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Formulation and Standardization of controlled release silymarin microsphere for effective management of Diabetes

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ABSTRACT
Diabetes mellitus is a metabolic condition characterised by high blood glucose levels and changes in carbohydrate, lipid, and protein metabolism caused by insulin production, action, or both. Without adequate insulin, the body's cells are unable to absorb enough glucose from the blood, causing blood glucose levels to rise, a condition known as hyperglycemia. Silymarin(drug used) displays antioxidant and membrane stabilizing activity. It protects various tissues and organs against chemical injury, and shows potential as an antihepatoxic agent. Over the past decades, the treatment of illness has been accomplished by administering drugs to the human body via various pharmaceutical dosage forms, like tablets, capsules, etc. Diabetes mellitus is a major burden for individuals, their families, communities, and the country as a whole. To avoid diabetes development and consequences, all kinds of diabetes should be recognised early and managed effectively with effective property of silymarin microspheres.As a result, it is clear that silymarin microsphere may play a significant role in the pharma sector. Furthermore, it is obvious that, with the exception of a few medications, it should be introduced for the benefit of the community at a national level.
Keywords : Diabetes mellitus, anti-diabetic, silymarin, microsphere, microencapsulation

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by elevated blood glucose with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia.[2] Type 2 diabetes (Non-insulin-dependent diabetes mellitus) is a chronic metabolic disease that results from defects in insulin secretion and insulin receptor kinase. Investigation of novel small active molecule that can potentiate insulin action or having a similar action as insulin is important in the treatment of diabetes.[3,4] It is a serious complex chronic condition that is a major source of ill health worldwide. Besides hyperglycemia several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro-vascular complications of diabetes, which are the major causes of morbidity and death. World ethnobotanical information on medicinal plants reports almost 800 plants used in the treatment of diabetes mellitus. However, only a small number of them have been studied thoroughly.[5]

1.1. Type 1 diabetes mellitus

T1DM also known as insulin-dependent diabetes, is the consequence of insulin deficiency arising from the progressive destruction of pancreatic β-cells through an autoimmune response. Histologic analysis of pancreas in a patient with T1DM showedinfiltration of various immune cells including T and B lymphocytes, macrophages, dendritic cells, natural killer cells, as well as islet-reactive autoantibodies and isletreactive T-cells in the islets of Langerhans [6,7].

1.2. Type 2 diabetes mellitus

T2DM is mainly linked to insulin resistance. The latter is majorly attributed to obesity, caused by poor dietary and lifestyle habits. The sensitivity of insulin fluctuates with intake of carbohydrate-rich foods, amount of physical activity, as well as stress signals. Obese individuals contain more adipose tissues, relating to the higher secretion of hormones and other substances that may increase the fluctuation of insulin sensitivity. Circulating non-esterified fatty acids (NEFA) in obese individuals is also associated with insulin resistance. A high fatty acid environment with

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hyperglycaemic conditions can lead to reduced insulin gene expression [8,9]

II. MATERIAL AND METHODS

2.1 Evaluation of Parameters

Drugs, polymers and excipients were characterized for their physical properties such as angle of repose, density, compressibility index, Hausner's ratio etc.

Silymarin(drug used) displays antioxidant and membrane stabilizing activity. It protects various tissues and organs against chemical injury, and shows potential as an antihepatoxic agent.

2.1.1 Angle of Repose

The angle of repose was determined by the funnel method. The accurately weighed powder was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powder. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured.[9,10,11]

The angle of repose was calculated by using the following equation.

$\tan(\theta) = h/r$

Where 'h' and 'r' are the height and radius respectively of the powder cone.

Table 2.1 Standard values of angle of repose (θ)[12]

Flowability	Angle of repose
Excellent	25-30
Good	31-35
Fair	36-45
Poor	45-55
Very poor	56-65
Very very poor	>66

2.1.2 Bulk Density

Both loose bulk density (LBD). A known amount of granules from each formula, previously lightly shaken to break any agglomerates formed was introduced into a graduated measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own height onto a hard surface from the height of 2.5 cm at 2 second intervals. LBD were calculated using the following formulas.[13] The powder was carefully leveled and unsettled apparent volume was noted, then the apparent bulk density in g/ml was calculated by the following formula:[14]

