1)$$

Or

Second order polynomial equation:

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (2)$$

Where, Y_i is the dependent variable, b_0 is mean response, b_1, b_2 are the determined coefficient of main factors X_1 and X_2, respectively, b_{12} is the coefficient of interaction of X_1 and X_2, b_{11}, b_{22} are the coefficient of polynomial terms X_1^2 and X_2^2 respectively, which were included to check non linearity. The code of independent variables and dependent variables are shown in Table 1.

Preparation of tablets

Nine different formulations (F1-F9) were prepared using a different proportion of FSM and MCC [Table 2] using wet granulation method. FSM powder was sieved through sieve no. 22 separately and mixed with glipizide and MCC. The mixture was granulated using sufficient distilled water. The granules were dried in a tray drier at 50°C. The granules were passed through sieve no. 20 and then lubricated using talc and magnesium stearate (1:1). The granules were compressed on 10 – station tablet compression machine (Shakti Machineries, India) using 10 mm punches. Full factorial design layout and the amount of FSM and MCC are shown in Table 2.

Evaluation of prepared tablets

Thickness

The thickness of five tablets was determined using a Vernier caliper and average was noted.

Weight variation test

20 tablets were weighed using electronic balance (Denver APX–100, Arvada, Colorado) and test was performed as per the official method of the United States Pharmacopeia (USP).

Drug content

Five tablets were weighed individually. The drug was extracted in phosphate buffer pH 7.4. The absorbance of the extract was recorded using a UV spectrophotometer (1800, Shimadzu, Japan) at 274 nm. The concentration of drug extracted and then drug content was determined using standard calibration curve.

Table 1: Full factorials design codes

Independent variables (factor)			Dependent variables (response)
Levels	Factors		
	X_1	X_2	
−1	40	150	Y_1=Percentage drug release at 1 h (Q_1)
0	80	200	Y_2=Percentage drug release at 12 h (Q_{12})
+1	120	250	Y_3=Time required for 50% drug release ($t_{50\%}$)

X_1: Amount of FSM (mg); X_2: Amount of MCC (mg).
FSM: Fenugreek seed mucilage, MCC: Microcrystalline cellulose

Hardness and friability

Hardness and friability of 5 and 20 tablets, respectively, of each formulation was determined using the Monsanto hardness tester (Cadmach, Ahmedabad, India) and Roche friabilator (Campbell Electronics, Mumbai, India), respectively.

Drug polymer compatibility studies

Drug polymer compatibility study was carried out using IR spectrophotometer (IR Affinity, Shimadzu, Tokyo, Japan). Prepared tablets were compressed with potassium bromide and transformed into disk. The disk was placed at the center of sample holder and scanned between 4000/cm and 400/cm at resolution 4/cm. The spectrum of formulation and glipizide [Figure 1] was compared to determine drug polymer compatibility.

In vitro release studies

Release of glipizide from tablets was studied in USP Apparatus II (Veego, Kolkata, India), paddles at 100 rpm and 37°C ± 0.5°C. Dissolution was carried out using phosphate buffer pH 7.4 as dissolution medium. 5 ml samples were withdrawn at every 5 min and analyzed using the UV spectrophotometer (UV – 1800, Shimadzu, Japan) 274 nm. Dissolution study was carried out in triplicate and average was considered. Percentage of drug release was calculated using PCP Disso v2.08 software.

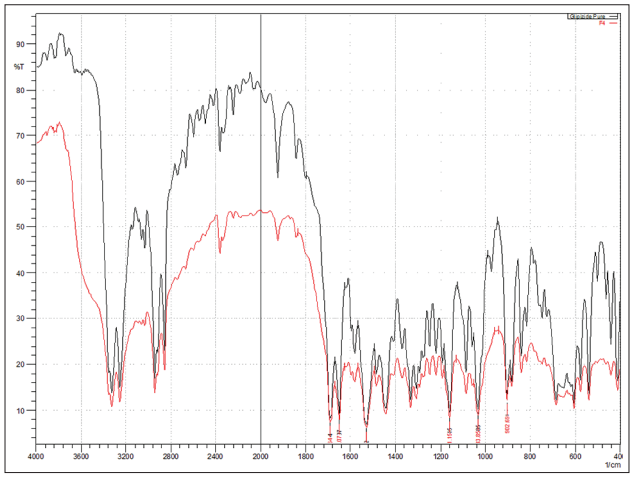

Figure 1: Comparison of Fourier transform infrared spectrum of pure glipizide and formulation

