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Research Article

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Potent Anti-Diabetic Activity of Polymeric Microsphere Formulated Metformin on Streptozotocin-Induced Diabetic Rat Model

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ABSTRACT: Metformin is an extensively used drug as frontline medicine for type II diabetes. However, the target-specific delivery and efficacy of Metformin in a microsphere formulated pattern has not been studied in detail so far. Therefore, the present study is aimed to develop an oral site-specific rate-controlled anti-diabetic drug delivery system to pacify systemic side effects and offer effective and safe therapy for diabetic diseases with the compressed dose duration of treatment. To formulate this, guar gum and sodium alginate was used, whereas ethyl cellulose was applied as a coating polymer. Subsequent drug entrapment efficiency (DEE) and drug release were performed which indicates the proper formulation and sustained release of Metformin in a microsphere. Thereafter, the formulated drug was applied in Streptozotocin-induced diabetic Swiss albino rats. Histopathology of the liver, kidney, and pancreas was performed. Also, the level of liver glycogen, Glucose-6-phosphate, dehydrogenase, succinate dehydrogenase, and malate dehydrogenase content were significant ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) with the metformin-loaded microsphere treated group. Moreover, the drug release from the optimized microsphere at 12 hr was found to be 72%. The significant control in blood glucose and inclined body weight, food, and water intake showed the potential of the formulated drug. A similar range of VLDL and the other lipid profile, especially HDL level, offers the combined metformin-loaded microsphere reversal effect on cholesterol and cardiovascular risk. Overall, this study depicts, the significant antidiabetic efficacy of metformin's polymeric microsphere using natural guar gum which can improve the blood glucose level, lipid profile level, and histopathological architecture of concerned organs.

KEYWORDS: Metformin; Anti-diabetic; microsphere; control release drug delivery, guar gum.

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1. INTRODUCTION

The oral administration route always implies prominence in any therapy due to its well-established advantages[1]. However, oral administration faces some physiological constraints due to the heterogenic nature of the gastrointestinal system[2]. Conventional methods are not enough to defeat all the difficulties imposed by the gastrointestinal tract. For instance, they are unsuitable for drugs that are preferentially absorbed in the lower part of the digestive system, since conventional formulations do not maintain the capacity to face gastric emptying; therefore, they cannot be delivered in the intestine or colon where they stay during the final period of their release time. To overcome these afflictions, technological researchers have developed pharmaceutical systems that control drug release to maintain a sustained effect, some of which are already available on the market. The failure in gastric absorption with conventional systems has led to the development of oral intestine release systems. Such delivery systems were designed to release the drug in the intestine for a prolonged period of time, during which they deliver the drug on a controlled basis. The extended contact of these systems with the absorbing membrane allows an increase in drug bioavailability[3-6]. Regarding this, several strategies have been investigated to formulate thriving controlled drug delivery systems that enhance effectiveness by controlled and sustained drugs, such as bio-adhesive mucoadhesive system, colon targeted microparticle system, liposome, nanoparticle, and intestinal targeted microsphere[7].

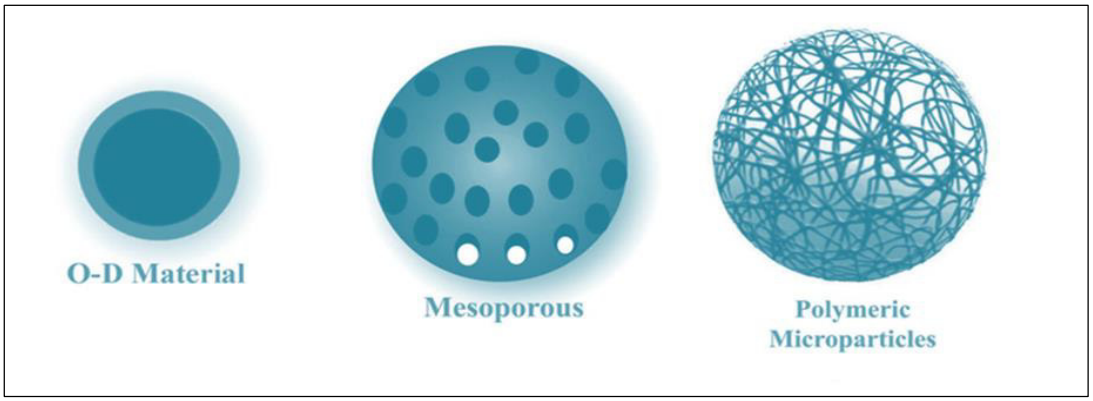

Fig 1: Different types of microcarriers used controlled delivery vehicles for cancertreatment[1].

Metformin is an anti-hyperglycemic agent, which improves glucose tolerance in type II diabetes. It has been reported that the absolute bioavailability of metformin, when given orally, is 50-60%[12]. The biological half-life of metformin is 1.5-1.6 h, and the leading site of its absorption in the proximal small intestine [13]. In our previous paper [14], a controlled release microsphere formulation of an antidiabetic drug and characterization of the microsphere has been sketched that a microsphere was formulated and characterized for the sustained release of metformin-loaded microsphere formulation, which was prepared by W1/O/W2 emulsion solvent evaporation technique. Here the article revealed that the increase in gum concentration in the W1 phase, which enhances viscosity in the 1hc W1 phase, results in an increase in the drag entrapment up to an optimum level and a decrease in the release rate. So, it can prolong the action. Metformin-loaded microsphere formulation would be a suitable pharmaceutical formulation for the treatment of diabetic patients in modern drug therapy for its prolonged action. The present object was to develop oral site-specific rate-controlled anti-diabetic drug delivery to pacify systematic side-effects and offer effective and safe therapy for diabetes with compressed dose and duration of treatment. To check the functionality of the microsphere, here we have observed in-vivo antidiabetic effects in Wistar albino rats. Further, we also assayed the hematological and biochemical parameters of the studied animals. Histopathology of the liver, kidney, and pancreas was also tested.

2. MATERIALS AND METHODS

2.1. *Chemicals*

Metformin was gifted from Stadmed Pharmaceuticals, Kolkata, India. Ethylcellulose was purchased from Quest chemicals Kolkata, India. Dichloromethane, tween-80, and span 80 were purchased from Merck India. All other reagents were of analytical grade. Guar gum was collected from the Bikaner district of Rajasthan, India. Streptozotocin (STZ) was purchased from Sigma Aldrich, India.

2.2. *Formulation Development*

2.2.1. *Preparation of stock solution*

Metformin was dissolved in 50 ml of distilled water and then made to 100ml by adding distilled water and thus 100 µg/ml stock solution was prepared[2].

2.2.2. *Determination of λ max*

10µg/ml of drug solution was prepared from the stock solution. The absorbance in the UV spectrum was determined by scanning a range of 200 nm to 400nm, and maximum absorbance was found at 233 nm, while distilled water was considered as blank.

2.2.3. *Preparation of standard curve*

A seven-point calibration curve was prepared by spiking appropriate amounts of the stock solution into the corresponding buffer to obtain a final concentration of 5,10,15,20,25,30.35 µg/ml for the analysis. Absorbance was studied at 233 nm, and a calibration curve was prepared by plotting the absorbance against the concentration.

2.2.4. *Swelling Index*

1gm of Guar gum was added in pH 6.8 buffer solution, 10 ml of distilled water, and 01N HCI solution. Then it was shaken

for 10 minutes in a magnetic stirrer at high speed and allowed to stand for 24 hours. Swelling capacity was measured using formula [1].

$$\%\ of\ weight\ change = \frac{Xv - Xi}{Xi} X\ 100$$

X_i – Initial weight,X_v– Final weight at swelling

2.2.5. ***Preparation of metformin loaded microsphere***

A small amount of sodium alginate was dissolved in 5 ml of distilled water and placed on a magnet stirrer. Guar gum was added to it with continuous stirring. 80 mg of Metformin was added and stirring was done for almost half an hour by a magnetic stirrer. Then the preparation was taken with a 20-garage needle. An organic solution was prepared with 1 gm of ethyl cellulose and 30 ml of dichloromethane (DCM). Then 300µl of span 80 was added to this organic solution. This preparation was transferred to the homogenizer tube. The homogenizer tube was then subjected to rotation at approx 4500 rpm, while the drug solution was poured drop by drop into it from the 20-gauge needle. A primary emulsion of the W/O type was produced[2].