LBD= Weight of the powder/volume of the packing

2.1.3 Determination of tapped bulk density

Accurately known amount of drug was taken, previously passed through 20 # sieve and was transferred in 100 ml graduated cylinder. Then the cylinder was mechanically tapped containing the sample by raising and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop at a nominal rate of 300 drops per minute. The cylinder was tapped for 500 times initially and measured the tapped volume (V1) to the nearest graduated units, repeated the tapping an additional 750 times and measured the tapped volume (V2) to the nearest graduated units. If, the difference between the two volume is found to be less than 2% then, final the volume (V2) was taken.[15,16,17]

TBD= Weight of the powder/tapped volume of the packing

2.1.4 Compressibility Index

The compressibility index of the granules was determined by Carr's compressibility index

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which was calculated by using the following formula:[18]

Carr's index (%) = [(TBD-LBD)×100]/TBD

2.1.5 Hausner's Ratio

Hausner found that the ratio DF/DO was related to interparticle friction and, as such, could be used to predict powder flow properties. It is calculated by using the following formula:[19]

Hausner's ratio = DF/DO

where,

DF is Tapped bulk density

DO is Loose bulk density.

Table 2.2 Standard values of Carr's index and Hausner's ratio[20]

Type of flow	Carr's index	Hausner's Ratio
Excellent	<10	1.00-1.11
Good	11-15	1.12-1.18
Fair	16-20	1.19-1.25
Passable	21-25	1.26-1.34
Poor	26-31	1.35-1.45
Very poor	32-38	1.46-1.59
Very, Very poor	>38	>1.60

2.1.6 Partition Coefficient

The octanol-water partition coefficient, Ko/w, describes equilibrium partitioning of pure drugs between octanol and water phases. It is an important parameter used in the assessment of environmental fate and transport of drugs because the octanol phase is a surrogate for the lipid phase or organic carbon content of the biological compartments...Ko/w is defined as an equilibrium ratio of the concentration of drug in the octanol phase, Co (µg mL-1) and its concentration in the aqueous phase, Cw (µg mL-1), calculated by using the equation: Ko/w = Co/Cw. The log Ko/w is referred as log partition value. A log partition value with "+" value indicates the drug is lipophilic and "–" value indicates the drug is hydrophilic in nature.[21,22,23]

2.1.7 Preparation of Silymarin microspheres by using different techniques

Silymarin and pectin micro beads were prepared by Solvent Evaporation Technique.[24,25] Different amount of pectin was dissolved in 10 ml acetone separately by using a magnetic stirrer. Silymarin was added to the polymer matrix and mixed for 15 minutes. The resulting dispersion was added to a mixture of 100 ml light liquid paraffin and contained 1% w/v span 80 in a 250 ml beaker, while stirring at 510 rpm using a mechanical stirrer. Stirring was continued for 45 minutes until the acetone evaporated completely. The micro beads formed were filtered using whatman no.1 filter paper. The residue was washed 4-5 times with 50 ml portions of n-hexane to wash the oil (LLP) completely. Silymarin loaded 100 microspheres were prepared by using same techniques as micro beads. Drug-embedded micro particles were rapidly formed in the dispersion when stirring was stopped, and the settled particles were collected and dried at room temperature and stored in desiccators at 25±1ºC. until further studies.[26]

2.1.8 Drug Entrapment Efficiency

Micro particles (25 mg) were pulverized and the powdered micro particles were suspended in 50 ml phosphate buffer (pH 7.4). After 24 h the solution was filtered and the filtrate was analysed by UV-VIS spectrometer at 276 nm. According to standard curve equation (y= 0.022x + 0.011), the percent drug entrapment efficiency (%DEE) was calculated as per the following formula[27,28]

Drug Entrapment Efficiency = (Drug content as per assay/ Drug content as per initial load)x100

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2.1.9 Determination of yield of the formulation

The drug content from the various formulations were determined by taking 25 mg of micro particles and crushed them to powder and dissolved in phosphate buffer of pH 7.4. Then the solution was filtered using membrane filter and analyzed for the drug content by UV-VIS Spectrophotometer at 276 nm.[29]

The micro particles yield was determined according to the formula:

Yield (%) = (Mass of micro particles/ Mass of drug + mass of polymer) x 100

III. RESULT AND DISCUSSION

Over the past decades, the treatment of illness has been accomplished by administering drugs to the human body via various pharmaceutical dosage forms, like tablets, capsules, etc. These traditional pharmaceutical products are still commonly seen today in the prescription and over-the-counter drug market place. To achieve and maintain the drug concentration in the body within the therapeutic range required for a medication, it is often necessary to take this type of drug delivery system several times a day.

