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DESIGN, FORMULATION AND EVALUATION OF GASTRO RETENTIVE FLOATING TABLETS OF NATEGLINIDE

Abhishek Yadav[1]*, Pranav Kumar Upadhyay[2] and Rajeev Shukla[3]

[1]Research Scholar Department of Pharmacy,

[2]Department of Pharmacy Dean (HOD) Sharswati Higher Education and Technical College of Pharmacy Varanasi.

[3]Department of Pharmacy Director Sharswati Higher Education and Technical College of Pharmacy Varanasi.

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***Corresponding Author**
Abhishek Yadav
Research Scholar
Department of Pharmacy.
Sharswati Higher Education and Technical College of Pharmacy Varanasi.

ABSTRACT

The global presence of diabetes has been rising day by day and it accounts for endangering 6.6% of the total population of the world. The increase in the blood glucose levels is the basic criterion of describing diabetes. It is also symptomized by glycosuria, hyper lipidaemia, ketonemia, etc. Insulin and oral hypoglycaemics are the two most widely used categories of drugs utilized for treatment of diabetes. Oral hypoglycaemics is the class of drugs associated with lowering the glucose levels, but many of them suffer from the drawback of smaller half lives, short residence time. The gastroretentive dosage forms help in overcoming these drawbacks and also increase the bioavailability of the drug administered. The gastroretentive dosage form has shown a better potential keeping in mind the research aspect as well as the commercial aspect of the formulation. Gastroretentive formulations consisting of oral hypoglycaemics have found a vast market and have been formulated and marketed by various MNCs. The present article tries to describe all the recent advances made and the current market status of the aforesaid technology.

KEYWORDS:- Gastroretentive, Hypoglycemics, Nateglinide, Glinate, Bio polymer chitosan.

1. INTRODUCTION

Diabetes mellitus (DM) is a term that refers to a variety of conditions defined by high blood glucose levels caused by an unbalanced oxidation and use of glucose, which is linked to the failure of insulin-producing pancreatic -cells. Diabetes is caused by a malfunction in insulin secretion (insufficient insulin synthesis), a decline in peripheral insulin response (insufficient insulin sensitivity of cells), or both.[1] The intensity of diabetic symptoms is determined by the kind of diabetes and how long it has been present. The majority of patients, especially those with TIIDM, are asymptomatic in the early stages of the condition. Patients may develop polydypsia, polyphagia, polyuria, ketoacidosis, and weight loss in addition to hyperglycemia. The severity of hyperglycemia increases the risk of microvascular consequences like diabetic neuropathy, retinopathy, and nephropathy, as well as macrovascular problems like ischemic heart disease, peripheral vascular disease, and so on.[2-5]

In the previous 16 years, the global prevalence of diabetes has climbed by 211 million people at an alarming rate. In 2017, an estimated 425 million persons aged 20 to 79 were diagnosed with diabetes, with that number anticipated to rise to 629 million by 2045. Diabetes has evolved over the last 30 years from a minor ailment of the elderly to one of the leading causes of morbidity and mortality among the young and middle-aged.[6,7]

2. MATERIALS AND METHODS

Nateglinide was collected from Sigma Aldrich, St.Louis, USA, as a gift sample.Chitosan bio polymer powder was collected from Sigma Aldrich, St.Louis, USA, as a gift sample.

Chemicals used during experiments

S. No	Chemicals	Manufacturers Name
1.	Nateglinide	Sigma Aldrich, St. Louis, USA.
2.	Chitosan	Sigma Aldrich Chemicals Pvt. Ltd.
3.	Chloroform, ethyl acetate	Dr. Reddy's Laboratories Ltd., India.
4.	Molish reagent, Fehling's reagent, Benedict's reagent, barfoed's reagents,	Span diagnostics Limited., India.
5.	Methanol	Sigma Aldrich, India.
6.	Ethanol	Sigma Aldrich, India.
7.	Reagent kit	Agappe Diagnostic Ltd (Kerala, India)
9.	Catalase	Sigma Aldrich, India.
10.	Hexane	Dr. Reddy's Laboratories Ltd., India
All other chemicals Were of Analytical grades.		