Effect on blood glucose level

The animal study was approved by the Institutional Animal Ethical Committee (CIP/IAEC/2015–16/070). Wistar albino rats (150-250 g) were used for this study. Solution of streptozotocin at 60 mg/kg body weight was prepared using freshly prepared citrate buffer pH 4.5. This solution was injected intraperitoneally to induce diabetes in rats. The animals were divided into four groups (n = 6): Group I was treated as normal group, normally fed, diabetes neither induced nor treated; Group II was treated as diabetic control group only with vehicle; Group III was treated with 5 mg/kg glipizide administered orally; Group IV was treated with formulation F7 containing 3 mg/kg equivalent of glipizide.

Statistical analysis

Data were statically evaluated using one-way analysis of variance (ANOVA). Wherever the ANOVA values were found to be significant, Duncan's new multiple range test was applied (SPSS statistics software). The values were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Nowadays, diabetes is considered as endemic disease. In its 2014 global report, the World Health Organization has reported that 1.5 million people died of diabetes alone. 422 million people were suffering from diabetes in 2014 compare to 108 million in 1980.[6] The aim of this research work was

Table 2: Factorial design layout with coded levels and their responses

Batch number	Independent variables		Actual responses			Predicted response			% Relative error		
	X_1	X_2	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
F1	−1	−1	20.22	86.47	4.84	19.06	86.14	4.80	−6.09	−0.38	−0.79
F2	−1	0	24.09	92.11	5.01	23.89	92.06	5.07	−0.85	−0.05	1.16
F3	−1	+1	24.49	97.13	5.31	25.85	97.50	5.29	5.27	0.38	−0.40
F4	0	−1	6.53	81.74	4.61	6.43	82.70	4.62	−1.56	1.16	0.26
F5	0	0	15.58	89.47	4.86	14.82	88.96	4.87	−5.12	−0.57	0.18
F6	0	+1	19.49	95.19	5.09	18.34	94.74	5.07	−6.27	−0.47	−0.42
F7	+1	−1	3.85	80.76	4.29	3.51	80.13	4.32	−9.69	−0.79	0.59
F8	+1	0	7.09	86.18	4.61	8.05	86.74	4.54	1.94	0.64	−1.49
F9	+1	+1	10.35	92.79	4.68	11.13	92.86	4.72	7.02	0.08	0.89

to formulate and evaluate glipizide tablets containing FSM applying 3^2 full factorial designs. FSM was extracted using cold maceration technique and mucilage was precipitated using ethanol. A statistical 3^2 full factorial design including interactive and polynomial terms were used to evaluate the responses. Two factors, amount of FSM (X_1) and amount of MCC (X_2), were coded and varied at 3 levels [Table 1]. The amount of drug released in 1 h, Q_1 (Y_1), amount of drug released in 12 h, Q_{12} (Y_2), and the time required to release 50% of drug, $t_{50\%}$ (Y_3) were selected as dependent responses. Glipizide tablet was formulated by wet variation method. The prepared tablets were subjected various evaluation parameters. Average thickness of tablets varies from 4.11 ± 0.03 to 5.04 ± 0.03 mm. Deviation in weight variation test and friability is well within the permission limit of tablets as per USP. Average hardness of the tablet was found to be 5.3 ± 0.11 to 5.8 ± 0.57 kg/cm^2. Results of various parameters are shown in Table 3.

Drug polymer compatibility was determined by comparing Fourier transform infrared spectrum of pure glipizide and formulations. The characteristic peaks of glipizide 1528/cm, 1690/cm, 1650/cm, 1159/cm, 1032, and 900/cm were observed both in pure glipizide sample[7] and the formulations [Figure 1]. This indicates that no chemical incompatibility between glipizide and the excipients used exists.

