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FORMULATION AND EVALUATION OF ANTI-DIABETIC ACTIVITY OF *Indigofera mysorensis* NANOPARTICLES

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Abstract:

Indigofera mysorensis is one of the most traditionally using hypoglycemic agents among tribes in India and it is not Scientifically validated. Based on the above mentioned reasons, one new research is required to develop one new drug with more anti diabetic activity The main aim is Formulation and evaluation of Indigofera mysorensis. Nanoparticles, To study the anti diabetic activity of developed activity. The body weight was decreased in alloxan diabetic rats. Administration of Ethanolic extract of Indigofera mysorensis and nano particles of Indigofera mysorensis at a dose of 200 and 50mg/kg increases the body weight in alloxan induced diabetic rats. The ability of Ethanolic extract of Indigofera mysorensis and nano particles of Indigofera mysorensis at a dose of 200 and (50mg/kg) to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia. From the results of the present experiments it may be concluded that formulation F3 showing formulation of Nanoparticle size, high percentage of entrapment and desired sustained release of Nanoparticle. Hence F3 formulation was optimized one. The optimized formulation F3 was found to follow zero order release pattern which was revealed by the linearity shown from the plot of time versus concentration. Nanoparticle formation were stable for 4 weeks at 4^0C and affirm the drug leakage — increased the higher temperature. Comparative study of Nanoparticle formulation with the marketed. The Nanoparticle formulation revealed that anti diabetic activity wasreleased from the Nanoparticle formulation in a sustained manner for 24 Hours.

Keywords: *Formulation, Evaluation, Anti Diabetic Activity, Indigofera Mysorensis Nanoparticles*

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INTRODUCTION:

Diabetic Mellitus (Hyperglycemia) is an endocrine disease and not a single disease which is a group of chronic metabolic or heterogeneous affliction due to the irregular secretions of insulin and action of insulin or both. Absence or reduced insulin in turn leads to abnormal high blood sugar level and glucose intolerance. Diabetes mellitus (DM) is a chronic illness due to inadequate insulin production by pancreatic β cells. Diabetes could be hereditary or acquired. The insufficiency of insulin is a consequence of high blood glucose levels, which cause damage to various body systems, especially the circulatory and nervous systems [1,2]. As evident from the data of the International Diabetes Federation (IDF), the incidence of diabetes is rising internationally. In 2021, 536.6 million people had diabetes worldwide, and by the year 2045, it is projected to increase by 46% to 783.2 million [3]. As previously estimated by the IDF and other surveys, almost 50% of all patients with diabetes are oblivious to their illness [4]. Changing one's lifestyle to include increased physical exercise, consuming low-calorie foods, and avoiding inactive habits is necessary for DM prevention and control [5]. Although synthetic drugs like miglitol and acarbose have high inhibitory effects against alpha-amylase and alpha-glucosidase, they have consequences like causing diarrhea, nausea, vomiting, and intestinal swelling [6]. Therefore, plant extracts that are active in lowering serum glucose levels with slight or no side effects are used as hypoglycemic medications. Various compounds extracted from plants are used in combinational treatment for diabetes, such as *Azadirachta indica*, *T. indica*, and *Ceilba pentandrat, which* are well-known for their hypoglycemic properties. Therefore, the green synthesis methods are an attractive option. Green nanotechnology refers to the use of nanotechnology to enhance the environmental sustainability of processes producing negative externalities [7]. It includes making and using nano-products in support of sustainability. Biologically developed chemicals are used in these methods, which are not harmful for the environment [8]. Green nanotechnology has two goals: producing nanomaterials and products without harming the environment or human health, and producing nano-products that provide solutions to environmental problems [9]. Due to the wide range of applications of NPs, researchers, including biologists, chemists, physicists, and engineers, are working in this fascinating area [10]. Green synthesis has many advantages compared to chemical and physical methods; it is non-toxic, pollution-free, environmentally friendly, economical, and more sustainable [11,12]. Therefore, accessing green principles offers a high degree of safety, eco-friendliness, and cost-effectiveness. On green pathways, nanoparticles can be fabricated using natural compounds extracted from various biomass precursors, such as bacteria, fungi, biomolecules, and plant extracts [13]. The most important feature of the biogenic approach is the utilization of biologically reducing and capping agents to replace toxic chemicals. This alternation makes the biosynthetic method environmentally friendly, benign, and inexpensive [14].