3.1 Physical properties

Silymarin was received as gift sample from Macleod's Pvt. Ltd .The powder was observed for colour, taste, and odour. The physical properties of the sample was observed and compared with the physical properties reported. These properties were found to be identical with standard.[30]

Physical Property	Observation	Standard
Appearance	Crystalline	Crystalline
Color	Brownish	Brown
Taste	Bitter	Bitter
Odor	Odorless	Odorless

3.2 Melting point

These tests are performed to judge the purity of crude drug. The melting point was determined using capillary method. Small quantity of sample was placed into a capillary tube. The tube was placed in melting point apparatus (Jyoti Laboratories, India). The temperature at which sample started to melt and the temperature when the entire sample gets melted were observed.

S.no	Observed Melting point	Average Melting point	Reference Melting point
1.	150°C - 160°C	154°C	158°C
2.	145°C - 155°C		
3.	155°C - 160°C		

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Table 3.1 Characterization of microsphere

S.no	Formulation	Entrapment efficiency (%)	Drug Content (%)
1.	SM-1	52.33	54.42
2.	SM-2	73.82	70.49
3.	SM-3	74.32	54.93
4.	SM-4	86.45	67.36
5.	SM-5	61.35	49.12

Fig 3.1 Graph of entrapment efficiency and drug content (%)

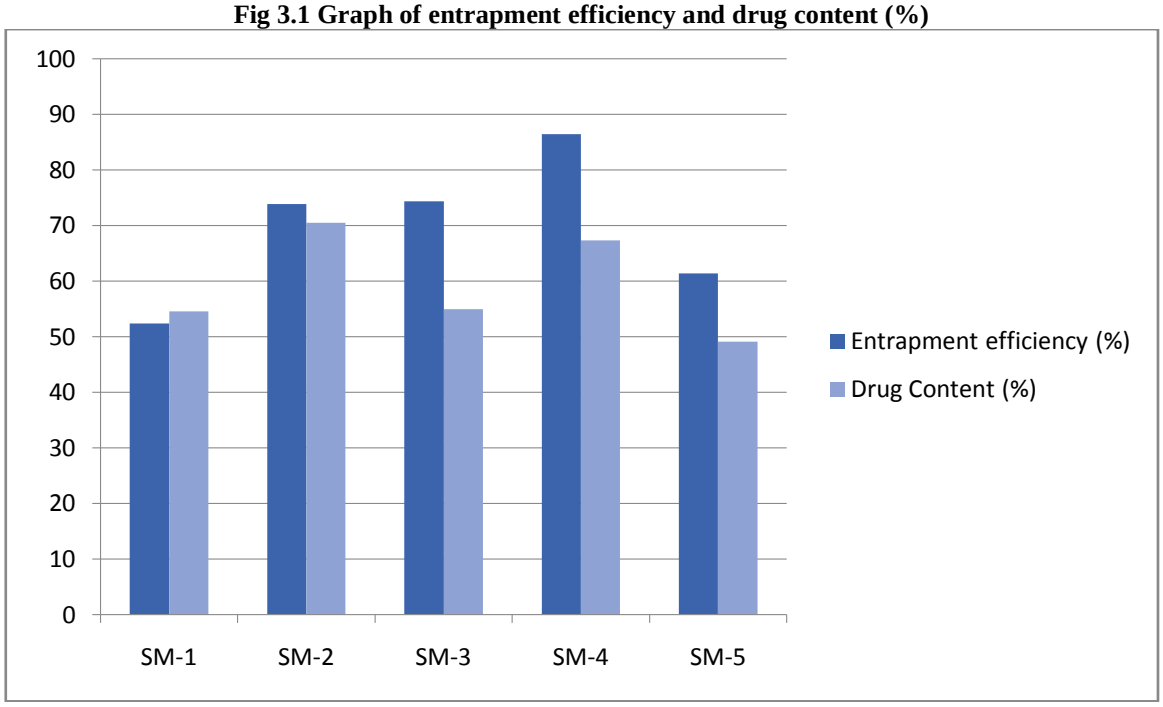

Table 3.2 Evaluation of Microspheres

S.no	Formulation	Zeta potential (mv)	Particle size (nm)
1.	SM-1	22.24	425
2.	SM-2	24.39	290