2.2.6. ***Characterization of prepared microsphere***

The percentage yield of the microsphere was calculated using the ratio of practical yield and theoretical yield. Practical yield is the weight of the microsphere obtained. The theoretical yield is the total weight of the raw materials[3].

$$Percentage\ Yield = \frac{practical\ yield}{theoretical\ yield\ theoretical\ yield} X\ 100$$

2.2.7. ***Drug entrapment Efficiency***

40 mg of prepared microspheres were correctly triturated and made into powder form. Then 100 ml of phosphate buffer (pH 6.8) was added into it and was subjected to a magnetic stirrer for 2 hours. Filtration of the solution was done by the Whatman filter paper. 10 ml of this stock solution was diluted with phosphate buffer (pH 6.8) and analyzed for metformin content at 233 nm[4]

$$Drug\ entrapment\ efficiency\ (DEE) = \frac{Experimental\ Drug\ content}{theoretical\ drug\ content} X\ 100$$

2.2.8. ***Particle size distribution and zeta potential***

The particle size distribution was obtained from the optical microscopic method. The mean average diameters of the microsphere particles were obtained in the SEM studies. A weighted quantity of the experimental sample was dispersed in Milli-Q water (Milli-Q, Merck Millipore, Billerica, MA, USA) by vertexing and then sonicated and placed in a cuvette for zeta potential measurement [5].

2.2.9. ***Scanning electron microscopy (SEM) analysis***

Particle size, shape, and surface morphology were detected by SEM analysis. SEM was done by CARL ZEISS EVO 18 special edition machine with the platinum coating. The platinum coating was done by QUORUM Q150 TES machine[6].

2.2.10. ***Fourier Transform Infrared Spectroscopy (FTIR) Study***

FTIR was done to find out the chemical interactions between the drug molecule and other active ingredients used in the microsphere preparation. It was done on IR-Prestige 21, Shimadzu, Japan[7]

2.2.11. ***X-ray diffraction (XRD) studies***

The samples of various batches were evaluated by X-ray diffraction studies. XRD studies were done by X-ray diffractometer of model no Ulcinia-111, Renuka (Japan), Cu target slide 10 nm. The possible drug-polymer interaction was detected by XRD studies of the drug and the drug-loaded microspheres[8].

2.2.12. ***Drug release study***

A drug release study was done in the dissolution test apparatus, LAB INDIA DS 8000 USP -type 2(paddle type) apparatus calibrated at 37°C and rotated at 50 RPM. So, the dissolution test apparatus initially, 750 ml of the acid buffer of pH-1.2 was added, and microspheres of 50 mg were added. Then after 2 hours, 150 ml of trisodium orthophosphate buffer solution was added into the acidic solution so that the solution's resultant pH Became 6.8. From the final solution, 5ml of the sample was withdrawn every 1 hr, and it was replaced by 5ml ml phosphate buffer of pH 6.8 every hr. The same thing was done at the initial 2 hrs, maintaining pH 1.2. This process continued for 12 hours[9].

2.3 In vivo study of the metformin loaded microsphere

2.3.1 Animals and the maintenance

Animal Study (both sex) with weight of 180 to 220 grams using wistar rats (n=6) were carried according to the OECD 407 guideline (OECD, 2008). The keeping of animal handling and care were conducted under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSFA). Considerations were taken to assure that for female animals were nulliparous and non-pregnant. The animals were acclimatized under the standard controlled conditions (temperature: 25±5^{0}C, relative humidity, 55±10%) with the light and dark cycle. A period of 7-10 days was afforded to the animals to acclimatize under stipulated

laboratory circumstances before the induction of experimentation. Throughout the experimentation phase, animals were housed in polypropylene cages given a standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum [10]. All experiments were carried out as per guidelines cleared by the Animal Ethics Committee of the Department of the Pharmaceutical Technology of Jadavpur University, India (Registration number: 147/1999/CPCSEA).

2.3.2 ***Induction of diabetes***

STZ was freshly dissolved in a citrate buffer (0.01 M. pH 4.5) and kept on ice before use. Rats in fasting conditions were injected intravenously with 50 mg/kg b.w. of STZ[11]. One week after STZ administration, the rats with FBG concentrations of over 150 mg/dl were considered diabetic. and used in the experiment. The oral glucose tolerance test (OGTT) is low in normal and STZ-induced diabetic rats. After overnight fasting, normal and diabetic rats were divided into five groups, each with six rats in each group. All animals were experimental by following orders.

Group-1 Normal rats were treated with distilled water.

Group -2 The group has diabetic control rats treated with distilled water.

Group-3 Standard drug metformin was given orally as 80 mg/kg b.w.[2]

Group-4 Metformin-loaded microsphere was given orally at a dose of 150 mg/kg. b.w.

Group-5 Metformin loaded microspheres 150 mg/kg. b.w. with thiamine 70 mg/kg b.w. was given orally.

Blood Samples (0.5-0.6 ml) were obtained from the tail vein in cold heparinized tubes at 0 hr, 1 hr, 3hr, 5 hr, 7 hr to estimate blood glucose level. Thereafter through centrifugation plasma was separated and put at -20°C. The plasma glucose concentration was evaluated by the method of glucose oxidase-peroxides using Span Diagnostic kits.

2.3.3. Effect of metformin loaded microsphere on blood glucose and lipid profile in diabetic rats

At the end of the experimental period, the animals were fasted overnight for eight hours, and blood samples were taken under mild ether anesthesia[25]. Plasma was separated, and the fasting blood glucose (FBG) level was measured using glucose oxidase peroxidase using Span Diagnostic kits. The cholesterol level was done by the enzymatic method [12], triglyceride levels by the enzymatic colorimetric method[13], and HDL by the phosphotungstate method [14] using span diagnostic kits.

2.3.4. Histopathology of liver and kidney tissue

Three randomly chosen mice from each group were sacrificed at the end of the investigation, and their liver and kidney tissues were separated. Post- investigation from adhering tissue matter, tissues were rinsed with Cold normal saline and weighed cut into small pieces, fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol. Cleared in xylene and planted in paraffin wax, Sections (5µm) cut, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Eclipse TS100. Nikon, Japan).