2.1 Preparation of the immediate-release layer

The feasible industrial technique, solid dispersion (SD) was chosen to enhance the solubility of model anti-diabetes agent nateglinide with Chloroform, ethyl acetate. SDs at various weight ratios were formulated by the suitable solvent evaporation method. Different weight ratios of 1:0.25, 1:0.5, 1:0.75, 1:1 drug, and carrier (Molish reagent, Fehling's reagent) were weighed individually and were dissolved in 10 mL ethanol to get a clear solution. The polymer solution was added to the drug solution with continuous stirring. Ethanol was completely removed by continuous heating on a heating plate at 40-50 ºC, which was carried out until a semisolid mass was obtained. The aerosol (0.02 % w/w) was added to make solid in nature and free-flowing. The powder was kept in a desiccator for further study.[21] The formulations were named F1-F8.

2.2 Percentage yield.

The practical yield was calculated in an appropriate method that signifies the efficiencyof any method.[6]

2.3 Drug content test

The percentage of drug content in SD's, was estimated by dissolving the SDs equivalentto 10 mg of nateglinide in 5 mL of ethanol. Each of these solutions was further diluted with 1.2 pH buffer, and nateglinide was analyzed at a wavelength of 400 nm to 200 nm by U.V. Visiblespectrophotometer (Model: cary 60, Agilent, USA).[6,18]

2.4 Fourier transforms in infrared spectroscopy (FTIR)

The prepared formulations, pure drugs, polymers/excipients were scanned at a resolution of 2 cm^{-1}, from 4000 to 400 cm^{-1} using FTIR (Cary 60, Agilent Technologies, USA). The FTIR spectra were obtained for the characterization of functional groups.

2.5 Differential scanning calorimetry (DSC)

DSC (Pyris Diamond, Singapore) thermograms of nateglinide, cremophor RH 40, and SDs were recorded. Operating conditions: heating rate (10°/min), temperature 30-280 °C, alumina powder as reference.

2.6 X-ray diffraction analysis (XRD)

XRD diffraction pattern of nateglinide, cremophor RH 40, and SDs were recorded using ULTIMA III, Japan (Cu target slit 10 mm).

2.7 Scanning electron microscopy (SEM).

Nateglinide and SDs were subjected to SEM analysis using JSM6360, Jeol SEM (UK), to investigate morphological characteristics. Operating conditions: probe current 45 nA, accelerating voltage 20 kV, counting time 60 sec.

2.8 In vitro dissolution studies

The dissolution was performed using USP II (paddle) dissolution apparatus (Electrolab, Mumbai, Model: TDT-08 L) in triplicate. The operation conditions of dissolution: temperature 37 ± 0.5 ºC and paddle rotation speed 50 rpm. Nateglinide and developed SDs equivalent to 60 mg nateglinide were individually placed in 1000 mL of 0.01 N HCl with 0.5 % w/v sodium lauryl sulfate (SLS). At predetermined time intervals 5, 10, 15, 30, 45, and 60 min, 5 mL of aliquots were withdrawn. The equal quantity was replaced with a preheated medium after each sampling. The aliquots were filtered using a 0.45 µm syringe filter and analyzed at a wavelength of 245 nm using a UV-Visible spectrophotometer (Agilent, Cary 60).

2.9 Compression of immediate-release and sustained-release layer tablets

Based on physicochemical characterizations, SDs containing nateglinide and cremophor RH 40 in the weight ratio of 1:0.25 and 0.02 % w/w aerosol (F5) (of drug equivalentto 60 mg of nateglinide) was selected to formulate into immediate-release tablets. All the ingredients of the immediate-release layer (IRL1-IRL3) (Table 1) and sustained- release layer (SRL1-SRL12) (Table 2) were weighed separately and passed through sieve 44#. Two layers of ingredients were taken in a mortar separately and mixed using a pestle. Magnesium stearate was added before punching tablets by the direct compression method.

Table 1: Formulation of immediate-release layer tablets by direct compression method. Each batch contains 25 tablets.

