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Formulation and evaluation of gastro-retentive floating microspheres bearing metformin HCl for treatment of diabetes mellitus

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Abstract
Objective: To develop and characterize oral gastroretentive floating microsphere of Metformin HCl to prolong gastric residence time for treatment of diabetes mellitus.
Methods: The Gastroretentive floating microspheres of Metformin HCl were prepared by emulsion solvent evaporation method. Eudragit RS-100 and RL-100 were used as polymer. Microspheres were characterized for their percentage yield, entrapment efficiency, particle size analysis, floating behavior, optical and scanning electron microscopy. The *in-vitro* release evaluation and floating behavior were studied in hydrochloric acid buffer (0.1N HCl, pH-1.2). Different drug release kinetic models were also applied for all the prepared batches of selected drug. Selected formulations were also subjected to *in-vivo* anti-hyperglycemic activity on albino rat model.
Results: Floating microspheres were successfully prepared by emulsion solvent evaporation technique. The maximum yield of microspheres was upto 84.20%. On the basis of optical microscopy (at 40X) and scanning electron microscopy particle size range of Eudragit based microsphere was found to be ranging from 50 to 200 *µm* and also spherical in shape and somewhat rough and smooth surfaces when seen at different magnifications. Buoyancy of microspheres were found to be maximum after 12 hr in hydrochloric acid buffer and all formulation shows buoyancy more than 50%. *In-vivo* studies showed that such drug delivery system of Metformin HCl could be a better alternative approach for the physicians over the existing immediate-release formulations.

Keywords: Oral gastroretentive floating microspheres, eudragit rs-100 and rl-100, metformin HCL, *in-vitro* drug release studies, *in-vivo* anti-hyperglycemic studies

Introduction
Metformin HCl is an anti-diabetic drug with oral bioavailability varying from 50-60% and its shorter half-life ($t_{1/2}$ - 1.5 to 4.5 hr) and given in 0.5-2.5 gm daily in divided doses (2-3 times/day). The side effects associated with Metformin HCl is that it may cause GIT upset, nausea, vomiting, mild diarrhoea, abdominal pain, anorexia etc. Therefore, this drug may be formulated as GRDDS in order to minimize frequent dosing with more uniform drug levels and to improve oral bioavailability.
Gastro-retentive floating microspheres are low density systems that have sufficient buoyancy to float over gastric contents of the stomach and avoid peristaltic movement to remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in constant blood level of drug with reduced fluctuations in plasma drug concentration.

Materials and Methods
Metformin HCl was obtained as a gift sample from Stadmed Private Ltd., Kolkata. Eudragit RL-100 and RS-100 (Rohm Pharma GmbH, Germany) were used as polymers. All other reagents and chemicals were of analytical grade and used as without further purification.

Preparation of Gastro-retentive floating microspheres
Floating microspheres were prepared by method reported by S. Haznedar *et al*. Nine formulations of floating microsphere were developed. Different ratio of Metformin HCl, Eudragit RL-100 and RS-100 were mixed in acetone (30ml) for 15 minute. The resulting organic mixture was added drop wise into liquid paraffin light (100ml) containing 1% span 80 (1ml) while stirring at 700-1000 rpm for 3 hr at room temperature then prepared microspheres were collected by filtration and washed repeatedly with n-hexane after evaporation of solvent. The collected microspheres were dried in desiccators and stored at room temperature for 24 hr.

Table 1: Formulation specification of different batches of microspheres prepared using different polymers

Batch No.	Drug (mg)	Eudragit RL-100 (gm)	Eudragit RS-100 (mg)
1.	200 mg	1 gm	200 mg
2.	200 mg	1 gm	400 mg
3.	200 mg	1 gm	600 mg
4.	200 mg	1.5 gm	200 mg
5.	200 mg	1.5 gm	400 mg
6.	200 mg	1.5 gm	600 mg
7.	200 mg	2 gm	200 mg
8.	200 mg	2 gm	400 mg
9.	200 mg	2 gm	600 mg

Characterization of floating microspheres

Morphology

The internal and external structure of the microspheres was investigated by optical microscopy (at 40X) and scanning electron microscopy (SEM).