2.3.5. Gluconeogenic enzymes in Liver and kidney

Glucose-6-phosphate dehydrogenase was estimated spectrophotometrically at 25°C as described by Beutler. Briefly, the enzyme sample was added to a 2.5 ml of final volume incubation mixture containing 0.1 M Tris-HCL 0.5 mM EDTA pH 8.0.10 mM $MgCl_2$ 0.2 mM $NADP^+$ and 0.6 mM glucose-6-phosphate. The activity measurement was performed by monitoring the rise in absorption at 340 nm because, it reduced $NADP^+$ at 25°C. One enzyme unit describes the reduction of 1 µmol of $NADP^+$ min^{-1} at 25°C, pH 8.0[15]. To estimate the Succinate dehydrogenase activity, 10% (w/v) homogenates of the liver tissues were prepared in ice-cold 0.25 M sucrose solution and centrifuged at 1000 g for 15 min 4°C. The supernatant fraction was utilized for enzyme assay. The reaction compound in a final volume of 2ml is added to 40 µmoles of sodium succinate and 100 µmol of phosphate buffer (pH 7.0), and 4 µmol of INT. The reaction was initiated by adding 0.2 ml of homogenate, 20 mg of tissue, as an enzyme source. The incubation was performed for 15 min at 37°C, and the reaction was arrested by the addition of 5 mL of glacial acetic acid. Zero Time controls (ZIC) were controlled by adding 5 ml of glacial acetic acid before adding the enzyme source to the incubation mixture. The form was extracted overnight into 5 ml of toluene at 5°C. The color exhibited was measured at 495 nm in a Spectrophotometer. Enzyme activity was expressed as nmol succinate oxidized/min and specific training as units/mg of mitochondrial protein [16]. Estimation of Malate dehydrogenase (MDH) (L-malate NAD+ Oxidoreductase); 10% (w/v) homogenates of the liver tissues were prepared in ice-cold 0.25 M Sucrose solution and centrifuged al 1000g for 15 min at 4°C. The supernatant fraction was utilized for enzyme assay. The total volume of 2 ml of reaction mixture comprised 100 µmol of phosphate buffer (pH 7.0). 0.1µmol of NAD, 40 µmol of sodium malate, and 4 µmol of INT. The reaction was caused by adding 0.2 ml. of homogenate, receiving 20 mg of tissue as an enzyme source. The incubation was performed at 37°C for 30 min, and the reaction was arrested by adding 5 ml of glacial acetic acid Zero time controls (ZTC) were managed by adding 3 ml. of glacial acetic acid before adding the enzyme source to the incubation mixture. The formazan formed was extracted overnight into 5 mL of toluene at 5^0C. The color exhibited was measured at 495 mm in a spectrophotometer against the toluene blank. The enzyme activity was revealed in µmoles of formazan formed /mg protein[17].

STATISTICAL ANALYSIS

All *in vitro* studies were performed on a triplicate represented by independent biological evaluation. Data were derived as mean±standard error of the mean (SEM) and were compared by one-way analysis of variance (ANOVA) followed by Kruskal Wallis Test utilizing SPSS statistical software of 20.0 version. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ was found to be statistically significant when compared with control.

RESULT AND DISCUSSION

3.1. ***Formulation Development***

The most broadly ratified traditions for microsphere preparation are the solvent extraction evaporation techniques, which formulate the aspired formulation with an adjusted drug release profile. However, the classical solvent extraction or evaporation techniques are restricted to incorporate lipid-soluble compounds through the process of emulsification of

drug and polymer solubilized organic solution in a continuous aqueous phase. Thus, modified techniques are in use to incorporate water-miscible drugs through incorporating in the aqueous phase as a saturated solution [18] or formulating multiple emulsions (W1/O/W2) [19] implicated pressing parameters play a vital role in this modern W1/O/W2 solvent evaporation technique fix microspheres preparation. Guar gum and sodium alginate were used as matrix building materials, whereas ethyl cellulose was applied as a coating polymer, and this produced metformin-loaded microspheres by W1/O/W2 emulsion solvent evaporation technique. Initial experiments showed that a higher volume of the organic phase and internal aqueous phase, and processing temperature considerably reduced the DEE of the microsphere. As a result, the DEE of the microsphere decreased (66.78 to 35.75). A previous report indicated that the entrapment efficiency of vitamin B_{12} in poly (C-caprolactone) microparticles decreased when the volume of the external aqueous phase was increased and vice-versa[2,9]. At the time of preparation of microsphere by W1/O/W2 emulsion-solvent evaporation method, the organic phase separated the internal and external aqueous phases and acted as a diffusion barrier for the drug between the two aqueous phases. Higher internal aqueous volume may increase the volume of W1 droplets in the oil phase and consequently may decrease the thickness of the organic polymer phase. This promoted more partitioning/leaching of the drug from the internal to the external aqueous phase. As a result, the DEE of the microcapsules decreased. The observation is in agreement with the results of other researchers [2, 10, 11]. In this research, DEE also varied due to changes in guar gums ratio, and these changes gave maximum DEE (66.78 %) at & a particular ratio (drug: guar gum = 1:0.62) And decreased DEE followed by an increase in gum ratio. The percentage of yield of the microsphere was proportional to the gum ratio (94.4 at 1.1 drug: guar gum) (**Table3**).

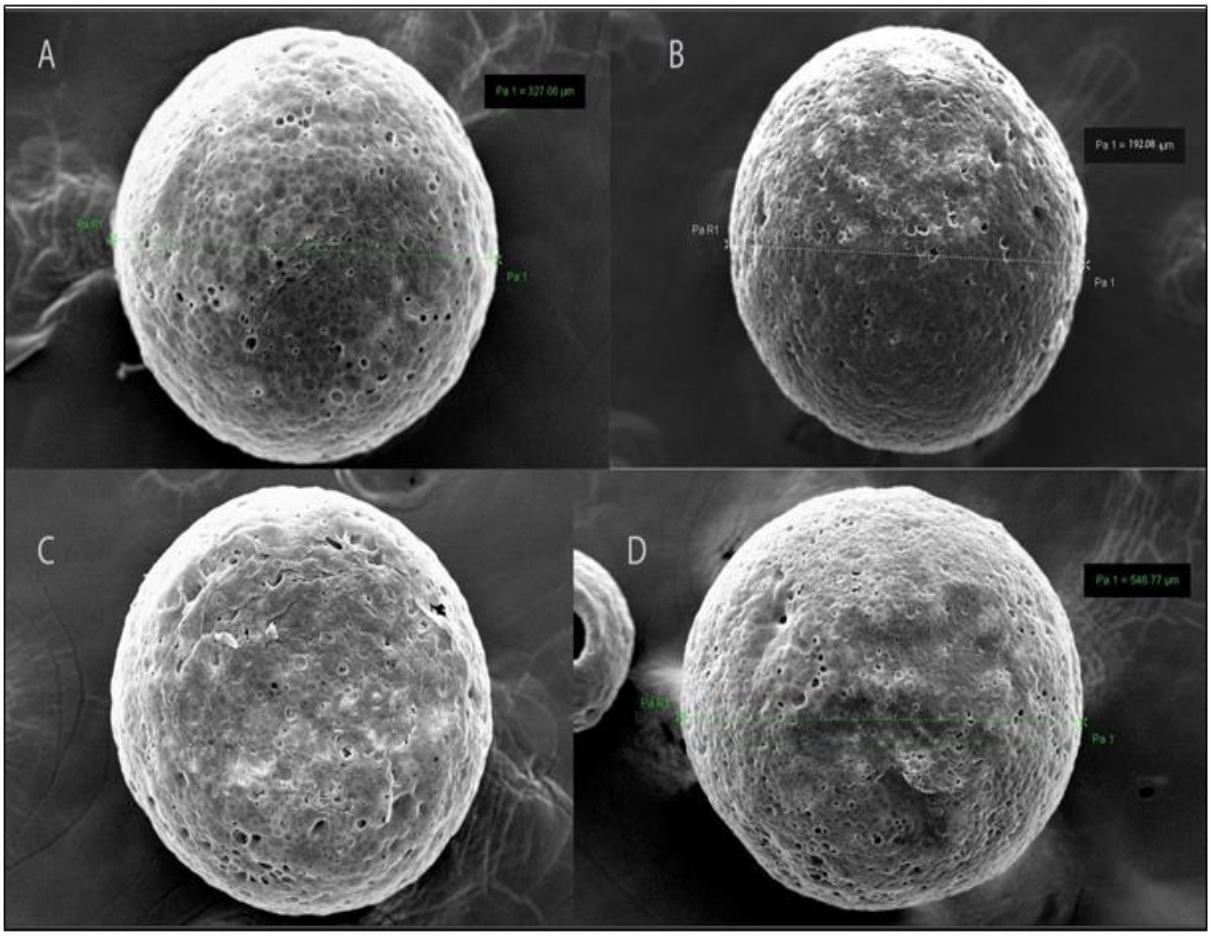

Fig 2: A- Drug-loaded microspheres showing apparently smooth surface, the existence of minute holes in the surface due to the rapid evaporation of the solvent in the magnetic stirrer. B- The best-optimized formulation (f2) showing smooth texture on the outer surface with a fine round shape in the appearance. Little roughness in the external surface helped its fine attachment with the intestinal lumen. C- The blank microsphere without the drug, having a spherical shape and rough in appearance. D-As the concentration of the gum increased in the internal phase so it produced a larger size due to the increase in viscosity in the W1 phase of W1/O/W2 micro emulsion [2].