Ingredients	Formulation code (Quantity in mg)								
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9
Nateglinide	25	25	25	25	25	25	25	25	25
Chitosan	40	38	40	35	30	42	45	35	45
Pvp	15	14	14	14.5	15	14	14.5	14.5	15
Sodium Bicarbonate	20	22	20	20	22	18	20	22	20
Magnesium Stearate	2	3	2	2.5	3	3	2	2.5	3
Talc	1.75	1.5	1	1.5	1	1.5	1.5	1	1.5
SLS Powder	1.75	1.5	1	1.75	1.5	1.5	1	1	1.75

2.10 Preparation of gastro-bilayer floating tablets (GBFTs).

Based on the dissolution profiles and solid-state characterizations, formulation IRL2 (immediate-release) and SRL6 (sustained-release) were taken for the preparation of GBFTs. A varying concentration of sodium bicarbonate was added in the sustained-release layer (Table 3). GBFTs were fabricated by feeding the sustained-release followed by immediate-release layer power to the die cavity. Tablets were compressed using a 12 mm flat punch with an acceptable hardness of 5.5-6.5 kg/cm^2.

Table 2: Compression of gastro-bilayer floating tablets.

Ingredients (mg)	**BLT1**	**BLT2**	**BLT3**
Immediate-release layer SD equivalent to 60 mg of nateglinide	75	75	75
F-Melt type C	72	72	72
Crospovidone	3	3	3
Sustained-release layer natiglinide	50	50	50
HPMC K15	150	150	150
Sodium bicarbonate	12.5	25	50
MCC	129.5	117	92
Magnesium stearate	8	8	8
Total	500	500	500

2.11 Post compression parameters of GBFTs.

The thickness of the ten randomly selected tablets from each batch was determined with the Vernier caliper scale (Model: 530-312, Japan).

2.12 Weight variation

Randomly twenty tablets were selected from each batch and calculated the percentage deviation of individual tablet weight from the average weight of tablets.

2.13 Crushing strength

Crushing strength was determined using Monsanto type hardness tester by selecting randomly six tablets from each batch.

2.14 Friability

As the weight of the formulation was 500 mg, 6.5 g of the tablet was used for the friability test as per IP (2010). The tablets were put into the Roche friabilator apparatus (Model: 40 FT A01). With a rotation speed of 25 rpm, it was rotated for 4 min while allowing the tabletsto fall from a height of 6 inches in each turn. Following this, the tablets were weighed again, and the

loss in weight was expressed in percentage as a parameter of friability following the official method of IP 2010.

2.15 Drug content.

The average drug content was analyzed after verifying the UV-Visible spectrophotometric method for the simultaneous estimation of nateglinide and from bulk (Fig. S1 and Table S1-S6). Briefly, 20 tablets were crushed, and powder equivalent to 50 mg of drug combination was transferred to a 50 mL volumetric flask and dissolved in 0.1 M HCl. This was sonicated to ensure a homogeneous solution. This was further filtered 1 mL of the filtrate was used for estimation of drug content at a wavelength of 232.7 nm.

2.16 In vitro floating ability

The floating or buoyancy ability to float tablets was determined following established protocols.[22] Briefly, the tablet was placed in a beaker containing 250 mL of 0.1 M HCl. Floating lag time (FLT) was noted as the time taken by the tablet to appear on the surface of the medium. Similarly, the time to constantly float on the medium was considered as the total float time (TFT).

2.17 Dissolution studies of GBFTs

The dissolution study was carried out by operating the USP type II apparatus (paddle method) at 50 rpm and 37 ± 0.5 ºC temperature. The developed GBFT was placed in 900 mL of 0.1 N HCl (pH 1.2 simulating gastric pH) dissolution medium. Aliquots of 5 mL from each were withdrawn at specified time intervals of 5, 10, 15 min, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h. Equal volume was replaced with fresh preheated medium after each sampling. The aliquots were filtered using 0.45 µm Millipore syringe filters. The filtrates were analyzed at a wavelength of 232.7 nm for the estimation of nateglinide and simultaneously.

2.18 Release kinetics

In vitro, drug release data were subjected to mathematical models like zero order, first order, Higuchi, Hixson Crowell, Korsmeyer, and Peppas in order to investigate the release pattern of formulated batches.[23,24]

3 RESULTS AND DISCUSSION

Nateglinide (BCS Class II) is practically insoluble in water (8.8 mg/L) [25]. In order to meet the aim of the study, an attempt was made to improve the solubility of nateglinide. There are

eight different formulations (F1-F8) that were prepared with cremophor RH 40 and with and without aerosol (0.02% w/v) by solvent evaporation technique in a weight ratio of 1:0.25, 1:0.5, 1:0.75, 1:1. The formulations were subjected to physicochemical characterization like FTIR, DSC, XRD, SEM, and *in vitro* dissolution studies.