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3.	SM-3	26.36	389
4.	SM-4	27.20	345
5.	SM-5	26.54	234
6.	SM-6	28.36	546

Figure 3.2Evaluation of zeta potential

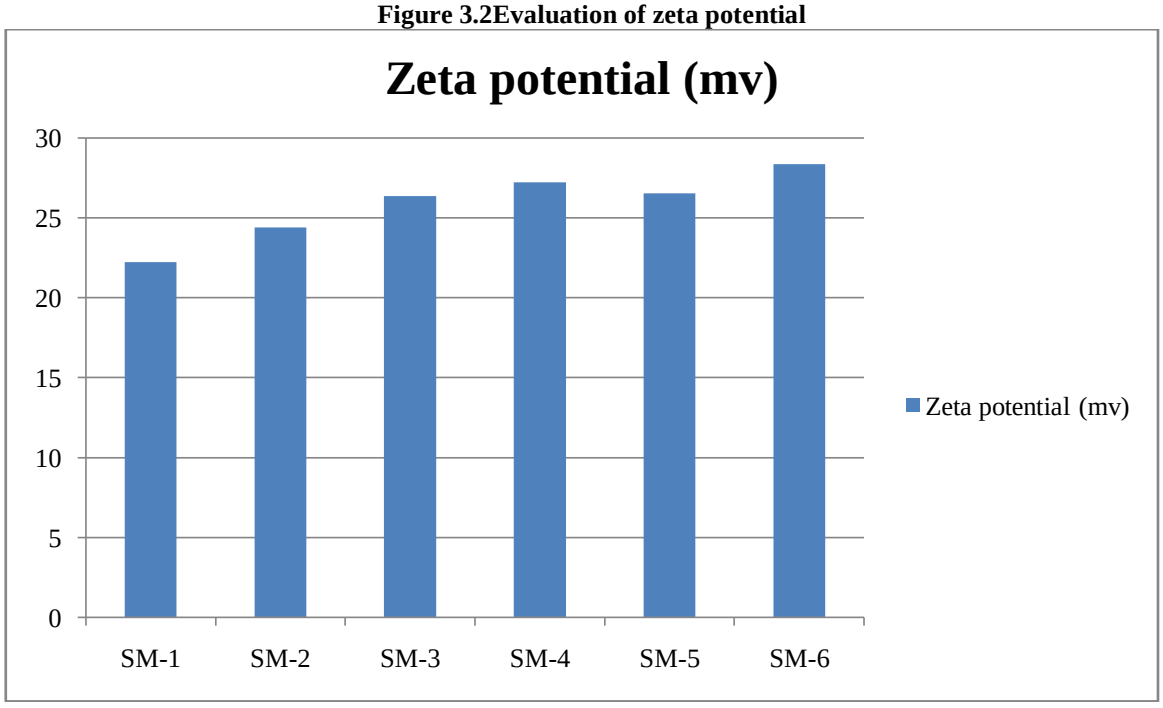

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Figure 3.3 Comparison of particle size