Indigofera mysorensis Rottl. ex DC., Fabaceae, commonly known as Konda vempali, is a glutinous shrub used for its antidiabetic activity in rural India.[5] It is common in dry deciduous forests, especially on exposed rocky slopes. It is common in dry deciduous forests, especially on exposed rocky slopes of Japalitheertham in Tirumala, way to water falls at Talakona, Punganur and border of Karnataka State. Leaves simple, trifoliate or imparipinnate the side leaflets usually opposite, but sometimes alternate, entire; stipules usually small shortly adnate to the petiole. Flowers are generally very small usually reddish or purple, in axillary panicles. Calyx minute campanulate, teeth sub equal or the lowest longest. Corolla more or less caduceus, standard ovate or orbicular, sessile or slightly clawed; wings oblong slightly adherent to the keel; keel petal erect, obtuse with a downward spur on each side near the base stamens didelphous the vexillary stamens free the other with connate filaments; anther uniform apiculate. Ovary sessile or subsessile 1-2 pod cylinder, pubscent, 3-5 in long 3-4 seeded.

Indigofera mysorensis is one of the most traditionally using hypoglycemic agents among tribes in India and it is not Scientifically validated. Based on the above mentioned reasons, one new research is required to develop one new drug with more anti diabetic activity The main aim is Formulation and evaluation of *Indigofera mysorensis*.L Nanoparticles, To study the anti-diabetic activity of developed activity.

MATERIALS AND METHODS:

Table 1: List of Instruments Used

S.No	Materials	source
1	*Indigofera mysorensis*.Flower	Chittor,AP
2	Hydroxyl propyl methl cellulose	S.D.Fine Chem Ltd.Boisar
3	Sodium lauryl sulphate	S.D.Fine Chem Ltd.Boisar
4	Methanol	S.D.Fine Chem Ltd.Boisar
5	Chloroform	S.D.Fine Chem Ltd.Boisar
6	Methanol	S.D.Fine Chem Ltd.Boisar

EXPERIMENTAL INVESTIGATION

Physicochemical Evaluation Flower of *Indigofera mysorensis*.L:

Analysis of Physiochemical constants of the powder flower has been done to evaluate the quality and purity of the drug. Various physiochemical parameters like moisture contents, foreign organic matters, Ash values and Extractive values ware calculated as per WHO guidelines. The information collected from these tests was useful for standardization and obtaining the quality standards.

Extraction Process of Flower *Indigofera mysorensis*.L:

Indigofera mysorensis .L flowers (500g) were extracted with1500 ml of Ethanol by the method of continuous hot extraction at 60 °C for 6 h and evaporated. The residual extract was dissolved in water and used in the study

Determination of Absorbance maximum

Indigofera mysorensis. L was dissolved in phosphate buffer saline7.4 pH, solution with 20 µg / ml concentration was prepared by suitable dilution The *Indigofera mysorensis* .L drug in solution was scanned in UV spectrophotometer from 200 to 400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 284 nm. The drug was later quantified by measuring by measuring the absorbance at 284nm in phosphate buffer saline pH 7.4.

Standard Curve for *Indigofera mysorensis* .L (by UV method)

Preparation of primary stock solution

Indigofera mysorensis .L 100 mg weighed and dissolved in phosphate buffer saline p H 7.4 in 100 ml volumetric flask. The flask was shaken and volume was made up to mark with phosphate buffer saline pH 7.4 to give a solution containing 1000µg /ml.

Preparation of secondary stock solution

From the primary stock solution, pipette out 2 ml and placed into 100 ml volumetric flask. The volume was made up to mark with phosphate buffer saline pH 7.4 to give a stock solution containing 20 µg /ml

Preparation of sample solution

Appropriate volumes of aliquots (1to10ml) from standard *Indigofera mysorensis*.L secondary stock solution were transferred to different volumetric flasks of 10 ml capacity The volume was adjusted to the mark with phosphate buffer saline p H 7.4 to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg /ml . Absorbance of each solution against phosphate buffer saline p H 7.4 AS blank were measured at 284 nm and the graph of absorbance against concentration were plotted and shown in Figure.

Table 2: Standard curve data for Indigofera mysorensis.L alcoholic extract(By U.V method)

Concentration in µg /ml	Absorbance at 284 nm
2	0.125
4	0.197
6	0.291
8	0.389
10	0.483
12	0.576
14	0.670
16	0.769
18	0.865
20	0.939

Formulation of *Indigofera mysorensis*.L Extract Nanoparticles:

Emulsion Solvent by Evaporation Method

The emulsion thus formed was further evaporated by flash rotatary evaporator for20 min. The nanoparticle was All batches of nanoparticles were prepared by Emulsion Solvent Evaporation Method. The required quantity of polymer was dissolved in 2.5 mlof water and 2.5 ml of chloroform in (1:1) ratio as organic phase. The organic phase was then mixed with an aqueous phase containing drug and 0.2% polyvinyl alcohol (4ml). The polymer concentration differs in various batches formulation as given in table

This mixture was homogenized by vortex mixture for 1 min and then sonicated using a probe set at 55w collected by ultra-centrifugation (15000rpm)