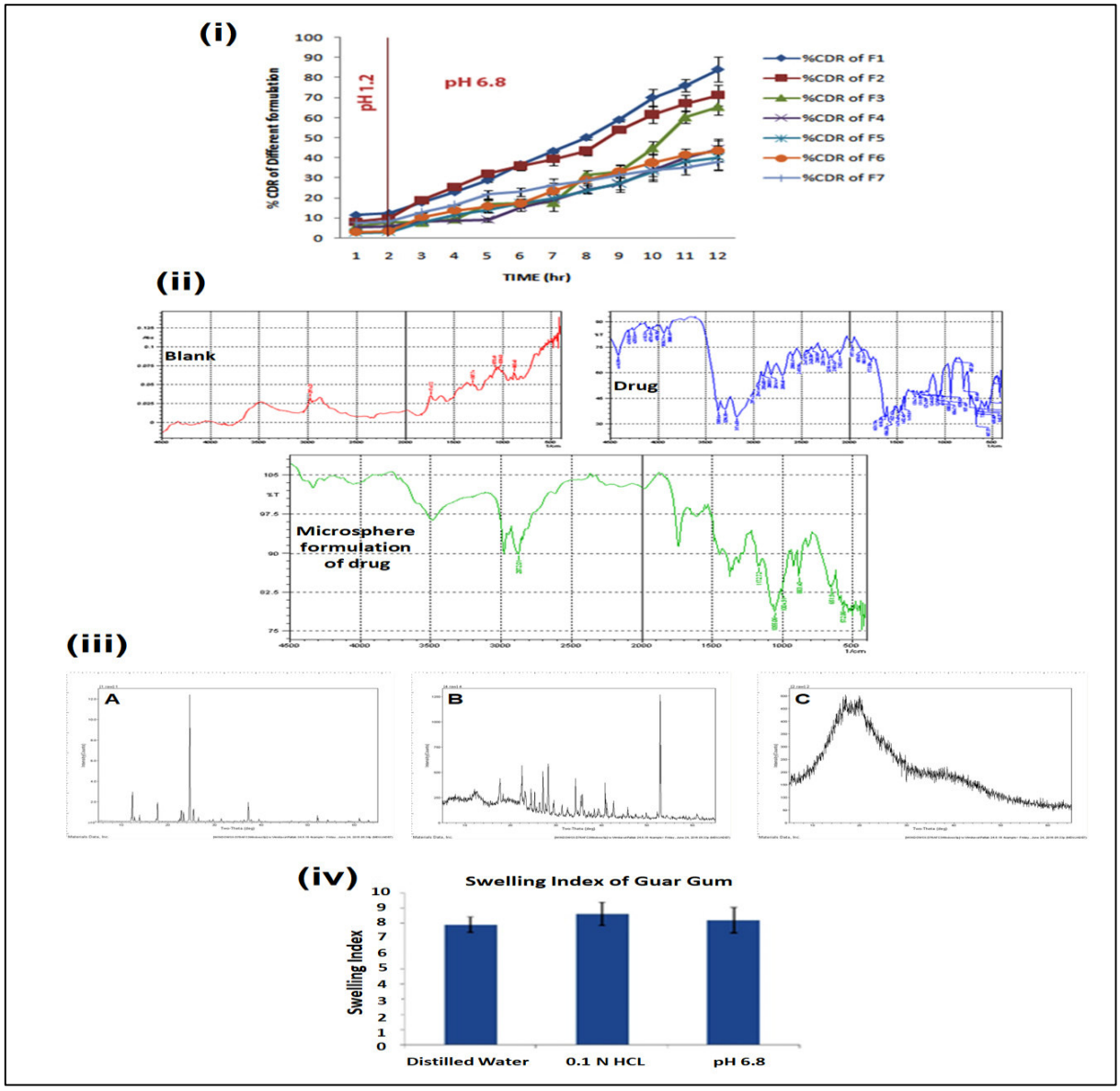

Figure 3: (i) Dissolution profile of F1 to F7 batches are depicted here in the figure. F1 batch having lowest concentration of gum showing highest percentage of drug released (85%). The optimized formulation F2 is showing 72% of drugs released in 12hrs. Fig. (ii) FTIR study of (A) blank microsphere (B) pure metformin and (C) metformin loaded microsphere. Fig. (iii) XRD study of (A) pure metformin (B) metformin loaded microsphere and (C) gum. Fig (iv) represents the swelling characteristic of guar gum at different pH (1.2 and 6.8). SD; Standard deviation of n=3 [2]**.** Increasing volume of the internal aqueous phase (W1) tended to increase the microsphere's size [12, 13].An increase in the volume of the W1 phase increased the number of dispersed droplets in a fixed volume of the organic phase, and the probability of coalescence between the dispersed droplets increased. This resulted in an increase in the size of the microsphere. Similar results had been reported by various workers[2,13]. The incorporation of guar gum concentration in the W1 phases also affected the matrix microsphere's size (327.08 um to 556.48um). Increases in the concentration of guar gum increases the size of the microsphere (**Table 3**)[2]. As the concentration of guar gums was increased, the viscosity of the W1 phase also increased. This hindered the easy breakdown of the W1 phase into smaller droplets. Besides, an increase in viscosity of the W1 phase made the primary W1/O emulsion more viscous and formed largerW1/O/W2, emulsion droplets. As a result, a bigger size of microspheres was formed (556.48 um) (**Figure. 2**)[2]. The release of the drug from the microsphere, prepared with the different concentrations of gum of the inner aqueous phase, was slow. Replacement of the dissolution medium after 2h with phosphate buffer (pH 6.8) produced a sudden increase in the release, which extended for different periods depending on the gun concentration in the W1 phase. Such a difference in release in the two-dissolution media may be attributed to the drug's pH-dependent solubility, which was poorly soluble in acidic solution and more soluble in an aqueous solution of higher pH. Besides, as the gun concentration in the W1 phase was increased, the release of drugs in both the dissolution media decreased. The time required for 50% (t_{50}%) and 80% (t_{80}%) drug release was determined from the cumulative percentage release versus time curves. Here, t50% was found to increase from 7.53 h in 10.82 h, and t_{80}% increased from 16:35 h to 11.85 h, as the gum concentration in the internal aqueous phase increased represented the microsphere could retard the release(**Table 2**)[2]**.** This suggest that, it can be used as a sustained drug delivery formulation. The higher the volume of the W1 phase, the porosity of the microcapsules wall and resulted in faster drug release[14]. SEM photographs showed the presence of pores on the surface of the microcapsules (**Figure 2**). The development of pores may be due to leakage of water through the organic phase. During the W1/O/W2, emulsion solvent evaporation method, organic liquid diffuses from W1/O droplets to the external aqueous phase, and simultaneously water from the external aqueous phase back