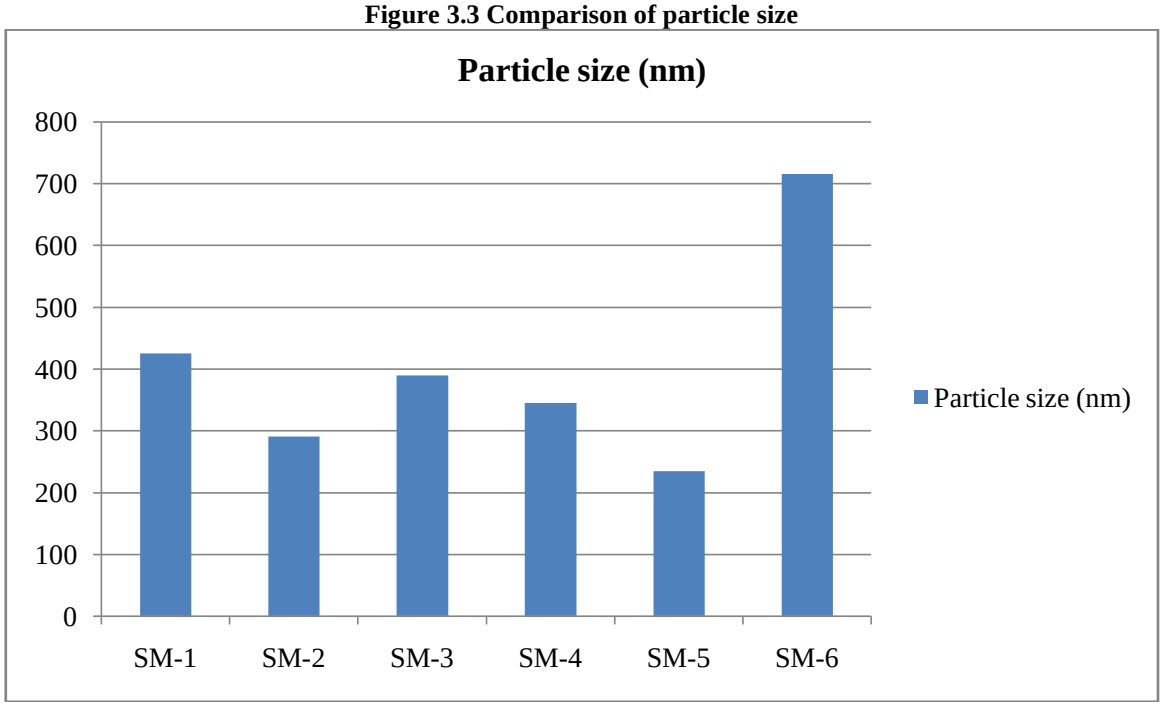

Table 3.3 Evaluation of Microspheres

Evaluation Parameters	Bulk density (g/cc)	Hausner`s ratio	Tapped density (g/cc)
Sm 1	0.44	1.12	0.49
Sm2	0.36	1.17	0.42
Sm3	0.55	1.19	0.65
Sm4	0.45	1.15	0.51
Sm5	0.47	1.18	0.55

Table 3.4 Evaluation of Microspheres

Evaluation Parameters	Compressibility index (%)	Angle of repose (θ)
Sm 1	10.20	21.16
Sm2	14.28	24.42
Sm3	15.23	23.36
Sm4	11.76	24.21