Table 3: Various Composition of nanoparticles formulation

S.NO	ORMULATIONCODE	INDIGOFERA MYSORENSIS.L	OLYMERHPMC	OLYMERHPMC
1	F1	200	300	-
2	F2	200	200	-
3	F3	200	100	-
4	F4	200	-	300
5	F5	200	-	200

Evaluation of Indigofera mysorensis.L nanoparticles:

Fourier Transformer Infrared (FTIR) spectral study:

IR study was carried out for identification of pure drugs. IR spectroscopy (using Perkin Elmer) by KBr Pellet method was carried out on drug. They are compressed under 15 tons pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1}in a spectrometer and peaks obtained were identified Infrared (I.R.) spectrum of drug, physical mixture of drug-polymer loaded microsphere gives information about the group present in that compound. Before I.R. spectra studies, Physical mixture of drug-polymer and microsphere were dried in vaccum for 12 hours. Potassium bromide (KBr) 200mg in 3mg test sample was used to prepared discs, scan under the range 4000 — 400 wave number (cm-1) and % Transmittance employing Perkin Elmer (USA). The above experiments were performed in triplicate manner to confirm the results.

Particle size analysis and Scanning Electron Microscopy (SEM) study:

The particle size of microspheres were determined using Scalar-USB Digital scale ver. EPhoto microscope, attached with canon camera system based on mean diameter and then calculated size distribution. The surface morphology and shape of microspheres were analyzed by a Scanning Electron Microscopy (SEM, Hitachi Model S-3000H, CECRI, Karaikudi, Tamilnadu, India). During the SEM examination, a drop of microspheres dispersion to be examined was mounted over a SEM stub and dried in desicator. Microspheres were coated with very thin coat of gold employing a vaccum evaporator to make electrically conductive. Then the size of the microspheres was recorded under SEM at a magnification ranging from 500X to 3000X and operated at an accelerating voltage of 20 kV.

Particle Size and Zeta Potential

Value of Particle size and Zeta Potential prepared nanoparticles determined by using Malvern Zetasizer (Ver. 6.11In SAS process liquid solvents are used, which should completely miscible with the supercritical fluid. The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid, the extractof the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, it results the formation of nanoparticles. In RESS high degree of super saturation occur by dissolving solute in a supercritical fluid to form a solution, followed by therapid expansion of the solution across an orifice or a capillary nozzle into ambient air by the rapid pressure reduction in the expansion which results in homogenous nucleation and thereby, the formation of well-dispersed particles.

Differential Scanning Calorimetry (DSC) study:

The thermal behavior of ACF, physical mixture of drug-polymer and drug-loaded microspheres were investigated employing differential scanning calorimeter (DSC-60 Instruments, Shimadzu Corporation,Japan). The samples (5mg) were accurately weighed, sealed hermetically into aluminum pans and heating run for each sample kept from 50°C- 300°C at a heating rate of 10°Cper min, using in atmosphere of air as blanket gas.

***In vitro* Drug Release Profile:**

The *in vitro* dissolution studies were carried out in phosphate buffer solution (PBS), 900 mL of pH 7.4,maintained at 37 ± 0.5°C temperature thermostatic controlled water bath, 100 rpm by

employing basket-type dissolution apparatus (United States Pharmacopeia XXIV) of eightstation (Electro-lab, Mumbai, India). Microspheres weighed contain 200 mg of were used as test sample. Withdrawn the sample solution (5ml) at predetermined time intervals over a period of 12hours, filtered through a 0.45 mm membrane filter,diluted suitably, and assessed for drug release at 284nm for ACF by using a UV spectrophotometer(Shimadzu UV-1700, Japan). After each withdraw, Immediately supplemented an equal amount of fresh PBS. Each determination was performed thrice and the percent cumulative drug release plotted as the percent drug release in dissolution media Vs time

Stability Studies

The optimized formulation F3 was subjected to stability study for one month at 4 ºC (refrigerator) room temperature and at 45 ºC / 75% RH. At the interval of 30 days, samples of nanoparticles formulation were taken and evaluated for the entrapment efficiency and *in vitro* release of drug. Entrapment efficiency of optimized formulation F9 kept at 9º C shows a release rate of 95.5% after 30 days of stability study .The entrapment efficiency of formulation got decreased on exposure to higher temperature. The percentage entrapment of formulation kept on room
temperature and at 45ºC /75% RH were 82% 81% respectively after 30 days of stability study.

Release Kinetic Studies

For estimation of the kinetic and mechanism of drug release, *invitro* drug release study of nanoparticles were fitted with various kinetic equation like were used to described the release kinetic. The zero order release states that drug release rate was independent of its concentration. The first order release describes, the releases rate from the system was concentration dependent. Higuchi described the releases of drug from insoluble matrix as a square root of time dependent process was based on Fickian diffusion.