Particle size analysis

A random sample of dried microspheres was placed on a glass slide with a drop of liquid paraffin, and the size was measured using an optical microscope and the mean diameter (MD) was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Percentage yield of microspheres

The prepared microspheres were collected and weighed. The actual weight of obtained microspheres divided by total amount of all drug and polymer solid materials that were used for preparation of the microspheres.

$$\% \text{ Yield} = \frac{\text{The amount of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

Percentage Incorporation efficiency

The drug content of drug loaded microspheres were determined by dispersing 100 mg microspheres in 100 ml hydrochloric acid buffer (pH-1.2) which was stirred with a mechanical shaker for up to 12 hr to dissolve the polymer and to extract the drug. The samples were diluted and analyzed spectrophotometrically at 233 nm for the drug content.

$$\% \text{ Incorporation efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoratical drug content}} \times 100$$

***In-vitro* buoyancy studies**

An *in-vitro* buoyancy study was carried out using an USP XXIII dissolution apparatus filled with 900 mL 0.1N acidic solution (HCl) containing Tween 80 (0.02% v/v) as a dispersing medium. The medium was stirred with a paddle, rotating at a speed of 100 rpm for 12 hr. After each time interval, two fractions of the microspheres were observed, one was floating on the surface of the medium and the other was the settled portion. The settled portion of the microspheres was collected and recovered separately at a predetermined time interval, dried in vacuum and weighed.

$$\text{Buoyancy } (\%) = \frac{Wf}{(Wf + Ws)} \times 100$$

W_f and W_s are the weights of the floating and settled microparticles respectively.

***In-vitro* drug release studies**

The drug release rate from floating microspheres was determined using USP XXIII paddle type dissolution apparatus. From each batch, a weighed amount of floating microspheres were taken and subjected to dissolution studies. 900 ml of hydrochloric acid buffer (0.1N HCl, pH-1.2) was used as dissolution medium and maintained at 37±1˚C at a speed of 100 rpm. Samples (5 ml) were taken at predetermined times and replaced with equal volume of fresh dissolution medium. The collected samples were diluted with 5 ml of dissolution medium and then analyzed spectrophotometrically at λ_{max} 233 nm to determine concentration of drug present.

***In-vivo* anti-hyperglycemic studies**

In-vivo ant-hyperglycemic studies of the selected formulation and pure form of drug were carried out on normal healthy male albino rats having average body weight of about 110–170 g. They were housed in separate cages, maintained under standard conditions (12-hr light and 12-hr dark cycle; 25±30°C; 35–60% humidity); the animals were fed with standard rat pellet diet and water *ad libitum*. This study was approved by the Institutional Animal Ethical Committee of Lala Lajpat Rai University of Veterinary and animal sciences, Hisar, India. Noninsulin-dependent diabetes mellitus (NIDDM) was induced in rats by giving 25% (w/w) fructose solution for upto 21 days. Hyperglycemia was confirmed by the elevated glucose level in plasma, determined at 14th day and then on 21th day. Each animal having a blood glucose concentration level above 120 mg/dL was considered to be diabetic and used in the experiments. Only those rats found with permanent NIDDM were used for *in-vivo* studies.

Animals divided into four groups of six rats each such as group I: normal control rats given only drinking water; group II: diabetic control rats administered with drinking water; group III: diabetic rats administered with pure metformin (i.e. 100 mg/kg body weight); and group IV: diabetic rats administered with selected formulation of microspheres equivalent to the dose of the pure drug (i.e 100 mg/kg body weight) using intragastric tube. For the control (groups 1 and 2), the fasting was done overnight and water (*ad libitum)* was allowed. For groups 3 and 4, pure drug and microspheres were given orally in the morning following overnight fasting. No food and liquid except water (*ad libitum*) were given to the animals during the experiment. After collection of zero-hour blood sample, selected formulation of microspheres was given orally through intragastric tube. Blood samples (0.1 mL) were withdrawn from the tail vein of the rat's upto 12 hr (0 min., 30 min., 1 hr, 2 hr, 4 hr, 6 hr, 9 hr, and 12 hr). Plasma glucose levels were determined using Dr Morepen Gluco one Glucometer BG-03, Blood Glucose monitoring system, Morepen laboratories Ltd.

Statistical Analysis: All values were expressed as mean ± SEM. The data were statistically analyzed using one way ANOVA followed by Tukey-Kramer Multiple comparisons test. The p value <0.001 was considered to be statistically significant.