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Sm5	14.54	22.43

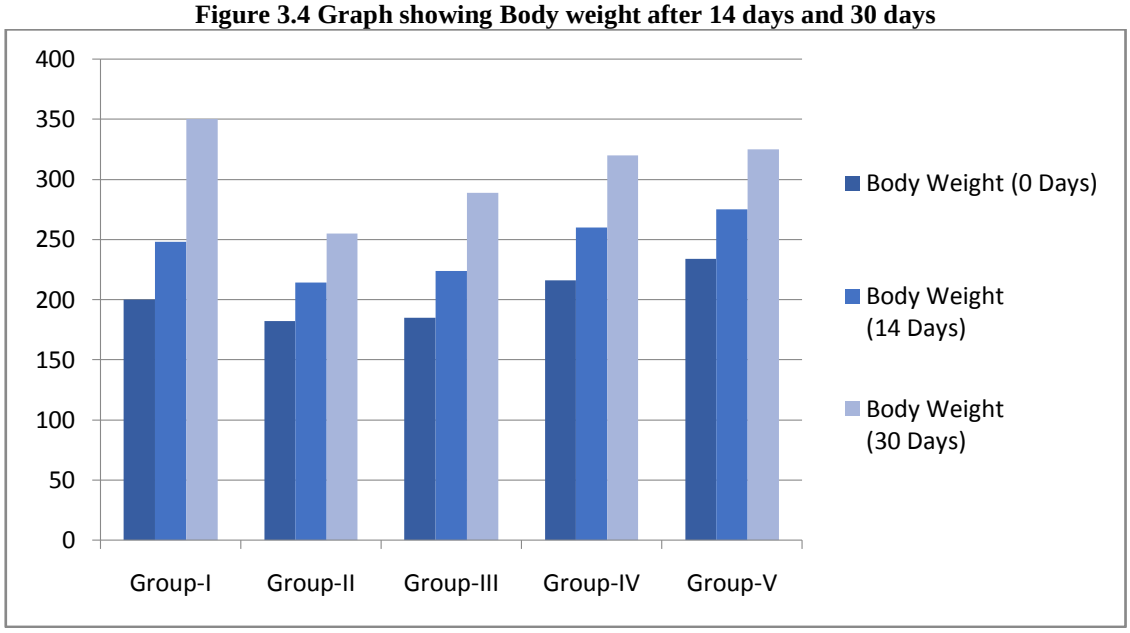
3.3 Weight gain

Regular Weight Of The Rat Was Measured During Dosage Period, (0 Days), (14 Days),(30 Days) Weight Was Considered To Calculate The Weight Gain. All The Rats In Groups I, III, IV, V Remained Healthy And Active With Normal Feeding Behavior. Their Body Weight Gain Was Found To Be The Lowest Among All Groups, Which Was Statistically Significant When Compared With The Control Group.

Table 3.5Weight gain in each group:

STUDY GROUP	Body Weight (0 Days)	Body Weight (14 Days)	Body Weight (30 Days)
Group-I	200	248	350
Group-II	182	214	255
Group-III	185	224	289
Group-IV	216	260	320
Group-V	234	275	325

Figure 3.4 Graph showing Body weight after 14 days and 30 days



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3.4 Measurement of biochemical parameters

Table 3.6 Effects of Drug on triglyceride and cholesterol levels of control and experimental groups of diabetic rat.

	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Group-1	51.23	39.30
Group-2	75.60	68.14
Group-3	69.31	36.31
Group-4	58.04	37.15
Group-5	56.21	39.25

Figure 3.5Graph showing effects of Drug on triglyceride and cholesterol levels of control and experimental groups of diabetic rat.

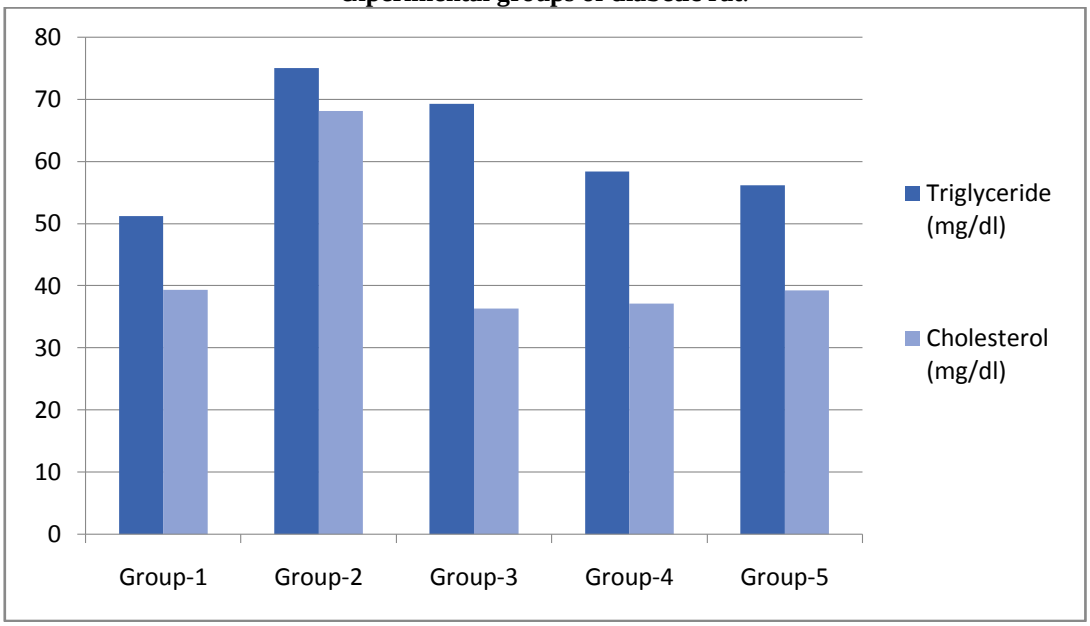

Table 3.7 Biochemical Parameters of different groups

	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Group-1	21.35	11.5	15.14
Group-2	29.78	19.4	19.5
Group-3	19.43	09.87	24.89