1. Zero order - Cumulative % drug release versus time.
2. First order -Log cumulative % drug remaining versus time.
3. Higuchi's model -Cumulative % drug released versus square root of time.
4. Korsmeyer equation / Peppa's model — Log cumulative per cent drug released versus log time.

Table 4: Diffusion exponent and solute release mechanism for cylindricalshape

S.No	Diffusion	Exponent (n) Overall solute diffusionmechanism
1	0.45	Fickian diffusion
2	0.45<n<0.89	Anomalous (non-Fickian)Diffusion
3	0.89	Case - 2transport
4	n>0.89	Super case-2 transport

Morphology:

The nanoparticle morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscoy (SEM) at different magnifications .A few mg of prepared nanoparticles was gold coated using a Hitachi HVSJGB vaccum evaporater. Coated samples were viewed and photographed in a Hitachi S-450 SEM operated at 20kv.

Anti-diabetic activity:

***In vitro* α- Amylase Inhibition Assay**

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 di nitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540nm. Calculation of 50% inhibitory concentration (IC50).The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract .

Experimental Models of *Indigofera mysorensis*

For the study of anti-diabetic an experimental model is selected in such a way that it would satisfy the following:

- The animal should develop hyperglycemia rapidly.
- Pathological changes in the site of induction should result from pancreatitis or damage of β-cells.

- The symptoms should be ameliorated or prevented by a drug treatment effective in human beings.

Treatment Protocol

- Group-I: (Normal control) consist of normal rats given with 10ml/Kg of normal saline, orally.
- Group-II: (Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate through I.P.
- Group-III: Diabetic control received glipizide at a dose of (10mg/Kg orally) for 28days.
- Group-IV: Diabetic control received Ethanolic extract of *Indigofera mysorensis* at a dose of (200mg/Kg orally) for 28 days.
- Group-V: Diabetic control received Nano particles of *Indigofera mysorensis* at a dose of (50mg/Kg orally) for 28 days.

Methodology:

Sample collection:

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocularpuncture. in EDTA coating plasma tubes for the estimation of blood parameters.

RESULT AND DISCUSSION:

Collection of Flower *Indigofera mysorensis*:

The fresh flower of *Indigofera mysorensis*.L.was collected in the month of November from vagaikulam, thoothukudi, tamilnadu. The plant was identified and authenticated by The American college, Madurai. The fresh bark was used for the study of macro morphological and microscopy, physicochemical characterization, and phytochemical analysis.

Formulation of *Indigofera mysorensis*. Nanoparticles

The entrapment efficiency (%) of the *Indigofera mysorensis*. Loaded nanoparticles formulations (F1 ,F2 ,F3, , and F4) and an optimized F3 was determined and tabulated.

Table 5: Entrapment efficiency of formulations with drug and copolymer

S.no	nulationCode	Amount of Drug entrapment(mg)	PercentageEntrapmentEfficiency
1	F1	57.86 ±0.45	42.14±0.12
2	F2	36.44±0.24	63.56±0.14
3	F3	6.57±0.19	93.47±0.15
4	F4	10.15±0.32	89.85±0.17

The formulation has showed entrapment efficiency of the formulation F3 showed % entrapment efficiencies of 93.47.The decrease in the % entrapment efficiency then compared to formulation F4 was due to increased copolymer concentration because of higher hydroscopic or steric interactions between the polymer and drug.

Hence the F3 formulation has shown highest % entrapment efficiency with higher drug loading content when compared with other formulations. So it was selected as anoptimized formulation.

Evaluation of Indigofera mysorensis NANOPARTICLES:

FT-IR Spectrum *Indigofera mysorensis*.

FT-IR Spectrum was taken to study the compatibility of the drug and other excipients. Results show that there is no significant interaction of excipients with the drug in the formulation

Frequency cm^{-1}	Functional Groups
894.93	C-H
1070.49	C-H
1450.47	O=H
1514.12	N-O
2937.59	C-H
3336.85	C-H

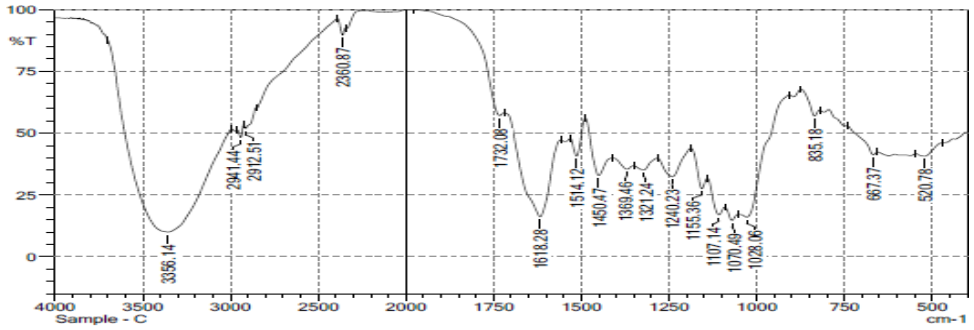


Fig:1. FT- IR of Indigofera mysorensis

Frequency cm^{-1}	Groups Assigned
894.97	C-H
1070.49	C-O
1450.47	O-H
1514.12	N-O
1562.06	N-O
2937.59	C-H
3336.85	C-H