Results and Discussion

Percent yield

Table 2: Percentage yield of all formulations are given in following table

S. No.	Batch No.	% Yield
1.	F_1	63.20
2.	F_2	73.33
3.	F_3	66.40
4.	F_4	79.45
5.	F_5	77.99
6.	F_6	64.39
7.	F_7	71.12
8.	F_8	84.20
9.	F_9	59.11

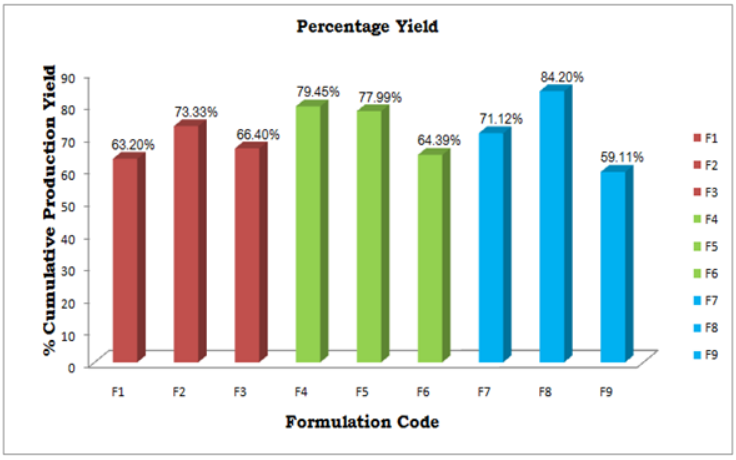

Fig 1: Showing histogram of % cumulative production yield of batches F_1 to F_9

All batches shows a percentage yield of greater than 60% whereas four batches showed a yield of more than 70%. Percentage yield is found to be maximum with formulation F_8 and minimum with F_9. Result shows that percentage yield increases with increase in amount of polymer.

Percent entrapment efficiency

Table 3: Percent entrapment efficiency of all formulations are given in following table

S. No.	Batch No.	% Entrapment efficiency
1.	F_1	62.60
2.	F_2	55.72
3.	F_3	65.69
4.	F_4	57.08
5.	F_5	60.00
6.	F_6	71.77
7.	F_7	60.76
8.	F_8	61.10
9.	F_9	63.55

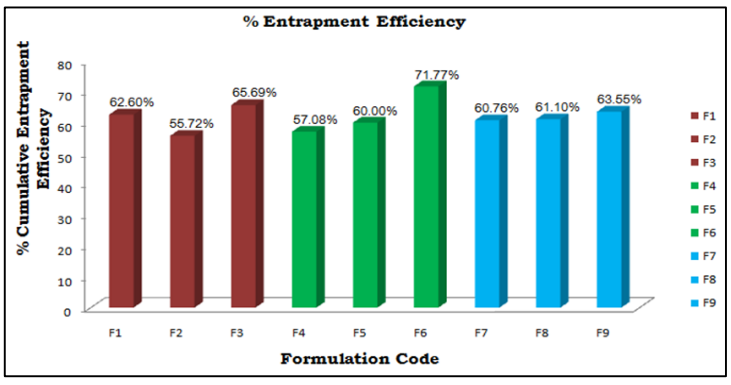

Fig 2: Showing histogram of % cumulative entrapment efficiency of batches F_1 to F_9

All batches show percentage entrapment efficiency more than 50% and it is found that drug entrapment increases with increase in the amount of polymer. According to Jain *et al*., 2005 when the loading was high, the proporation of larger particle formed was also high. Formulation F_6 shows maximum entrapment efficiency whereas F_2 shows minimum entrapment of the drug in the polymer. In our study we see that particle size, percentage yield and percent entrapment efficiency of prepared gastroretentive floating microspheres increased with increase in polymer concentration.

Particle size analysis

The prepared floating microspheres were examined for the particle size distribution using an optical and scanning electron microscopy.

Table 4: The size in µm range of all batches are shown in following table

S. No.	Batch No.	Particle size (µm)
1.	F_1	38.75
2.	F_2	42.53
3.	F_3	43.95
4.	F_4	45.37
5.	F_5	48.73
6.	F_6	52.20
7.	F_7	58.27
8.	F_8	63.47
9.	F_9	71.37