3.1 FTIR

The FTIR study provides evidence of the chemical/physical interaction and the shiftingof the bonds. The FTIR spectrum of the nateglinide, cremophor RH 40, and its developed SDsare shown in Figure 1. Nateglinide has a C=O group, which may participate in intermolecular interactions. Solutol HS 15 and cremophor RH 40 has functional groups which could favor the interaction with nateglinide by hydrogen bonding. The characteristic peaks of nateglinide [26] were observed at 3307.92 cm^{-1} for N-H stretching and 3068.75 cm^{-1} for aromatic C-H stretching. C-H symmetric and asymmetric stretching were observed at 2964.59 and 2918.30 $cm^{-1,}$ respectively (Figure 1A). C=O stretching was assigned to 1687.71 cm^{-1}. SDs showed a slight shifting of the peaks corresponding to N-H and carbonyl stretching groups (Figure 1D). This may occur due to hydrogen bond formations (non-covalent interactions) with polymeric groups, which might contribute to the enhancement of solubility of nateglinide in the SDs.

There was no chemical interaction between the nateglinide and chitosan. The optimized formulation showed characteristic peaks of the drugs and excipients (Figure 1E) thatindicate the compatibility between them.

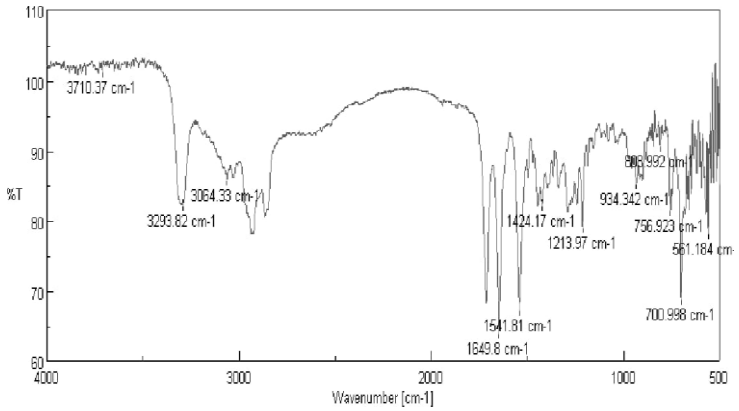


Figure 1: FTIR absorption spectra of A) Nateglinide, b) Chitosan powder, c) Nateglinide SD (F5),and d) Optimized bilayer tablet (BLT3).

3.2 DSC

The DSC thermographs of nateglinide, cremophor RH 40, and SDs are depicted in Figure 2. Nateglinide recorded a melting point at 136.67 ºC (ΔH = 89.83 J/g) suggesting its

crystallinity (Figure 2A) [23, 26]. The endotherm of cremophor RH 40 displayed a melting point at around 32.2 ºC (Figure 2B). The nateglinide melting peak disappeared in SD when prepared with cremophor RH 40 (Figure 2C). This indicates the amorphous state of the drug and that the amount of cremophor RH 40 used was sufficient to solubilize nateglinide.

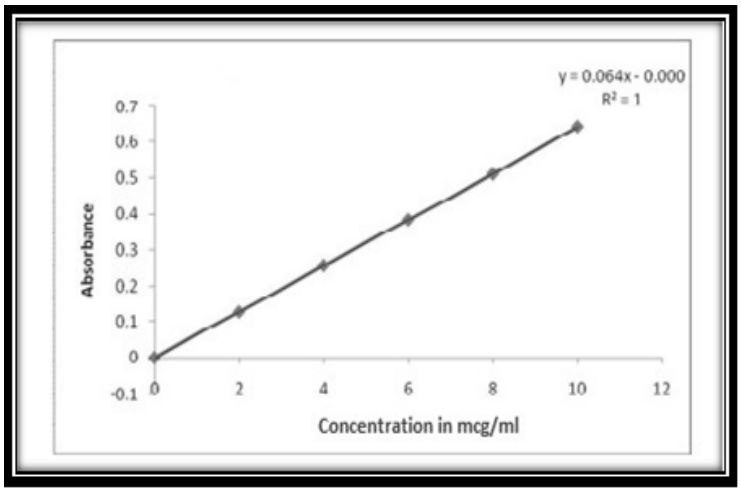


Figure 2: DSC thermograms of A) nateglinide, B) cremophor RH 40 and C) nateglinide SD (F5).