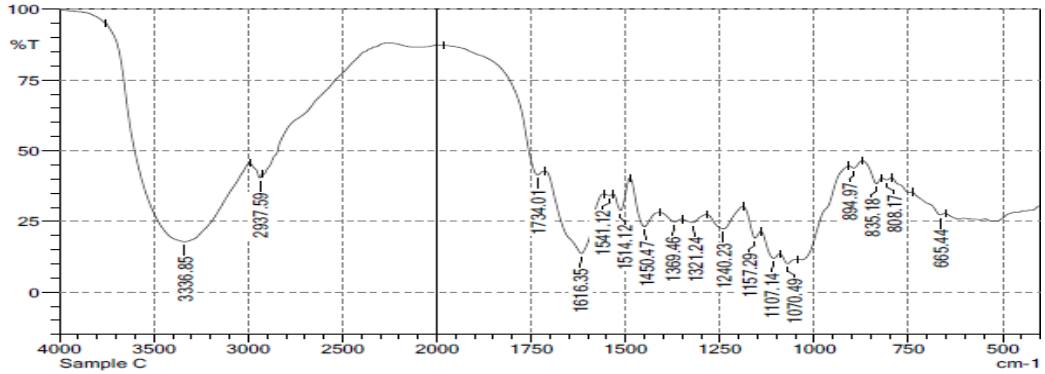


Fig: 2. FT-IR of *Indigofera mysorensis* nanoparticle

Important peaks, seen in the FT-IR spectrum of extraction of Indigofera mysorensis are exhibited in the nanoparticle of Indigofera mysorensis formulation. Hence there was no significant interaction between the drug and the excipients.

***In vitro* Drug release profile of *Indigofera mysorensis* Nanoparticles**

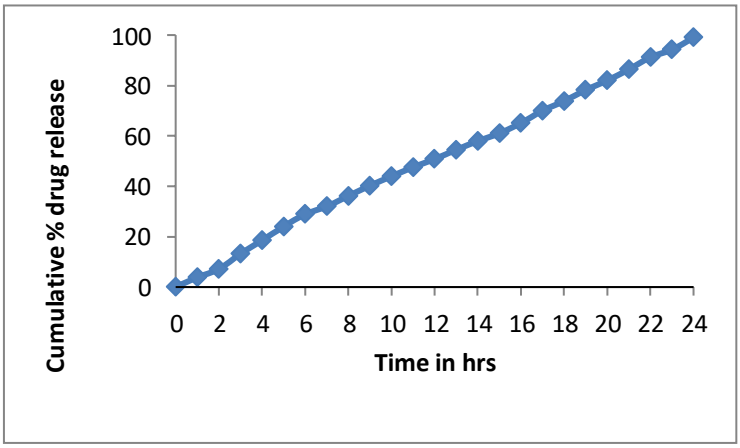
The in vitro drug release studies were performed for the the prepared for the prepared Nanoparticles formulations (F1, F2, F3, and F4). Results were tabulated and graphs were made by plotting cumulative % drug release against time in hours on the y axis and x axis respectively. The *in vitro* drug release characteristics for prepared Nanopariticle formulations were evaluated with the help of release profiles in graphical plots.

The formulation F3 has showed a drug release of in 93.47 % in 24 hours. Where as the F4 Showed a drug release of 89.85 %.The decrease in drug release when compared with the F3 formulation was found to be due increased hydrophobic or steric interactions between drug and polymer. Hence the F3 formulation was selected as the best formulation with highest percent drug release and also having the higher % encapsulation effienency then compared to the other formulations.

Table 6: *In vitro* drug release data of F3 formulation

S.No	Time in hrs	Percentage Drug release	lative % drugrelease
1	1	3.8	3.8
2	2	7.1	7.1
3	3	13.1	13.1
4	4	18.5	18.5
5	5	23.9	23.9
6	6	28.9	28.9
7	7	31.92	31.92
8	8	36.12	36.12
9	9	40.19	40.19
10	10	43.9	43.9
11	11	47.5	47.5
12	12	50.75	50.75
13	13	54.42	54.42
14	14	57.92	57.92
15	15	60.95	60.95
16	16	65.01	65.01
17	17	69.95	69.95
18	18	73.75	73.75
19	19	78.12	78.12
20	20	81.95	81.95
21	21	86.25	86.25
22	22	91.17	91.17
23	23	94.11	94.11
24	24	98.98	98.98