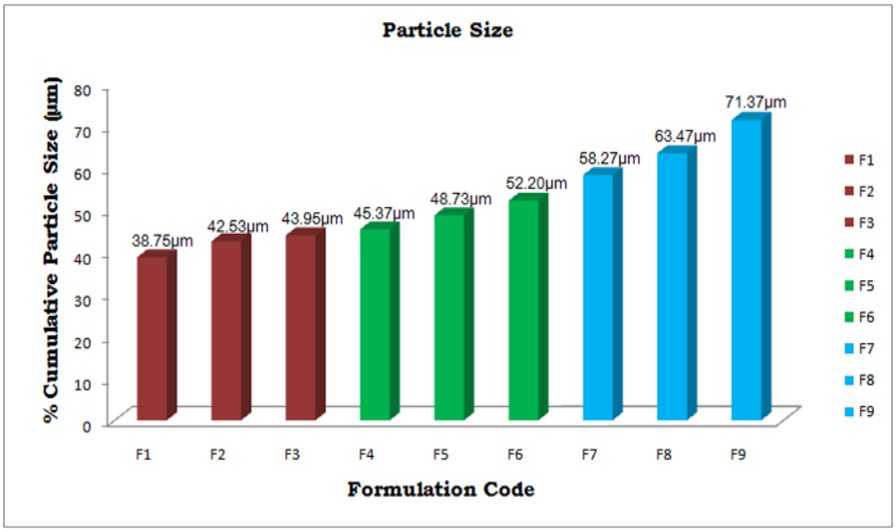

Fig. 3: Showing histogram of % cumulative particle size of batches F_1 to F_9

Results shows that particle size of prepared floating microspheres was found in the range of 38.7 to 71.37 µm. Our study shows that with increase in polymer concentration, particle size of prepared floating microspheres increased. Haznedar *et al*., 2003 prepared Eudragit (RL and RS) microspheres of acetazolamide and found mean particle size in the range of 261 to 294 µm.

Morphology

The external and internal structure of the microspheres was studied by optical microscopy (at 40X) and scanning electron microscopy (SEM). The optical microscopic images and photomicrograph of prepared microspheres are presented in figure 4,5,6,7.

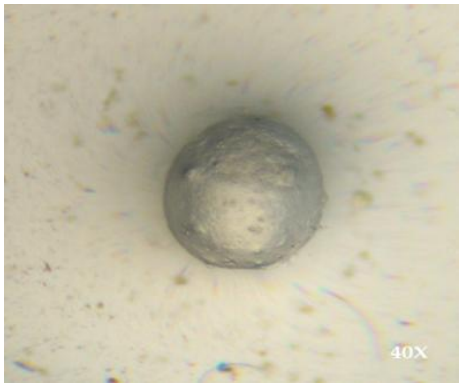

Fig 4: At 40X, Optical microscopic images of Eudragit RL+RS 100 polymer microsphere

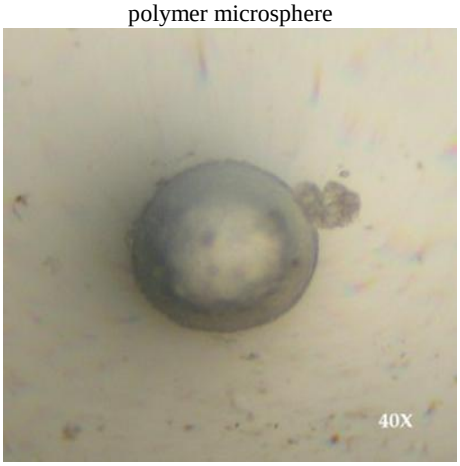

Fig 5: At 40X, Surface morphology of floating microsphere of Metformin HCl (stirring speed: 700-1000 rpm)

Result shows that Eudragit based floating microspheres were predominantly rounded or spherical in appearance. The spherical in shape of the microspheres are evident from their optical microscopic images.

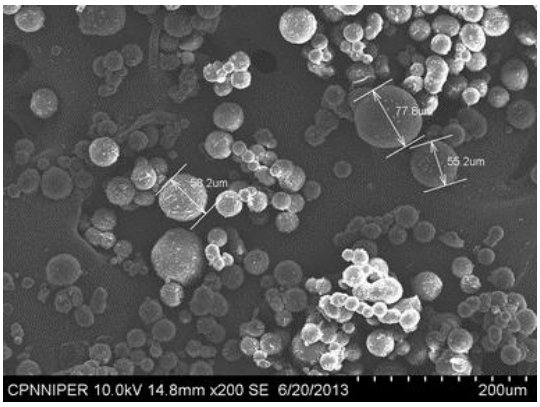

Fig 6: Scanning electron photomicrographs of Eudragit based floating microspheres (X 200)