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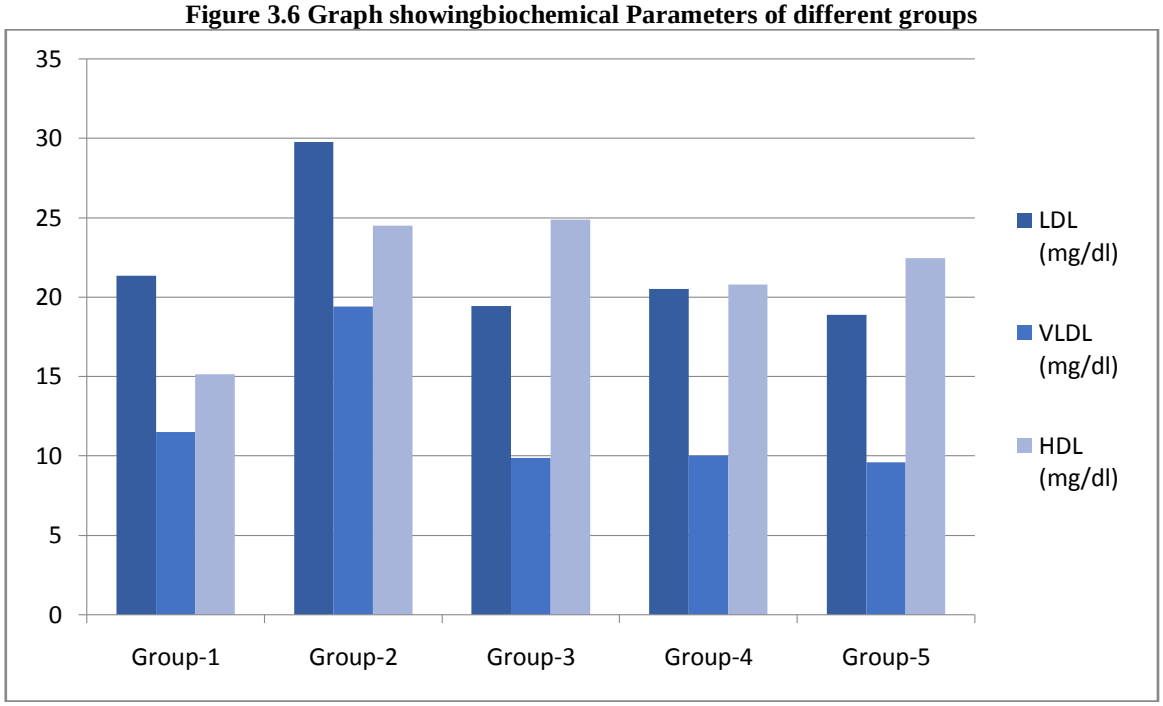
Group-4	20.52	10.01	20.78
Group-5	18.88	9.59	21.46

Figure 3.6 Graph showingbiochemical Parameters of different groups



3.4 Acute Toxicity Study

The biochemical parameters such as total protein, creatinine, alkaline Phosphatase, were within same ranges with a slight difference between control and experimental rats. P value for all markers was not significant among the groups.

Table 3.8Acute toxicity study of different parameters

S.no	Parameters	0 Min	6 Hrs	24Hrs
1.	Skin & Fur	Normal	Normal	Normal
2.	Respiratory rate	Normal	Normal	Normal
3.	Clinical signs	Normal	Normal	Normal
4.	Mortality	No	No	No
5.	Body weights	Normal	Normal	Normal
6.	Lethargy	Normal	Normal	Normal

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IV. CONCLUSION

Diabetes mellitus is a major burden for individuals, their families, communities, and the country as a whole. As mentioned earlier in the introduction chapter the all aims and objectives of this study were achieved. The achievements of this study can be concluded in short as follows:Silymarin micro beads were prepared by Solvent Evaporation Technique. Different amount of pectin was dissolved in 10 ml acetone separately by using a magnetic stirrer.Evaluation of silymarin microspheres 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 table in above. The angle of repose could not be measured by the above method for powders.The micro particles yield was determined according to the formula and the percent drug entrapment efficiency (%DEE) was calculated and recorded in the above chapter.Acute oral toxicity studies on were carried out using rats, to evaluate the toxic effects of drug. Data indicated that treatment of diabetic rats by low dose and high dose had no inhibitory effect on body weight reduction in diabetic rats.

As a result, we may infer that Silymarin composite might be employed as a prolonged drug release carrier, which would be useful for the treatment of diabetes mellitus with less frequent dosage. This shows that the formulation might help reduce vascular problems linked with diabetes.

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Conflict of Interest

The authors confirm no conflict of interest

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