diffuses into the droplets. The back diffusion was related to the difference in the Osmolarity between the internal and external phases. The greater the back diffusion, the greater is the leakage of water[15], and hence, the wall of the microcapsules became more porous, providing faster drug release. The microspheres' drug release properties showed a regular release pattern of the drugs for twelve hours. The f2 batch had the lowest guar gum content showing a reasonable release of drug for sustained delivery. However, as the guar gum concentration increases, the drug release becomes lower, possibly due to the more viscous nature in the W1 phase, with the increase in the concentration of guar gum in the W1 phase hindered the release of the drug[16,2]. The resultant formulations were produced by optimizing the process through the W1/O/W2 solvent evaporation technique; the resultant microsphere's diameter was found 392.08. 1.96. Here, within the limit of our experimentation, the percentage yield and DEE were 49.16 1.27 and 53.1+1.52, respectively. Moreover, the release of drugs from the optimized microsphere at 12 hrs was found to be 72%. The swelling capacity of the optimized microsphere in distilled water, 0.1NHCl, and pH 6.8 buffers were represented in **(Figure 3)**. The optimized microsphere resulted in good swelling behavior at pH6.8. From the FTIR studies, it was also found that no chemical interaction occurred between the drug metformin and exercise excipients. The conducted XRD experiment also demonstrated that metformin was compatible with gum and other exercise excipients. The SEM study revealed that the optimized microspheres have a good spherical appearance and anticipated surface morphology[2]. The best-fitted model was analyzed by comparing the correlation coefficient values of different mathematical models in **(Table 1)** Drug release profile in dissolution media from different drug-loaded microsphere formulations. The correlation coefficient value of the optimized microsphere formulation (r^2=0.99) was found to be higher and more suitable than the other drug-loaded microsphere formulations from the recorded data. The Zero Order kinetic model was the best satisfactory mathematical model for the microsphere formulation[f2][2].

Table1: Drug release profile in dilution media from different drug loaded microsphere formulation.[2]

Formulation code	Zero order		First order		Higuchi		Korsmeyer –Pepas			Hixon crowell	
	K_0	R^2_0	K_1	R^2_1	K_h	R^2_h	N	K_{kp}	R^2_{KP}	K_{hc}	R^2_{hc}
F1	6.983	0.9833	-0.064	0.8986	29.39	09175	0.8929	0.9008	099419	0.211	0.949
F2	5.90	0.9905	-0.045	0.957	26.87	0.9607	0.941	0.8245	0.9767	1.4521	0.9579
F3	5.423	0.8888	-0.0369	0.8161	20.65	0.7689	0.9646	0.5794	0.8503	0.206	0.9557
F4	3.6936	0.9411	-3.6936	0.9411	16.24	0.8537	0.926	0.5307	0.871	0.1745	0.9804
F5	3.547	0.9885	-0.02	0.9009	16.00	0.9436	1.1988	0.3045	0.9757	0.1873	0.9618
F6	3.924	0.9879	-3.924	0.9879	17.77	0.951	1.18	0.3742	0.9619	0.214	0.9765
F7	2.837	0.9831	-0.0161	0.9917	13.117	0.9855	0.7119	0.8061	0.9702	0.1274	0.9384

Table2: parameters of the release of drug from metformin loaded microsphere [2]

Formulation code	$T_{50\%}$(h)	$T_{80\%}$(h)
F1	**7.53**	**11.85**
F2	**8.38**	**12.23**
F3	**10.82**	---[b]
F4	---[a]	---[b]
F5	---[a]	---[b]
F6	---[a]	---[b]
F7	---[a]	---[b]

---[a] drug release was less than 50% in 12 h. ---[b] drug release was less than 80% in 12 h.

Table3: the average particle size of drug loaded microsphere with a difference drug –gum ratio[2]

Formulation code	Drug: guar gum	% of yield	% of DEE	Average particle size (m)	Zeta potential (-Mv)
F1	1:0.22	42.20±0.83	30±1.45	327.08±2.45	11.13±0.11
F2	1:0.37	49.16±1.27	53.1±1.52	392.08±1.96	13.21±0.92
F3	1:0.50	49.58±0.63	59.5±1.07	397.78±3.61	10.35±0.12
F4	1:0.62	49.65±0.56	66.78±2.1	540.42±2.55	9.98±0.23
F5	1:0.75	52.14±0.28	50.15±2.23	548.77±1.39	9.32±0.45
F6	1:0.87	89.38±1.32	32.5±1.89	553.48±5.38	8.91±1.07
F7	1:1	94.84±1.88	35.75±1.47	556.48±4.15	8.21±0.15

Each point represents the mean ±SEM (n=6). Values are expressed as mean ±SEM mean values are significantly different from each other (**p<0.01).

3.2. *In-vitro antidiabetic activity*

Metformin has been a well-known anti-diabetic drug from times immemorial. It reduces blood sugar, and so is an important drug in the anti-diabetic group of drugs. While provided in microsphere form as the spherical shape it produces a larger surface area, the probability of adsorption increases bioavailability. At the same time, it provides controlled release of the drug's effect, so the drug's effect is for a longer duration. The gastro-irritant side effect of the drug is countered as the drug is released in the small intestine. All these positive results favors further study in preparing a microsphere.

2.4. *Effect of drug-loaded microsphere treatment in blood glucose*

The blood glucose and lipid-lowering impression of metformin on Streptozotocin-induced diabetic mice was previously well documented [20, 21]. In the present investigation, the blood glucose level of metformin-loaded microsphere and metformin-loaded microsphere with thiamine group animals were observed to be 238.14±1.42 mg/dl and 234.25±12.03 mg /dl. on the initial treatment (0^{th} day). The significant decline in the blood glucose level of 186.07± 3.51 mg/dL metformin loaded microsphere treated group and 185.0±2.11 mg/dL in metformin loaded microsphere with thiamine-treated animals were seen on the 14th day of oral drug administration. On the 21st day, oral drug administration of metformin-loaded microsphere, the notable decline in the blood glucose level to 121.10 ± 5.18 mg/dL whereas 120.02±1.04 mg/dL in metformin loaded microsphere with the thiamine-treated group was witnessed(**Table4**).

Table4: Effect of metformin loaded microsphere on fasting blood glucose level on streptozotocin induced rats after a single dose (n=6)

Drug	(Mg/kg)	Day 0	Day 7	Day 14	Day 21
Normal Control	-	77.0±1.82	78.25±0.95	77.75±2.21	79.50±2.64
Diabetic control	150	246.25±9.53**	284.0±5.47**	309.50±8.73**	319.75±7.80**
Metformin free drug	80	233.01±12.03**	216.75±8.65**	190.50±4.18**	126.15±4.07**
Metformin loaded microsphere	150	238.04±1.42**	211.50±4.65**	186.07±3.51**	121.10±5.18**
Metformin loaded microsphere & thiamine	150	234.25±12.03**	210.0±4.10**	185.0±2.11**	120.02±1.04**

Values are expressed as mean ± S.E.M. mean values are significantly different from each other (**P<0.01).

This propitious remission of diabetic condition was significant with the Metformin 80 mg/kg treated group value of 126.154 4.07 mg/dL **(Table 4).** Throughout the 21 days study period, the increasing level of blood glucose was seen in diabetic control animals due to the untreated situations. The control group animals displayed no adverse modification. The reverse blood glucose level was observed on the 7th day of the Metformin 80 mg/kg treated animal group. Table **(Table 4)** showed the blood glucose level changes in all the experiment animal groups. The treatment of metformin-loaded microsphere and metformin-loaded microsphere with thiamine, restrain the diabetic condition meaningful with the standard Metformin 80 mg/kg treated group.

2.5. *Oral glucose tolerance test (GTT)*

In animal experimentation, the GTT is practiced appraising the degree of diabetes and testing the wanted effects of insulin or other drugs on the body's capability in processing glucose. It can also be utilized to recognize the unintended side-effects of medicines intended to treat other unrelated diseases. All the animals were treated with glucose (2gm/kg b.w.). Normal control, diabetic control. Metformin powder standard group metformin microspheres test and group, metformin microsphere test group, and metformin microsphere with thiamine group.

Table5: Effect of metformin loaded microsphere on blood glucose level of streptozotocin induced diabetic rats.