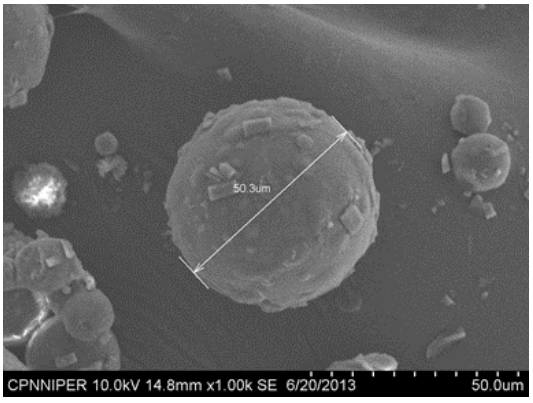

Fig 7: Scanning electron photomicrographs of Eudragit based floating microspheres (X 1000)

Result shows that Eudragit based floating microspheres were predominantly spherical in appearance and somewhat rough and smooth surface when seen at different magnifications. The spherical in shape of the microspheres are evident from their SEM photomicrographs.

***In-vitro* buoyancy studies**

Table 5: Various batches showed different floating behaviour as given in following table

S. No.	Batch No.	% Buoyancy
1.	F_1	69
2.	**F_2**	**55**
3.	F_3	67
4.	F_4	68
5.	F_5	60
6.	F_6	61
7.	F_7	51
8.	F_8	65
9.	F_9	53

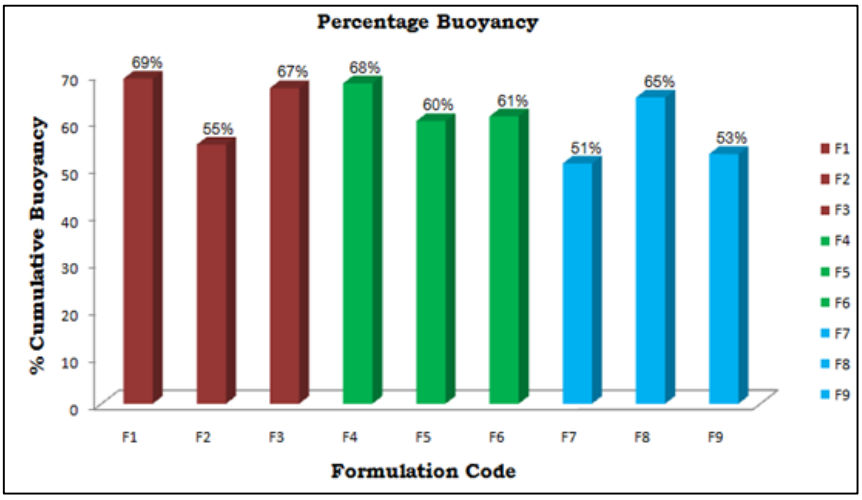

Fig 8: Histogram showing % cumulative buoyancy of batches F_1 to F_9

Result shows that after 12 hr, buoyancy was found to be maximum with formulation F_1 and all formulation shows buoyancy more than 50%. Kamila *et al.*, 2009 prepared multiunit floating drug delivery system i.e. floating microspheres of rosiglitazone maleate, an oral hypoglycemic agent and they found that all microspheres formulations showed good floating ability in the range of 65.32 to 96.34% at the end of 12 hr.

In-vitro drug release studies

Table 6: The data of release rate studies of formulation F1 to F9 and marketed formulation in 0.1N HCl acid buffer (pH-1.2) given in following table

S. No.	Time (Hrs)	F_1 (%)	F_2 (%)	F_3 (%)	F_4 (%)	F_5 (%)
1.	0.25	25.26	25.95	23.18	28.41	26.14
2.	0.5	31.56	27.61	27.63	34.72	29.01
3.	1	37.74	37.95	29.25	38.62	39.72
4.	2	41.42	40.97	31.76	43.64	46.83
5.	3	44.63	43.26	37.24	47.77	49.38
6.	4	52.40	44.87	40.91	51.43	54.40
7.	5	54.03	48.08	51.39	59.64	61.70
8.	6	60.19	51.96	54.63	65.14	63.79
9.	12	66.15	54.72	63.30	69.27	66.31
10.	18	70.29	64.98	66.53	70.89	68.14
11.	24	73.50	68.91	67.00	71.35	69.29