Fig: 3. *In vitro* drug release data of F3 formulation



Stability study

The optimized formulation F3 was subjected to stability study for one month at 4 ºC (refrigerator) room temperature and at 45 ºC / 75% RH. At the interval of 30 days samples of nanoparticles formulation were taken and evaluated for the entrapment efficiency and *in vitro* release of drug. Entrapment efficiency of optimized formulation F9 kept at 9º C shows a release rate of 95.5% after 30 days of stability study .The entrapment efficiency of formulation got decreased on exposure to higher temperature. The percentage entrapment of formulation kept on room temperature and at 45ºC /75% RH were 82% 81% reapectively after 30 days of stability study

The in vitro release data of optimized formulation f 9 shows that the nanoparticles formulations are more stable at 45 ºC / 75% RH (stability chamber), .The Nanoparticles formulation kept at 4 ºC showed a cumulative release of 89.89% after 30 days of

stability studies

Release Kinetics Studies

The release kinetics was studied for an optimized formulation F3 by plotting the graphs for different kinetic models by using the in vitro drug release data

Zero order plot:

Zero order plot of a freeze dried formulation F3 was fond to be linear with a regression value of, 0.996 which signifies that the drug was released in a controlled manner form the nanoparticles during the release study.

First order plot:

The first order plot was made by plotting log remaining cumulative % drug release against time and the regression value was found to be 0.75 which indicates that drug release was not followed the first order rate kinetics.

Higuchi plot:

Higuchi plot was found to be of linear with a regression value of ,0.948 which indicates that diffusion was one of the mechanisms of the drug release from Nanoparticles matrices.

Korsmeyer - Peppas plot:

The type of *invitro* mechanisam of drug release was best explained by Korsmeyer — Peppas plot. The plot was found to be liner with R^2 value

And diffusion exponent n value was 0.626, According to Korsmeyer — Peppas equation, mechanisam of drug release based on (given in table no:4), which indicates that mechanism of drug release from copolymer matrices was followed

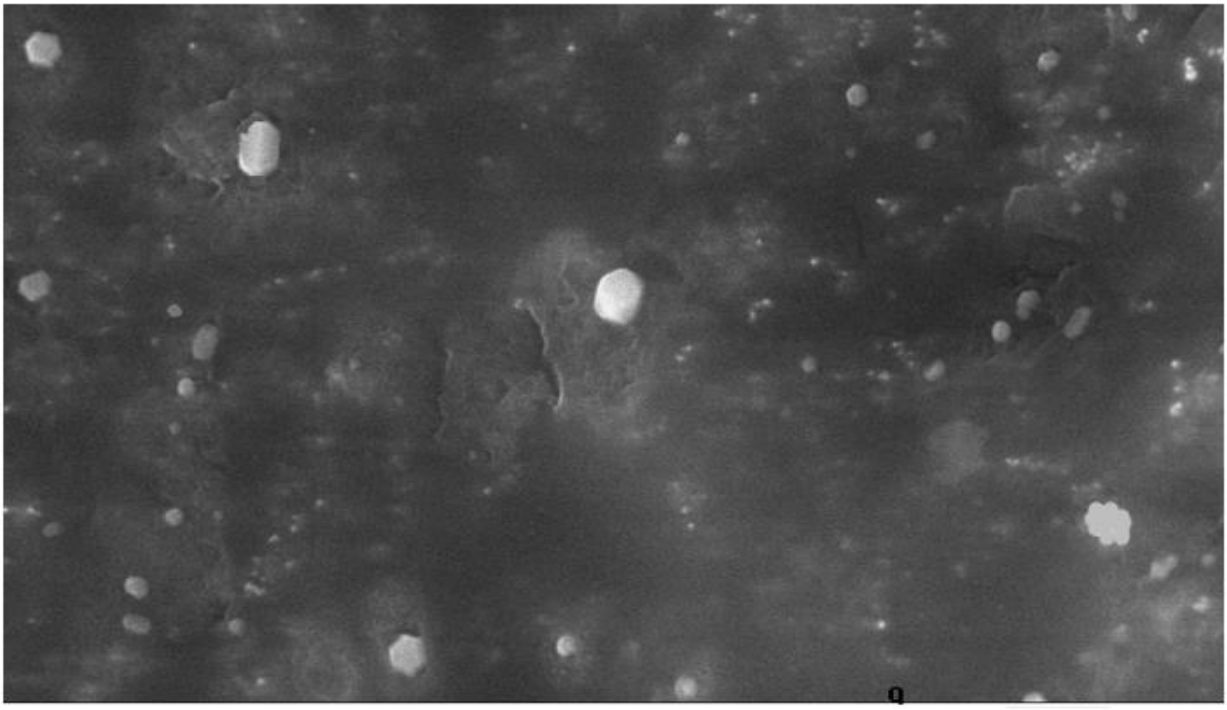
Anomalous (non-Fickian) diffusion. So the kinetic of an optimized F3 formulation with different kinetic models has showed that the *Indigofera mysorensis*.L . Release mechanism was found to be Anomalous (non-Fickian) based on n value from Korsmeyer – Peppas plot.