Table 3

No	P/V	Wavelength(nm)	Abs.
1	Peak	464	0.635

Table 4

No.	Formulation	Abs.
1.	Nateglinide 1	0.124
2.	Nateglinide 2	0.254
3.	Nateglinide 3	0.380
4.	Nateglinide 4	0.521
5.	Nateglinide 5	0.635

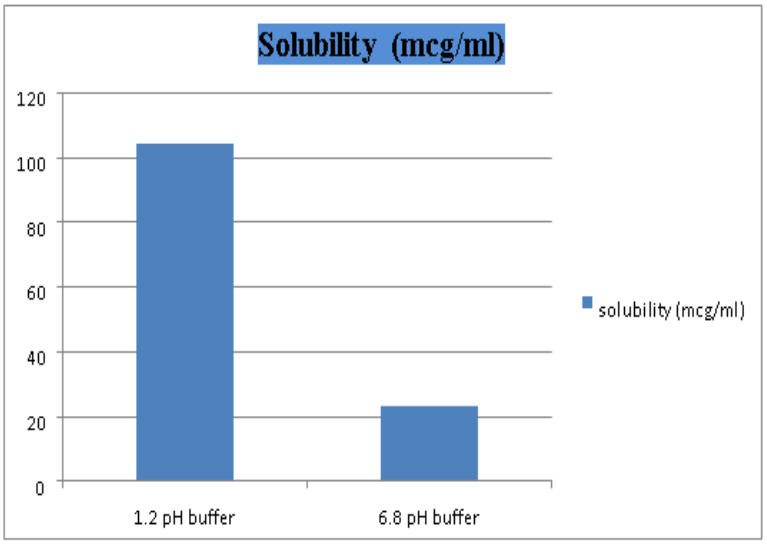
Table 5

No.	Parameters	Nateglinide
1.	λ-max	464
2.	Linearity	2-10
3.	slope(m)	0.063
4.	Regression equation	Y=0.063X-0.000

Solubility Study of Nateglinide

Table 5,4: Solubility data of nateglinide in various buffers.

Medium	Solubility (mcg/ml)
1.2 pH buffer	104
6.8 pH buffer	23



Evaluation of granulations

Granulation is the key process in the production of many dosage forms, involving the sustained release of a drug from coated or matrix type particles. A granule is an aggregation of component particles that is held together by the presence of bonds of finite strength. Although matrix tablets could have been made by direct compression method, to ensure good content uniformity and avoid flow related inter tablet weight variation process, a wet granulation (non-aqueous) is preferred in routine commercial production. Wet granulation method was, therefore, used in the present study. Physical properties of granules such as specific surface area, shape, hardness, surface characteristics and size can significantly affect the rate of dissolution of drugs contained in a heterogeneous formulation. The granules of drug formulations were evaluated for angle of repose, loose bulk density (LBD), tapped bulk density (TBD), Carr's index (CI) and Hausner's ratio (HF). The results were obtained are shown in tablets below**.** The angle of repose could not be measured by the above method for Nateglinide powders. The powder was too cohesive to flow through the funnel, where as the angle of repose values for granules were ranged from 21.99° to 23.70° for Nateglinide.

The Hausner's ratio values of the prepared Nateglinide granules were ranged from 1.154 to 1.348. The latter was thought to indicate good flow properties of the prepared granules as a result of increasing particle size owing to granulation. Also, the granulation has lowered the

tapped density as a result of relative increase in particle size compared with untreated powder. The percentage compressibility, an indirect method of measuring powder flow ability from bulk densities developed by Carr.

The percentage compressibility of Nateglinide was found to be 54.05. This result was in good agreement with the results of angle of repose and HF, supporting the idea that granulation improved both flow ability and compressibility. The percentages of fines in the granules for Nateglinide was found to be minimum. The immediate release powder blend showed good flow property (Angle of repose more than 27 and less than 30). Results revealed that immediate release powder blend can be directly compressed into tablets.