In vitro drug release study was done using phosphate buffer pH 7.4. The samples were withdrawn in 1 h interval for 12 h and fresh phosphate buffer pH 7.4 was added to maintain the sink condition. The withdrawn sample was then analyzed using the UV spectrophotometer at 274 nm. The result of dissolution study is shown in Figure 2a and b. The *in vitro* release study of the 9 batches showed a variation (drug released in 1 h, Q_1, ranges 3.85-24.49%, drug released at the end of 12 h, Q_{12}, ranges from 80.76% to 97.13%, and time taken to release 50% drug, $t_{50\%}$, ranging from 4.29 to 5.31 h). The responses of the dependent factor are shown in Table 2. The data indicate that the release pattern of the drug is dependent on selected independent factors. A low relative error value indicates that there exists a reasonable agreement between the independent and dependent factors. This establishes the validity of model and ascertains the effects of selected independent factors on dependent factors.

The fitted equation of full and reduced models, relating the experimental responses with the transformed factors are shown in Table 4. Conclusion can be drawn using polynomial equation. Magnitude of coefficient of polynomial equation and its sign (positive or negative) is also considered for drawing conclusion. ANOVA was performed to identify insignificant factors [Table 4]. For all the responses, level of significance of coefficient b_{12}, b_{22}, and b_{11} was more than 0.05 and hence insignificant, whereas

Table 3: Properties of compressed tablets

Batch code	Thickness* (mm)	Deviation in weight variation† (%)	Drug content* (%)	Hardness* (kg/cm^2)	Friability† (%)
F1	4.22±0.04	2.89±0.04	94.39±0.03	5.5±0.21	0.54±0.02
F2	4.21±0.05	2.14±0.03	96.44±0.02	5.3±0.11	0.67±0.04
F3	4.33±0.03	1.98±0.02	97.29±0.02	5.6±0.18	0.48±0.07
F4	3.98±0.02	2.16±0.01	97.67±0.03	5.4±0.37	0.39±0.04
F5	4.18±0.04	2.23±0.02	97.58±0.05	5.5±0.26	0.67±0.03
F6	4.37±0.01	2.26±0.04	98.26±0.09	5.8±0.57	0.51±0.03
F7	4.11±0.03	2.87±0.03	97.67±0.04	5.7±0.22	0.28±0.07
F8	4.48±0.02	2.68±0.03	98.62±0.11	5.7±0.34	0.36±0.10
F9	5.04±0.03	2.17±0.02	97.28±0.18	5.6±0.27	0.29±0.09

*All values are expressed as mean±SE, *n*=5. †All values are expressed as mean±SE, *n*=20. SE: Standard error

Table 4: Summary of regression analysis of measured response

Regression coefficient	Y_1				Y_2				Y_3			
	FM	P	RM	P	FM	P	RM	P	FM	P	RM	P
b_0	14.82	0.005	14.63	-	88.96	-	89.09	-	4.87	-	4.81	-
b_1	–7.92	0.005	–7.92	-	–2.66	0.005	–2.66	-	–0.26	0.002	–0.26	-
b_2	3.96	0.036	3.96	0.005	6.02	-	6.02	-	0.22	0.004	0.22	-
b_{12}	0.56	0.705	-	-	0.34	0.494	-	-	–0.02	0.587	-	-
b_{22}	–1.43	0.504	-	-	–0.24	0.726	-	-	–0.02	0.651	-	-
b_{11}	1.15	0.586	-	-	0.44	0.531	-	-	–0.06	0.267	-	-

FM: Full model, RM: Reduced model, P: The significance level, -: Value not calculated

level of significance of coefficient, b_0, b_1, and b_2, was found to be significant at $P < 0.05$. Insignificant levels of coefficient were omitted from full models and significant levels of coefficient were retained to generate reduced models. The reduced model was tested in portions to determine if the insignificant coefficients, b_{12}, b_{22}, and b_{11}, contribute significantly to the prediction of dependent responses.

The summary of models test in portion is shown in Table 5. At $\alpha = 0.05$, the calculated values of F is less than its critical values. It can be concluded that the terms X_1X_2, X_2^2, and X_1^2 do not contribute significantly to the prediction of dependent responses. The result of regression analysis of all the responses Y_1, Y_2, and Y_3 showed that the coefficient b_1 bears a negative sign, whereas b_2 bears a positive sign. It can be concluded that increasing the concentration of X_1 (amount of FSM) is expected to decrease the value of responses and increasing the concentration of X_2 (Amount of MCC) is expected to increase the value of responses.