F_6 (%)	F_7 (%)	F_8 (%)	F_9 (%)	Marketed Formulation (%)
19.31	28.19	21.82	20.68	20.71
27.61	33.12	23.53	28.53	27.43
30.16	36.79	26.95	34.26	37.72
37.23	39.08	31.98	38.84	43.70
42.27	43.19	34.96	42.96	44.64
47.08	51.40	43.40	47.76	54.67
49.83	55.54	46.85	49.84	58.83
52.58	62.39	51.20	54.85	60.22
60.10	65.62	57.82	57.84	70.71
64.01	68.59	63.54	61.49	74.87
66.08	71.11	66.08	63.56	77.17

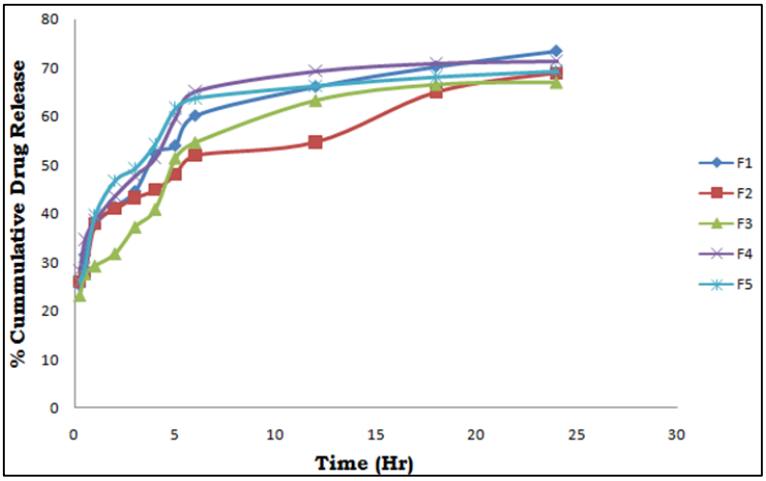

Fig 9: Comparison of drug release rate from formulations (F_1-F_5) showing zero order kinetics

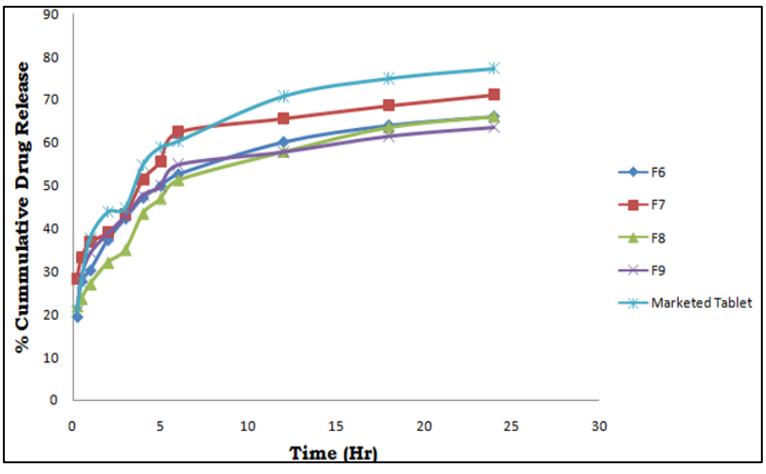

Fig 10: Comparison of drug release rate from formulations (F_6-F_9) and marketed tablet showing zero order kinetics.

Ideal property of floating microspheres includes high buoyancy and sufficient sustained release of drug in acidic buffer (pH-1.2). Result shows that formulation F_6 and F_8 showed a better controlled release and F_1 showed the maximum cumulative release. The formulation F_6 showed a kinetic release profile just similar to the theoretical controlled release profile of the drug and could be regarded as optimum formulation for *in-vivo* anti-hyperglycemic studies.

In-vivo anti-hyperglycemic studies

Table 7: Effect of F_6 formulation on blood glucose level in fructose induced diabetes in albino rats.