Morphology:

The nanoparticles morphology, surface, appearance and shape of the nanoparticles was analysed by Scanning Electron Microscopy (SEM) at different magnifications. A few mg of prepared nanoparticles was gold coated using a Hitachi HVSJGB vaccum evaporter. At 20 kv. It was found that the nanoparticles were mostly spherical in shape

and some nanoparticles are slightly elongated, discrete and almost uniform in size . Themorphology on the nanoparticles is shown in Fig

Fig: 4. SEM Photograph



Morphology:

SEM photograph of the prepared nanoparticles reveal that they are discrete, spherical in shape, amorphous in nature and had a matrix type structure. No drug crystals have been identified.

Antidiabetic activity:

In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered. Persistent hyperglycemia is a major contributor to such metabolic alterations, which lead to the pathogenesis of diabetic complications. The study was designed by L Pari, M Latha in 2002, to study the effect of *Indigofera mysorensis* flower extract on hepatic glycolytic and gluconeogenic enzymes and STZ-diabetic rats were given the plant's extract per os for 30 days. In conclusion, the observations showed that the aqueous extract possessed an antihyperglycemic effect and suggested that enhanced gluconeogenesis during diabetes is shifted towards normal and that the extract enhanced the utilization of glucose through increased glycolysis. The effect of the extract was more prominent than that of glibenclamide

Estimation of blood glucose:

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method. [93]

Table no: 20 illustrates the levels of initial and final blood glucose, and change in body weight, in normal rat, and treatment control animals in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and 50mg/kg was increased the body weight non-significantly as compared to normal control animals.

Fasting blood glucose level was significantly increased 219.48 ± 6.96 in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range in diabetic rats treated with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg).

Estimation of blood glucose:

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method.

Table 7: Effect of Ethanolic extract of *Indigofera mysorensis* on initial and finalbody weight and blood glucose in normal and treated animals.

GROUP	Body weight (g)		Blood glucose (mg / 100ml) Initial	Blood glucose (mg / 100ml) Final
	Initial	Final		
G1	234 ± 7.25	242± 7.60	92.60 ± 3.34	92.75 ± 3.80
G2	236 ± 7.48	176 ± 4.46**(a)	90.94 ± 3.30	222.75 ± 6.90**(a)
G3	238 ± 7.52	240 ± 7.55	88.95 ± 3.22	123.35 ± 4.30**(b)
G4	232± 7.18	242 ± 7.45	84.25± 3.16	141.50± 5.32**(b)
G5	230 ± 7.10	244 ± 7.50	94.36 ± 3.75	131.15 ± 4.40**(b)

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed byNewman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.
  ** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rat and treatment control animals in each group.

The levels of total hemoglobin, and plasma insulin levels were decreased significantly where as glycosylated heamoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin andplasma insulin, returned to near normal range in diabetic rats treated with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg).

Plasma insulin:

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit[with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of total haemoglobin and glycosylated haemoglobin:
Total haemoglobin was determined by the method of Drabkin and Austin (1932) [5] and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman (1981).

Table 8: ffect of Ethanolic extract of *Indigofera mysorensis* on plasma insulin,Hemoglobin & Glycosylated hemoglobin in normal and treated animals.

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA_1 (%)	Plasma Insulin (µU/ml)
G1	12.90 ± 1.68	0.47 ± 0.06	40.58 ± 2.85
G2	6.34 ± 0.82**(a)	0.96 ± 0.16**(a)	13.85 ± 1.90**(a)
G3	14.4 ± 1.48**(b)	0.42 ± 0.07**(b)	29.48 ± 2.52**(b)
G4	12.79 ± 0.90**(b)	0.49 ± 0.14**(b)	26.70 ±2.50**(b)
G5	11.98 ± 1.22**(b)	0.44± 0.05**(b)	28.90± 2.80**(b)

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table . shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL),Low density lipoprotein(LDL) and phospholipids of normal and experimental animals in each group.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, where as HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with Ethanolic extract of *Indigofera mysorensis* andnano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) for 28 days resultedin marked decrease in total cholesterol, triglycerides, Low density lipoprotein (LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

Estimation of lipid & lipoprotein:
Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970) (total cholesterol), Gidez and Webb (1950) (HDL-cholesterol),
Zilversmith and Davis (1950) (phospholipids) and Rice (1970) (triglycerides).

the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL) and phospholipids of normal and experimental animals in each group.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, whereas HDL-C level was decreased in alloxan induced diabetic rats as compared to normal rats. Treatment of normal and alloxan induced diabetic rats with Ethanolic extract of *Indigofera mysorensis* andnano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) for 28 days resultedin marked decrease in total cholesterol, triglycerides, Low density lipoprotein (LDL) and phospholipids levels and increase in HDL-C levels as compared to alloxan induced diabetic rats.