The relationships between the independent and dependent factors are further elucidated by using contour plot [Figure 3a-c] and response surface plots [Figure 4a-c]. On analyzing the contour plot and response surface plots, it

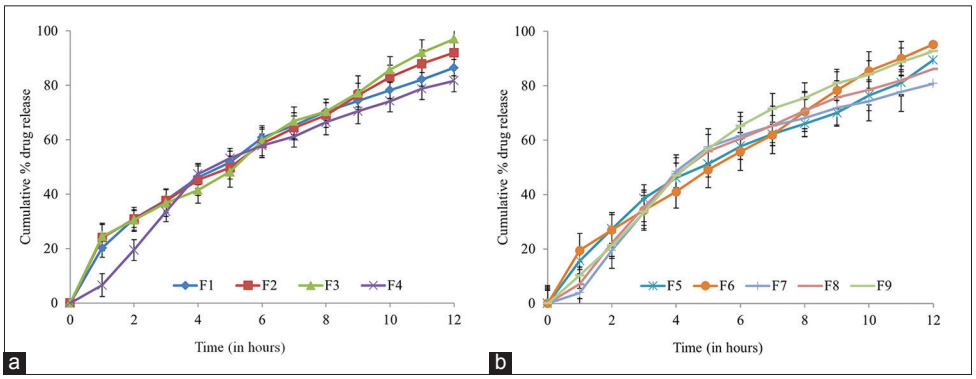

Figure 2: *In vitro* release profile of formulations, (a) F1-F4, (b) F5-F9

Table 5: Summary of models tests in portions

	DF	SS	MS	*F*	R^2	*P*
For percentage drug release at 1 h (Q_1), Y_1						
Regression						
FM	5	478.03	95.61	13.38	0.9571	0.0290
RM	2	470.05	235.03	47.94	0.9411	0.0002
Error						
FM	3	21.44	7.15			
RM	6	29.42	4.90			
For percentage drug release at 12 h (Q_{12}), Y_2						
Regression						
FM	5	261.21	52.24	67.11467	0.9911	0.002809
RM	2	260.24	130.12	236.09	0.9875	0.00000197
Error						
FM	3	2.34	0.78			
RM	6	3.31	0.55			
For time required for 50% drug release ($t_{50\%}$), Y_3						
Regression						
FM	5	0.73	0.15	33.40	0.9824	0.007835
RM	2	0.72	0.36	90.34	0.9679	0.0000332
Error						
FM	3	0.01	0.00			
RM	6	0.02	0.00			

DF: Degree of freedom, SS: Sum of squares, MS: Mean of squares, R^2: Regression coefficient, FM: Full model, RM: Reduced model, *P*: Significance *F*

can be concluded that increasing the amount of FSM had a negative effect on % drug release at 1 h, % drug release at the end of 12 h and $t_{50\%}$. On the other hand, increasing the amount of MCC leads to increase in values of dependent responses.

Blood glucose levels of rats are shown in Table 6. Diabetes was induced by single intraperitoneal injection of streptozotocin at 60 mg/kg. Blood glucose level of Group I was normal (79.57 ± 3.89) on the day of commencement and remained normal throughout the completion of the experiment. Group II was treated as diabetic control and did not receive any treatment. It exhibited hyperglycemia on the first day and continued to be hyperglycemic throughout the study. Group III rats were administered with glipizide and blood glucose level was hyperglycemic (337.74 ± 4.57) on day one. However, it was reduced to nondiabetic levels on completion of the experiment. Hyperglycemic status of Group IV rats was reduced to normal on completion of experiment. It was observed that the formulation was exhibiting promising result and could reduce elevated glucose level to lower levels than in Group III. This may be due to the fact that fenugreek seed has antidiabetic activity and the mucilage may have acquired the property. This could have potentiated the activity of glipizide.

CONCLUSION

It has been demonstrated in this study that FSM can be used as binder for formulation of glipizide tablets. The formulation F7 exhibits better hypoglycemic effect than the pure glipizide. This can be due to the fact that fenugreek seed also possesses antidiabetic activity. The mucilage may have acquired hypoglycemic activity that potentiated the activity. This calls for recognizing the dual role of mucilage, excipients, and potentiating of antidiabetic activity. However, it is suggested that human studies with the mucilage and combinations with glipizide must be