Time (hr)	Dose (mg/kg)	Glucose Level (mg/dL) Normal Control	Glucose Level (mg/dL) Diabetic Control	Glucose Level (mg/dL) Standard Control	Glucose Level (mg/dL) Test Formulation (F_6)
0	100 mg/kg	85.42±1.28	190.02±0.23	190.00±0.32***	195.83±0.38***
0.5		85.63±1.28	191.00±0.35	134.05±1.73***	141.71±1.38***
1		86.58±1.16	190.20±0.24	122.22±0.29***	133.36±0.50***
2		86.55±1.22	190.52±0.19	81.50±0.27***	120.92±0.15***
4		86.66±1.18	191.83±0.21	159.60±0.21***	84.21±0.35***
6		85.91±0.99	191.03±0.24	177.91±0.44***	82.51±0.51***
9		86.49±1.15	191.00±0.25	184.09±0.14***	82.20±0.39***
12		86.51±1.06	192.00±0.33	187.87±0.49***	82.57±0.32***

[a]Values are expressed as Mean ± SEM, n=6, ***P < 0.001 when compared to diabetic control using one-way ANOVA Tukey-Kramer Multiple Comparisons test

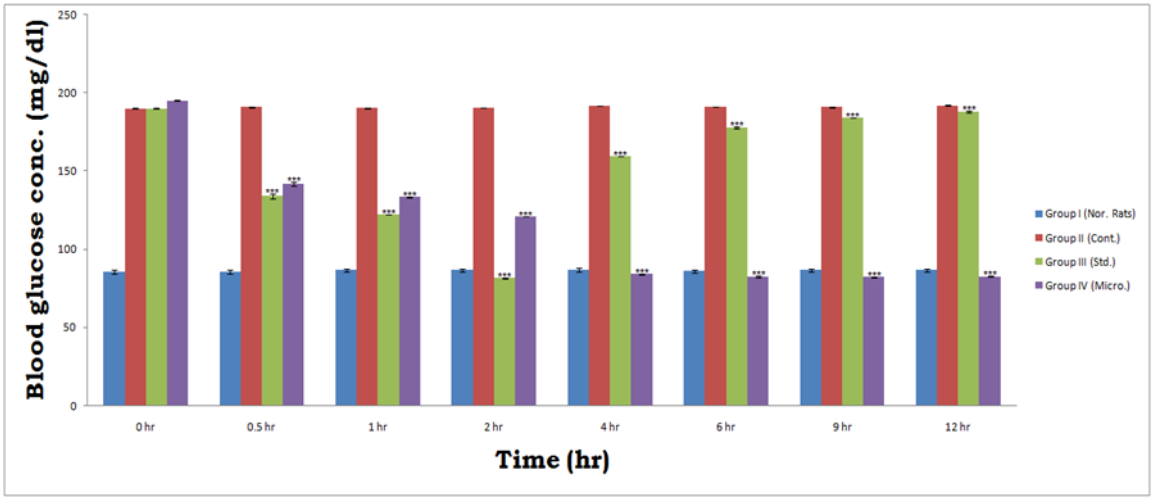

Fig 11: Comparison of *in-vivo* plasma glucose level in fructose-induced diabetic albino rats following oral administration of pure drug (group III) and Metformin HCl microspheres (group IV) with plasma glucose level of normal rat (group I) and fructose-induced diabetic rat without drug (group II)

Result shows that when Metformin solution (standard solution) was given orally, the blood glucose level started to decrease from the half an hour. After the second hour, blood glucose level reached to almost normal level but after the fourth hour blood glucose level started to increase again. On the contrary, the selected formulation (F_6) of Metformin HCl blood glucose level started to decrease from the first hour and this decrease continued up to the ninth hour until blood glucose reached to normal level. This was maintained upto the 12^{th} hour and blood glucose was found to be 82.57±0.32. The lowering of blood glucose level was slower, as expected, in case of Metformin HCl microspheres than pure Metformin drug due to its higher dissolution rate in pure form in gastric fluid of the rats.

In-vivo results suggested that such drug delivery system of Metformin HCl could be a better alternative for the physicians over the existing immediate-release formulations.

Conclusion

Metformin HCl is a drug of choice in treatment of type-II diabetes. Solvent evaporation method was used to prepare gastro retentive floating microspheres employing acetone as solvent to dissolve the drug and the polymer. Liquid paraffin light was used as the outer oil phase with span 80 (1% w/v) as the emulsifying agent. The prepared formulations were characterized for their particle size distribution by optical and scanning electron microscopy, surface morphology by SEM, percent yields, drug entrapment efficiencies, *in-vitro* buoyancy studies, *in-vivo* anti-hyperglycemic studies. The oral bioavailability of the drug was improved by more than two times due to prolongation of gastric retention time. The stability was also improved by formulating it into microspheres. This is important because the pure drug by itself showed several stability problems.

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