Evaluation of granulations pre-compression studies

Evaluation Parameters	Bulk density (g/cc)	Hausner`s ratio	Tapped density (g/cc)
F-1	0.45	1.2	0.54
F-2	0.47	1.04	0.49
F-3	0.57	1.05	0.54
F-4	0.51	1.19	0.56
F-5	0.46	1.16	0.49
F-6	0.48	1.12	0.54
F-7	0.42	1.16	0.49
F-8	0.44	1.22	0.54
F-9	0.47	1.25	0.59

Evaluation of granulations pre-compression studies

Evaluation Parameters	Compressibility index (%)	Angle of repose (θ)
F-1	8.92	22.14
F-2	4.08	25.62
F-3	5.26	24.56
F-4	16.66	23.71
F-5	6.12	21.56
F-6	11.11	26.83
F-7	14.25	24.65
F-8	18.59	23.87
F-9	20.33	26.76

Determination of parameters of drug (Post-compression studies)

The force applied to the edge of the tablet is gradually increased by moving the screw knob forward until the tablet breaks. The reading is noted from the scale which indicates the

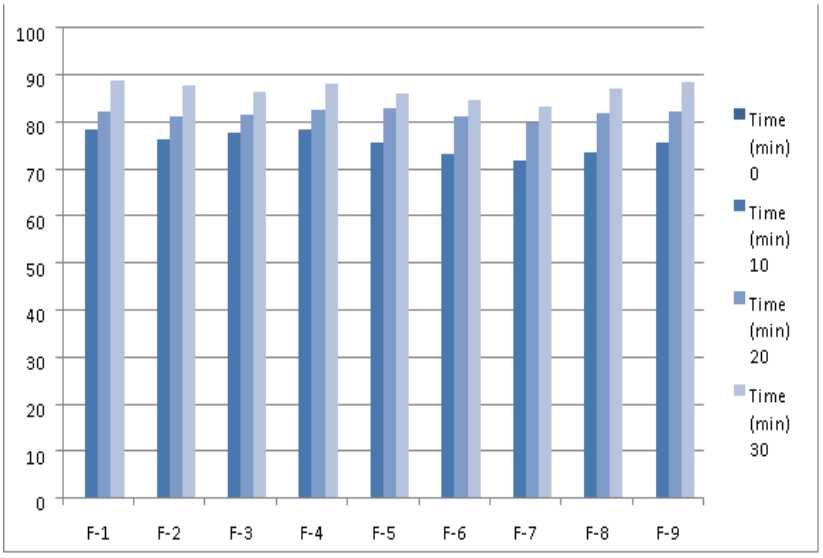
pressure required in kg/cm2 to break tablets. Pre-weighed sample of tablets was placed in a friabilator and were subjected to 100 revolutions.

Evaluation Parameters	Hardness (%)	Friability (%)	Drug Content (%)
F-1	5.8	0.4	97.45
F-2	4.2	0.3	93.36
F-3	6.3	0.3	94.74
F-4	4.5	0.5	96.75
F-5	6.1	0.4	92.45
F-6	4.2	0.4	91.15
F-7	4.9	0.3	93.32
F-8	5.2	0.5	94.15
F-9	5.7	0.4	90.85

Evaluation Parameters	Thickness (mm)
F-1	2.4
F-2	2.3
F-3	2.4
F-4	2.5
F-4	2.2
F-5	2.4
F-6	2.1
F-7	2.4
F-8	2.3
F-9	2.4

Table: Dissolution data of optimized batches of tablets in 6.8 pH buffer.

Evaluation Parameters	Time (min) 0	Time (min) 10	Time (min) 20	Time (min)30
F-1	0	78.45	82.15	88.75
F-2	0	77.46	81.24	87.65
F-3	0	76.45	81.67	86.47
F-4	0	78.43	82.54	88.13
F-5	0	75.48	82.96	86.14
F-6	0	73.05	81.05	84.58
F-7	0	71.65	79.86	83.16
F-8	0	73.45	81.96	86.98
F-9	0	75.43	82.09	88.46



Dissolution data of optimized batches

Evaluation parameters of batch which was kept for stability study:

Evaluation Parameters	Hardness (%)	Friability (%)
Before stability Storage	6.3	0.25
After 1 month Storage	6.2	0.5
After 2 month storage	6.3	0.35
After 3 month storage	6.2	0.4

After storage the formulations were withdrawn periodically and analyzed for various physical parameters, results were reported in Table above. No major difference was found between evaluated parameters before and after ageing storage of formulations, all were found to be in the acceptable limits