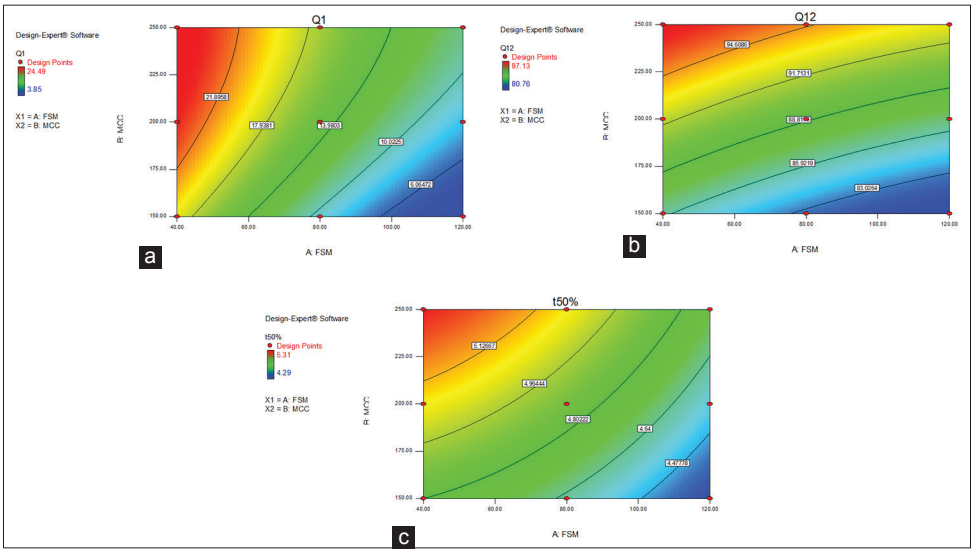

Figure 3: (a-c) Contour plot for response Q_1 (Y_1), Q_{12} (Y_2), and $t_{50\%}$ (Y_3), respectively

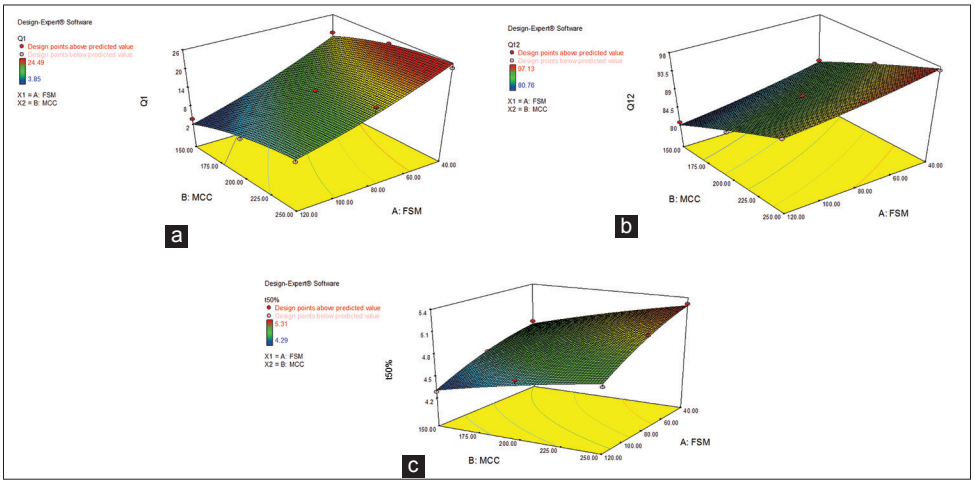

Figure 4: Response surface plots for (a) Q_1 (Y_1), (b) Q_{12} (Y_2), and (c) $t_{50\%}$ (Y_3)

Table 6: Effect on blood glucose level of the experimental groups

Treatment	Blood glucose profile post treatment (mg/dl) (day)				
	0	7	14	21	28
Group I	79.57±3.89	80.36±4.24	81.55±5.27	80.17±2.87	82.58±2.45
Group II	356.48±3.87	401.25±3.31	410.59±4.14	423.55±4.12	434.35±3.98
Group III	337.74±4.57	248.55±2.19	213.28±3.36	178.65±2.28	126.21±2.29
Group IV	329.24±3.36	276.38±2.76	193.82±4.12	121.22±3.14	87.62±4.18

Values are mean±SE from 6 rats of each group. All values are significant at $P<0.05$ compared with Group II (diabetic control). SE: Standard error

studied further. This may lead to the development of useful formulation with lesser amount of oral hypoglycemic agents and thereby better therapeutic applications and lesser side effects.

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