Drug	mg/kg b.w.	Initial	1Hr	3hr	5 hr	7 hr
Diabetic control	150	220.0 ±1.05	245.2±1.39	262.9±0.75	264.7±0.98	266.2±0.95
Metformin free drug	80	228.4±1.77	175.3±2.18***	170.0±2.18***	151.4±1.65***	131.3±0.60***
Metformin loaded microsphere	150	226.8±1.55	174.2±2.18***	160.2±2.18***	148.3±1.12***	129.8±1.17***
Metformin loaded microsphere & thiamine	150	222.6±1.77	169.3±2.18***	165.0±2.18***	160.0±4.39***	130.2±0.60***

Data are represented as mean± S.E.M. ***p<0.001 when compared to control. n=6 per group.

In the normal control rats, there was an almost minimal increase in the blood glucose level. In the diabetic control groups, there was a significant rise in the blood glucose level in 60 min, and then it was sustained at 120 min and 180 min (**Table5**). The standard (metformin powder) group significantly restrain the rise in blood glucose and the same thing was found in the test - I (metformin-loaded microsphere) and test 2 (metformin leaded microsphere - thiamin) groups. The insulin response curve in type I (absolute insulin deficiency) diabetes describes the inability of the pancreas to release insulin in reply to the glucose load. The deficiency of an insulin response, which is accountable for the failure of the diabetic to utilize the added glucose, prolonged hyperglycemia happens. A crucial factor uniting hyperglycemia occurs in the overproduction of glucose by the liver. The test dose of glucose stays in effect, added to the already being oversupply of glucose. Because the steady-state level at which the liver stops to supply or withdraw glucose is elevated in diabetes, the liver continues to oversupply glucose, contributing to the tolerance curve's slow return to its original level.

Table 6: Effect of metformin loaded microsphere on blood glucose level of streptozotocin induced diabetic rats.

Drug	(mg/kg b.w.)	Day 1	Day 7	Day 14	Day 21
Normal Control	-	202.1± 1.9	204.83±1.02	210.00±1.05	216.83±1.52
Diabetic control	150	229.2±2.1**	145.00±5.2**	122.33±2.51**	110.31±1.62**
Metformin free drug	80	228.15±1.62**	209.02±1.3**	215.20±2.4**	225.07±3.6**
Metformin loaded microsphere	150	227.25±0.73**	218.66±0.63**	222.83±0.55**	226.56±0.82**
Metformin loaded microsphere & thiamine	150	228.19±0.28**	211.58±0.30**	220.39±0.30**	227.37±1.83**

Values are given in average body weight (g) ±SEM for groups of six animals each. **p<0.001.

The study affirmed that the normal control rats' weight gradually increased with time: the diabetic control rats lost weight and a regular interval throughout the experiment. The metformin-loaded controlled group became healthier with time. The same thing happened in the metformin-loaded microsphere with thiamine and the standard drug metformin powder. In contrast with normal control (non-diabetic) group animals, the induced diabetic condition, weight loss, and raised water and food intake were observed in all the other groups (**Table 6**). The typical symptom of type II diabetes condition of the animal body weight loss, food, and water intake increase was witnessed, similar to the studies of [22-24]. The disease conditions' progression drives excessive degradation of a structural protein and inflated food and water intake. The metformin-loaded microsphere treated groups exhibited reduced water, food intake level, and elevated body weight after the first week of oral treatment. The microsphere treated group effects were significant with the Metformin powder treated group. On the final day of treatment, metformin-loaded microsphere treated animals had increased body weight, and food and water administration were significant with control group animals, which was not seen in the normal control rats. In the Metformin 80 mg/kg treated group animal water intake and body weight was reverted and normal. Moreover, food intake was decreased lower than the normal control animals.

2.6. Drug-induced modulation of the hepatic maker (liver function) enzyme and kidney function

The serum glutamate-pyruvate transaminase (SGPT) or ALT, Serum Glutamic-Oxaloacetic Transaminase (SGOT) or AST, LDH are the essential liver marker enzymes to reveal the ability of liver function. The type II diabetic condition of STZ induced rat serum contains an elevated level of the intracellular liver enzymes such as SGOT, SGPT, LDH, and the kidney functional indicator of Urea and Creatinine, the stress condition of the body metabolism

Table7: The effect of metformin loaded microspheres on liver function markers in streptozotocin induced diabetic albino rats.

Drug	(mg/kg b.w.)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Normal Control	-	14.55±0.63	18.91±0.56	116.16±2.6
Diabetic control	150	17.35±0.15***	33.60±7.75***	314.50±5.9***
Metformin free drug	80	12.70±1.38***	19.62±3.35***	119.38±2.8***
Metformin loaded microsphere	150	12.52±1.24***	19.17±0.66***	117.64±3.4***
Metformin loaded microsphere & thiamine	150	12.10±1.26***	18.30±2.42***	117.15±1.9***

Values are given in average body weight (g) ±SEM for groups of six animals each. ***p<0.001.

The toxic STZ digestion leads to secrete the enzymes at a higher level and leads to liver cell damage and kidney functions. (**Table 7**) shows the SGOT, SGPT, LDH enzyme levels of the experimental animal groups. The elevated level of SGOT. SGPT, and LDH in the diabetic control group show the liver's stressed condition. In the normal control group and the other treated groups, serum enzymes have not observed much variation. The metformin-loaded microsphere and metformin-loaded microsphere with thiamine treated group's hepatoprotective enzymes showed the significance with diabetic control group level of enzymes, which represented tut microsphere treated group to give some hepatic protection capability may slow the release properties of microsphere at the 21st day of treatment.

Table 8: The effect of metformin loaded microsphere on kidney function markers in streptozocin induced diabetic albino rats

Drug	(mg/kg b.w.)	Creatinine (mg/dl)	Urea (mg/Dl)
Normal Control	-	0.54±0.3	31.83±2.2
Diabetic control	150	57. ±0.1***	69.5±1.8***
Metformin free drug	80	0.59±0.2***	31.38±3.5***
Metformin loaded microsphere	150	0.56±0.3***	32.19±1.5***
Metformin loaded microsphere & thiamine	150	0.57±0.2***	31.30±2.1***

Values are given as mean ± SEM for groups of six animals each ***p<0.001.

The analysis of urea and creatinine in serum settles the kidney functionalities. An elevated level of urea and creatinine shows the impairment condition of organs. The Metformin powder treated group and metformin-loaded microsphere treated group show the control level of urea viz., 31.38±3.5. v mg/dL and creatinine 0.59 ± 0.2, 0.56±0.3 mg/dL are (*p<0.05) significant with the normal control group 31.83± 2.2, 0.54 ±0.3 mg/dl **(Table 8).** The increased urea and creatinine levels in the diabetic control group show adverse damage to the organs. The formulated drug-treated great results have potentially recovered the kidney and liver from the damaged condition. However, the retrieval of liver and kidney marker enzymes and kidney function indicators shows the treated formulation can control the diabetic condition. A similar study that the novel chitosan was recommended to administrate with metformin to improve the drug efficacy and reduction of overdose lethal effects [25].