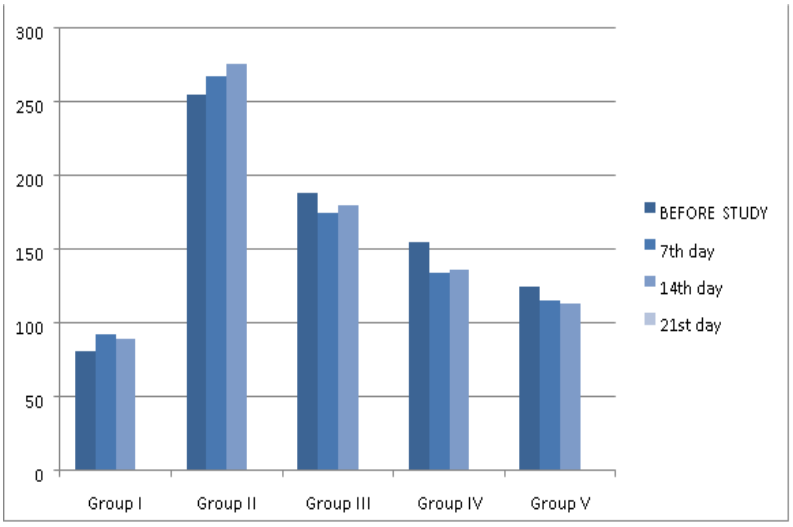
In-Vivo study: Measurement of biochemical parameters

Estimation of blood glucose

The blood glucose level of the normal control group remained almost constant. The blood glucose level of diabetic control group kept increasing significantly till last day compared with normal group. The group treated with extract showed significantly decrease in the blood glucose levels at all time intervals. During the study period of 28 days, Animals were treated at 0, 7, 14, 21 and 28 day and effect of vehicle, standard drug and all solvent fractions on blood glucose were determined.

Effect of extract on glucose level

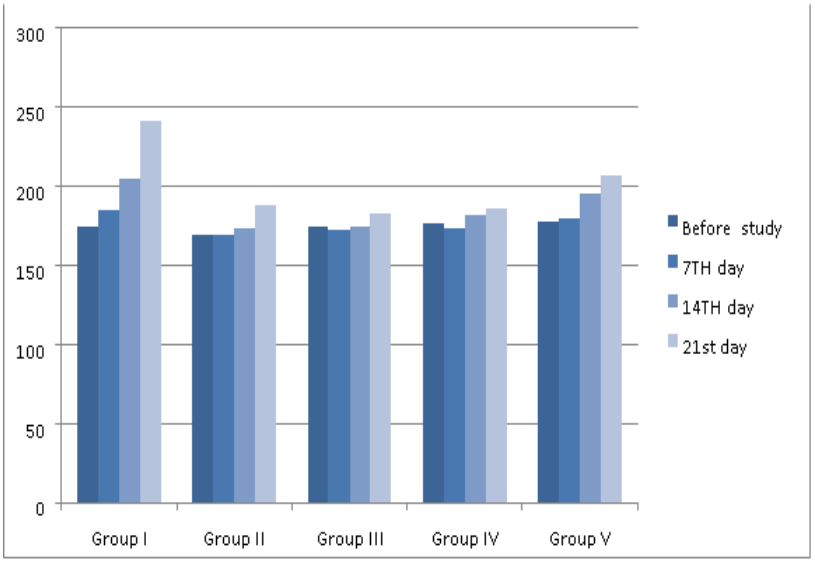
Groups	Time(days)			
	Before study	7th day	14th day	21st day
Group I	80.22	92.02	89.03	93.23
Group II	254.20	267.65	275.25	278.21
Group III (F-1)	188.22	174.25	179.85	184.26
Group IV (F-2)	154.21	134.21	136.32	146.61
Group V (F-3)	125.56	114.56	113.25	109.11



Determination of body weight of animals

Body weight in our study, the body weight of untreated diabetic group was reduced significantly. This reduction of body weight was also seen in Group III rats as compared to the normal rats in control groups. This data indicated that treatment of diabetic rats by Formulation 1 had no inhibitory effect on body weight reduction in diabetic rats. Our results were in accordance with the results of similar previous studies.

Groups	Time(days)			
	Before study	7TH day	14TH day	21st day
Group I	175	185	205	241
Group II	170	170	174	188
Group III (F-1)	175	173	175	183
Group IV (F-2)	177	174	182	186
Group V (F-3)	178	180	196	207



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