Table 9: Serum lipids of Normal and experimental groups.

GROUPS	Total Cholesterol (mg/dl)	riglyceride(mg/dl)	HDL-C (mg/dl)	hospholipids(mg/dl)	LDL (mg/dl)
G1	88.30 ± 2.62	93.75 ± 2.68	61.65 ± 1.70	131.80 ± 2.42	16.50 ±1.52
G2	231.35 ± 6.72**(a)	163.60 ± 4.62**(a)	31.72 ± 1.40**(a)	222.60 ± 6.45**(a)	38.65 $\pm_{2.45}$**(a)
G3	123.84 ± 3.40**(b)	99.65 ± 2.54**(b)	48.90 ± 1.43	155.48 ± 3.80	28.05 $\pm_{1.89}$**(b)
G4	133.58 ± 3.70**(b)	120.75 ± 2.88**(b)	39.40 ± 1.37**(b)	163.70 ± 4.18**(b)	29.24 $\pm_{1.83}$**(b)
G5	123.20 ± 3.40**(b)	96.80 ± 2.64**(b)	43.70 ± 1.64**(b)	160.45 ± 3.85**(b)	24.44 $\pm_{1.78}$**(b)

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.

** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Alloxan causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. The mechanism of alloxan action was fully described elsewhere (Lazarow, 1964; Colca et al., 1983). In our study, we have observed a significant increase in the plasma insulin level when alloxan induced diabetic rats were treated with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) this could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β- cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of several proteins including haemoglobin and α-crystalline of lens (Alberti and Press, 1982). Glycosylated haemoglobin (HbA_1C) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) and the amount of increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979). During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan induced diabetic rats (Sheela and Augusti, 1992). Administration of Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) for 28 days prevents a significant elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg).

The body weight was decreased in alloxan diabetic rats. Administration of Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and 50mg/kg increases the body weight in alloxan induced diabetic rats. The ability of Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

The level of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coronary heart disease (CHD). Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. [18] The abnormal high concentration of serum lipids in diabetic subject is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

In the alloxan-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low density

lipoprotein (LDL) levels were brought to near normal by the treatment with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg)in alloxan induced diabetic rats.

DISSCUSION:

The effect of Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and 50mg/kg on diabetic hyper triglyceridemia could be through its control of hyperglycaemia. This is in agreement with the facts that:

1. The level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations.
2. Improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides.
   The main 'anti-atherogenic' lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver and thereby it acts as a protective factor against coronary heart disease (CHD).

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats. [123] Our results clearly show that the level of HDL-cholesterol was increased in alloxan induced diabetic rats when treated with Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg). These results suggest that Ethanolic extract of *Indigofera mysorensis* and nano particles of *Indigofera mysorensis* at a dose of 200 and (50mg/kg) has protective effect against alloxan- induced diabetes and its complications

CONCLUSION:

Medicinal plant species constitute important alternatives to conventional medicine in a large number of developing countries, especially within poor communities that inhabit rural areas and lack access to health services. *Indigofera mysorensis.*L flowers might be a potential alternative agent for antidiabetic activity. Hence it is anticipated that *Indigofera mysorensis* flower would be a useful pharmaceutical material to treat diseases. This investigation may focus research field to develop clinical studies which might be of great scientific contribution for the society. The importance of medicinal plants in traditional health care practices clues to new areas of research and in biodiversity conservation.

The two *invitro* antidiabetic methods have been performed and found to be α-amylase inhibition and glucose uptake of different extracts. To identify the active constituents. Further studies are required to purify the active principle and study the molecular mechanism of the exact pathway. This information's will be useful for the development of alternative method rather than insulin and hypoglycemic agents for the treatment of diabetes mellitus.

From the results of the present experiments it may be concluded that formulation F3 showing formulation of Nanoparticle size, high percentage of entrapment and desired sustained release of Nanoparticle. Hence F3 formulation was optimized one.

The optimized formulation F3 was found to follow zero order release pattern which was revealed by the linearity shown from the plot of time versus concentration.

Nanoparticle formation were stable for 4 weeks at 4^0C and affirm the drug leakage — increased the higher temperature. Comparative study of Nanoparticle formulation with the marketed. The Nanoparticle formulation revealed that anti diabetic activity was released from the Nanoparticle formulation in a sustained manner for 24 Hours.

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