2.7. Effect of total cholesterol and triglyceride

The serum triglycerides and cholesterol were analyzed after the 21 days of the treatment period in all the experimental groups **(Table 9)**. Study results revealed increased triglycerides and cholesterol in the diabetic control group. The significant recovery and the controlled level of the triglycerides and the cholesterol were noted. A modest reduction in the microsphere treated groups was found Metformin 80 mg/kg administered animal group displays the complete recovery of the adverse condition. The metformin-loaded microsphere oral drug administered groups (p<0.05) were significant with the control group, and the Metformin 80 mg/kg treated groups to the treatments of the 21day period, animals were recovered from

the deterioration condition of heart functionality cholesterol. The Cetin & Sahin experiment has reverted the expected level of total cholesterol, pancreatic islets by the combined microparticulate and nanoparticulate drug delivery due to the controlled release[26]

Table 9: Effect of metformin loaded microsphere on serum on total lipid profile in streptozotocin induced diabetic albino rats

Drug	(mg/kg b.w.)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Normal Control	-	145.36±3.2	86.83±5.5	3.83±2.5	91.32±1.2
Diabetic control	150	271.16±10.5**	200.83±1.6**	20.05±1.9**	189±12.4**
Metformin free drug	80	148.65±5.6**	90.21±2.9**	36.63±2.1**	93.65±3.6**
Metformin loaded microsphere	150	145.61±3.6**	88.12±5.1**	36.47±2.6**	95.21±3.7**
Metformin loaded microsphere & thiamine	150	145.18±2.9**	87.20±5.2**	34.17±2.5**	93.54±3.8**

*Values are given as mean ± SEM for groups of six animals each **p<0.01.*

The HDL-C and LDL-C are the various blood cholesterol which is a reliable indicator of heart functionalities. The increase in the Non-HDL and the decrease of HDL range in the blood leads to cardiac arrest and fat accumulation in the blood vessels. The tests were made on the condition of heart function and an indication of diabetic heart disease in the experimental animal groups **(Table 9).** The decreased level of HDL-C 20.05 ± 1.9 mg/dL. and the higher LDL-C 27.72± 1.1 mg/dL and VLDL. 20.05 ± 1.9 mg/dL; 189 ± 12.4 mg/dL level shows the development of heart functional disease in the diabetic control group of animals at the 21-day study period. Whereas 36.47 ±2.6 mg/dL HDL-C and the lesser amount of LDL-C 95.21±3.7 mg/dL; in the metformin-loaded microsphere treated group is signed with the metformin 80 mg/kg administered group ($p<0.05$). In the normal control group, the normal range of HDL and the other cholesterol were noted. In the microsphere formulation, lower administered group cholesterol values showed a decreased acute heart disease level **(Table 9).** Rani *et al*., reported that thymoquinone nanocapsules (actually containing half of the dose of thymoquinone) produced a better antihyperglycemic effect in type 2 diabetic rats as compared to thymoquinone alone[27]. Type II diabetic condition of insulin resistance is interrelated with the abnormalities of the lipid and lipoprotein abnormalities in blood plasma. This condition reflects the reduced HDL cholesterol level and the predominance in Non-HDL, VLDL, and LDL combined triglyceride elevation [28]. The increased dis-lipidomic features are associated with cardiovascular risk and lead to heart disease [29].

2.8. Histopathology of liver, kidney and pancreas

In metformin and optimized microsphere administered groups of animal liver, kidney, and pancreases histology, the regenerative changes have been observed in the hepatocytes and in beta cells regeneration[30]. The liver cell morphology and their changes were seen **(Figure 4).** Histology of control liver exhibited normal hepatic lobules of central vein and portal vein. The vein wall structures remain firm, and no dilation was witnessed. Sinusoidal hepatocytes from central to portal boundaries are expected in the control group. In the diabetic control group, the random shapes of hepatocytes in the vein wall and the congestion were observed. The metformin-loaded microsphere treated group cells gave the portal's recovered state. The central vein and the clumping cell of the sinusoid boundary remained normal and significant with the Metformin 80mg/kg treated group. The metformin-loaded microsphere treated group also exhibited a sturdy recovery of cell damage. The tissue investigation was performed with 10X in the light microscope.

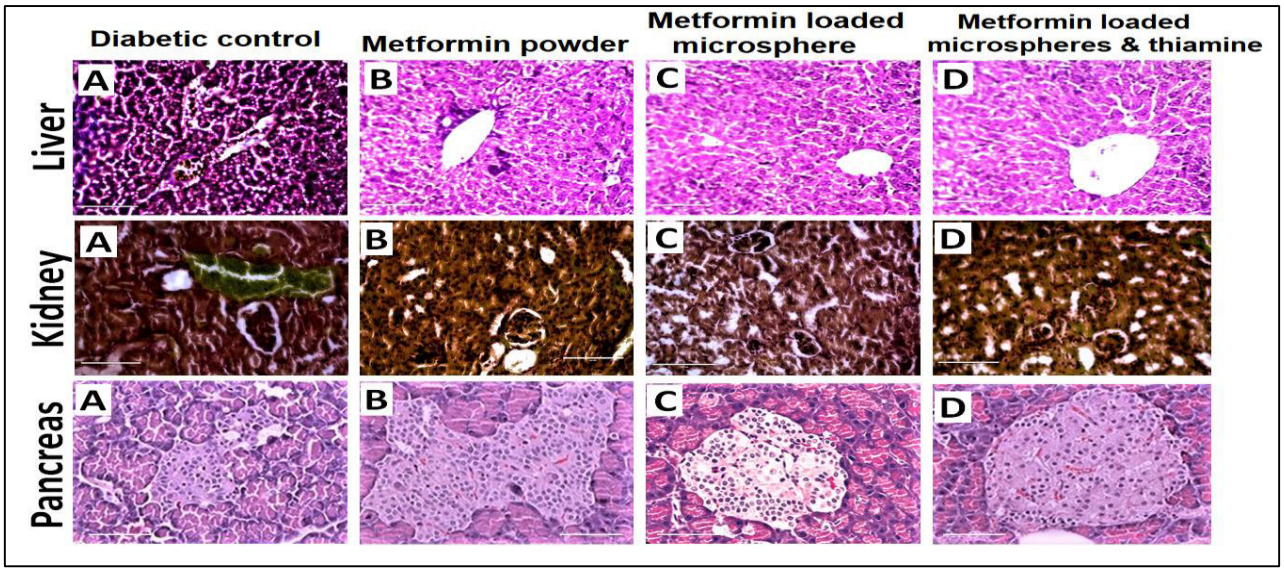

Fig 4:H & E stained section of liver, kidney, and pancreas of treated rats showing cell morphology of groups (A) diabetic control (B) metformin powder (C) metformin loaded microsphere and (D) metformin loaded microsphere with thiamine

The histology of pancreas cells was 10X if the light microscope **(Figure 4).** The clumping structures in the Islets of Langerhans were seen. The Acne beta cell structure was shrunken, necrotic lobular, and improper arrangements were observed in the diabetic control group. The 21 days oral drug-treated group exhibited the proliferated islets of Langerhans with recovered lobular cell arrangements observed in the metformin-loaded microsphere group metformin-loaded microsphere with thiamine treated groups. The observations were significant with the regenerated cell of Metformin 80 mg/kg treated group. The Langerhans islets were shrunken, and the cell structure was destroyed, including their numbers reduced. The histology of kidney cells was 10X of the light microscope **(Figure 4).** The kidney cell of disease control animals revealed the disturbing arrangements of glomerular vessels and the reconstructed shapes in the Bowman's capsule. In the normal control group, the usual structure of nephrons and cup-shaped Bowman's space was examined. The treated animal groups (metformin-loaded microsphere group and metformin-loaded microsphere with thiamine) give the reconstructed nephrons and the proper arrangement of glomerular vessels. The endocytic vacuole development was lost in the control group. A significant absence was mentioned in the metformin-loaded microsphere group, metformin-loaded microsphere with thiamine treated group and metformin 80 mg/kg treated group animals. The endocytic vacuoles were missing in the control group, and recovered cells were inspected in metformin and microsphere treated groups compared with the diabetic control group. The 21 days of any administration and the recovered hepatic lobules of the central vein and portal vein were perceived in the metformin-loaded microsphere treated group. The hepatocytes of the sinusoidal cell pattern, the dilated central and portal boundaries were reported at the alloxan-induced non-obese diabetic rat and STZ induced non-alcoholic rat[31, 32]. Metformin 80 mg/kg and metformin-loaded microsphere groups were conferred significant stricture due to the oral administration of the treated drug with slow drug release capability. Particularly in the metformin-loaded microsphere group, results were comparable to the histology of kidney, liver, and pancreas of metformin 80 mg/kg. Langerhans' scattered islet, pancreatic acne cell abundant prominent nuclei arrangement on the lobular duct arrangement of metformin-loaded microsphere treated group significant with control group tissue.

2.9. Gluconeogenic enzymes in the liver

The gluconeogenic enzyme increases the glucose level in the blood and serum. The increased Glucose-6-phosphate and Fructose 1-6 bi-phosphatase in liver and kidney enzymes were found in the induced type II diabetic animal groups. These enzymes control carbohydrate metabolism as reported in diabetic in-vitro cell culture in STZ induced experimental animals[33-35]. The increased level of Glucose-6-Phosphatase in the liver 11.02±1.09 was observed in the diabetic control group **(Table 10)** and the succinate dehydrogenase and malate dehydrogenase were 2.07±0.39 and 1.16±0.07 in the liver were noted. In the metformin-loaded microsphere treated groups and the Metformin 80 mg/kg administered group, decreased enzyme level is significant to the diabetic control group **(Table 10).** The treated group of metformin-loaded microsphere shows a reduced level of gluconeogenesis. The administered oral drug potentially reduced the carbohydrate conversion rate by inhibiting the gluconeogenic cycle significantly with Metformin at 80 mg/kg of dose. On the other hand, the 21 days of the standard drug (Metformin at 80 mg/kg) oral administration increased the glucokinase level in diabetic rats.

Table 10: Effect of Metformin loaded microsphere on enzymes of glucose metabolism in Streptozotocin induced diabetic rats.

	Normal control	Diabetic control	Metformin free drug	Metformin loaded microsphere	Metformin loaded microsphere & thiamine
Glucose-6- phosphate dehydrogenase liver (nmol NADP+ reduced /min/mg protein) lactate dehydrogenase	19.33±1.31	11.02±1.09**	18.96±0.76**	19.04±0.24**	19.16±0.38**
Liver (nmol pyruvate formed/min/mg protein)	63.16±2.58	78.49±2.84**	62.80±5.93**	63.25±4.47**	63.07±4.82**
Succinate dehydrogenase Liver (umol NADH oxidized /min/mg protein)	4.16±0.40	2.07±0.39**	5.89±0.68**	4.63±0.29**	4.15±0.21**
malate dehydrogenase Liver (umol NADH oxidized /min/mg protein)	1.56±0.14	1.16±0.07**	1.45±0.21**	1.50±0.21**	1.54±0.12**
Plasma (umol NADH oxidized/min/ml)	0.88±0.13	0.52±0.10**	0.82±0.19**	0.84±0.15**	0.86±0.18**

Values expressed as mean SEM (n=6). **p<0.01, in comparison with normal and diabetic control

The decreased level of Glucose-6-phosphate dehydrogenase enzyme in the diseased group shows oxidative stress due to diabetic conditions[36-38]. The Metformin group, followed by metformin-loaded microsphere treated groups, has positively controlled the carbohydrate metabolic enzyme's secretion. The glycogen decreased due to diabetic stress indicated by the diabetic control group. Notably, an increased level of glucose conversion in the metformin-loaded microsphere group significantly with the Metformin-treated group was observed in 21 days of the study period.

2.10. Carbohydrate metabolic enzymes & glycogen

Glucokinase is the potential antihyperglycemic enzyme that plays a significant role in controlling blood glucose homeostasis on hepatic glucose disposal. The lower level of Glucose-6-phosphate dehydrogenase indicates the high prevalence of diabetic conditions and hypertension. The glycogen increased in liver tissue, indicating the diabetic diseased condition. **(Table 10)** shows the declined level of Glucose-6-phosphate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase activity in the

diabetic control group liver homogenates. A significant increase in metformin-loaded microsphere treated groups with the Metformin 80 mg/kg treated groups was observed on the 21 days of the study period. The recovery of the liver metabolic enzyme results from the tissues' development potential against induced type 1 diabetic conditions. The results of Glucose-6-phosphate dehydrogenase in the diabetic control group, metformin 80 mg/kg, metformin loaded microsphere, and metformin loaded microsphere with thiamine treated groups are viz., 11.02±1.09, 18.96±0.76, 19.04±0.24, and 19,16±0.38 IU/h/mg protein **(Table 11),** and the succinate dehydrogenase was 2.07±0.39,5.89±0.68, 4.63±0.29 and 4.15±0.2 IU/mg protein **(Table 11);** malate dehydrogenase in liver tissue are 1.16±0.07, 1.45±0.21, 1.50±0.21 and 1.54±0.12 IU/h/mg protein and liver glycogen in liver tissue 1.07±0.93, 4.52 ±1.82, 4.50±1.29 and 4.53±1.30 mg/g wet tissue. Earlier Metformin when given in a nanoparticle form has shown such antidiabetic efficacy[39]. However, whole results of the present study are more significant in its anti-diabetic activity and drug efficacy level as it was found to be effective in a very lower dose against diabetic rat.

Table 11: Effect of Metformin loaded microsphere of liver glycogen and glycogen synthase in Streptozotocin induced diabetic albino rats.

Drug	(mg/kg)	Liver glycogen (mg/g wet tissue)	Glycogen synthase (mol UDP formed/min/mg protein)
Normal control	-	4.95±0.87	3.01±0.19
Diabetic control	150	1.07±0.93***	0.77±0.18***
Metformin free drug	80	4.52±1.82***	2.81±0.11***
Metformin loaded microsphere	150	4.50±1.29***	2.75±0.16***
Metformin loaded microsphere & thiamine	150	4.58±1.30***	2.81±0.17***

Values expressed as mean SEM (n=6). ***p< 0.001

CONCLUSION

The results of the whole study indicates that metformin-loaded microsphere prepared with guar gum as matrix material could be a suitable way to have target specific anti diabetic activity. At the same time, it may provide prolonged release in the intestine to achieve better drug therapy. Metformin in a microsphere form has not been evaluated in detail so far. In this study, Metformin in a microsphere form has shown controlled release and sustained delivery and also better efficacy at lower doses when compared to metformin in a free form. Variation in the histopathology of liver, kidney, and pancreas of the treated animals resulted from the control released design having a slow-release rate, giving less trace of liver and kidney function. Hematological and biochemical analysis revealed that ALT and AST, creatinine, and urea level of metformin-loaded microspheres was better tolerated when compared to the diabetic control. Also, the level of liver glycogen, Glucose-6-phosphate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase content were found to be modulated significantly with the metformin-loaded microsphere treated group. The significant control in blood glucose level and inclined body weight, food, and water intake was observed in rat body after 21 days of the drug induction. A similar range of VLDL and the other lipid profile, especially HDL level, offers the combined metformin-loaded microsphere reversal effect on cholesterol and cardiovascular risk. Finally, we foretell that metformin's polymeric microsphere using natural guar gum could be a promising micro-carrier for efficient intestinal targeting delivery tools with improved anti-diabetic efficacy and lesser side effects against diabetic disease. Hence, further studies are highly solicited to explore the molecular release behavior, enhancement mechanism, and the individual effect of this novel microsphere activity against diabetes and consequent metabolic disorders.

ETHICAL APPROVAL AND CONSENT FROM PARTICIPATE

The study protocol was approved by the institutional animal ethical committee of Jadavpur University, Department of Pharmaceutical Technology, Kolkata, India. This research work was approved by the Ethical Review Committee of Research cell of Jadavpur University of Science and Technology, Kolkata-700,032, India (Registration number:147/1999/CPCSEA).

AUTHORS CONTRIBUTION STATEMENT

Biplab Kumar Chakra and Dr. Souvik Debnath conceived the presented idea, conceptualized and planned for the research in addition to contribution in data analysis, results discussions and writing the article. Dr. Avinaba Mukherjee, Mayur Chakraborty contributed to the planning of data collection and in writing the article. Dr. Ketousetou Kuotsu, and Prof. Tapan Kumar Chatterjee designed the manuscript. The final manuscript was prepared by incorporating inputs from all the authors.

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CONFLICT OF INTEREST

Conflict of interest declared none

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