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Development and characterization of polymeric microparticles containing *Murraya koenigii* leaves extract for management of diabetes mellitus

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Abstract
Diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism. There are many undesired effects related with the synthetic drug molecules and in case of diabetes there is drug regimen which means more synthetic drug and more associated side effects. Current work is based on the extraction of roots of *Glycyrrhiza glabra* Linn. Which is of natural origin; this will help to get rid of side effects and after that the problem was dosing frequency which is again a drawback of diabetes treatment. We had selected the particulate carrier system for the drug release modification. Characterization and performance evaluation of particulate system loaded with herbal plant extract of the *Glycyrrhiza glabra* roots was done. Solid lipid nanoparticles was formulated and characterized for the particle size, shape and its distribution, percentage drug entrapment and *In-vitro* drug release profile along with the stability studies. *In-vivo* tissue distribution studies on albino rats suggested the accumulation of formulations in the different organs. These results suggest that the solid lipid nanoparticles are not only effective in rapid attainment of high drug concentration in body and also maintain the same over prolonged period of time.

Keywords: Solid lipid nanoparticles, plant extract, *Glycyrrhiza glabra*, diabetes mellitus

Introduction
Mulethi, sweet liquorice (*Glycyrrhiza glabra*) is another Indian medicinal plant, which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant [1]. *Glycyrrhiza glabra* commonly known as mulethi belongs to family Leguminosae [2]. *Glycyrrhiza glabra* Linn, is a commonly used herb in Ayurvedic medicine. Although it possess phytochemical and pharmacological activities, such as antibacterial, antioxidant, antimalarial, antispasmodic, anti-inflammatory and anti-hyper glycemic properties [3].

Plant profile
- Botanical Name: *Glycyrrhiza glabra*
- Hindi Name: Mulethi
- Sanskrit Name: Yastimadhu
- English Name: Liquorice
- Family: Leguminosae
- Parts of Plant used: Roots [3]

Material and Method
Roots of *Glycyrrhiza glabra* were collected from agriculture college, Indore. Their identification and authentification was confirmed by Department of Botany, Holkar Science College, Indore by correlating their morphological and microscopic characters with those given in literature. The roots were collected, washed well to remove all the dirt and were shade dried and then powdered transferred into airtight containers with proper labeling for further use. Chloroform, ethanol, methanol, petroleum ether and ethyl acetate were purchased from S.K. Traders, Indore. All solvents were analytical grade.

Preparation of plant extract
Petroleum ether extract
The coarsely powdered, dried roots (50 g) were extracted with 300-500 ml petroleum ether by hot extraction process (Soxhlet) for 4 hours. After completion of extraction the solvent was removed by distillation and concentrated in vacuo. Chloroform Extract: The marc left after petroleum ether extraction was dried and extracted with 300 ml -500 ml chloroform by hot

extraction process (Soxhlet) for 4 hours. After completion of extraction the solvent was removed by distillation and concentrated in vacuo. Ethyl acetate Extract: The marc left after the extraction of the chloroform extraction was dried and extracted with 300 ml -500 ml ethyl acetate by hot extraction process (Soxhlet) for 4 hours. After completion of extraction the solvent was removed by distillation and concentrated in vacuo. Likewise methanol and ethanol extraction performed.
The above extracts were used for phytochemical studies. The extractive values for each extract were calculated and recorded.

Isolation Method

Preparation of ethanolic extract

The drug powder was taken in the soxhlet extractor and was extracted using ethanol for 72 hours. After the extraction was over the solvent was recovered by distillation and the residue was concentrated in vacuo. The extract obtained was then stored in dessicator.

Fractionation of the ethanolic extract

50gm of ethanolic extract obtained was suspended in distilled water (200ml) in small amounts. It was extracted successively and exhaustively with solvents in increasing order of polarity viz. petroleum ether (60-80° C) (200ml X 5), solvent ether (200 X 5) and ethyl acetate (200X 3). Each fraction was washed with distilled water (5ml), dried over anhydrous sodium sulphate and freed of solvent by distillation. The aqueous remnant was freed of organic solvent by distillation under reduced pressure and then evaporated to dryness on a water bath. The yield of each extract was recorded and subjected to chemical investigation.

Table 1: Successive extractive values of the powdered roots of *Glycyrrhiza glabra*

S. No.	Extracts	Yield (% W/W)
1.	Petroleum ether extract	2.52
2.	Chloroform extract	2.76
3.	Ethyl acetate extract	3.21
4.	Methanol extract	5.12
5.	Ethanol extract	4.45

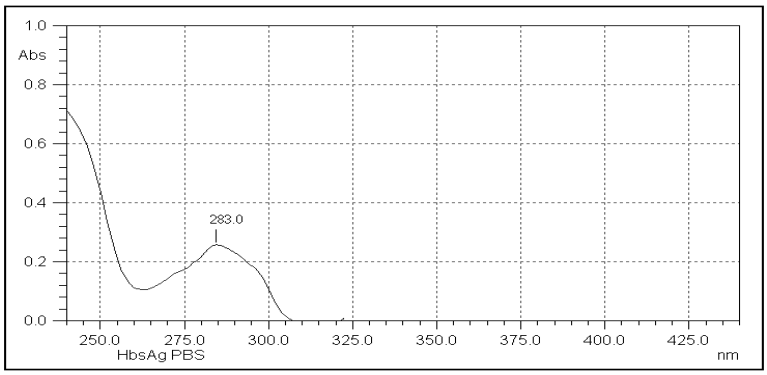

Fig 1: UV Spectra of isolated glycyrrhizin

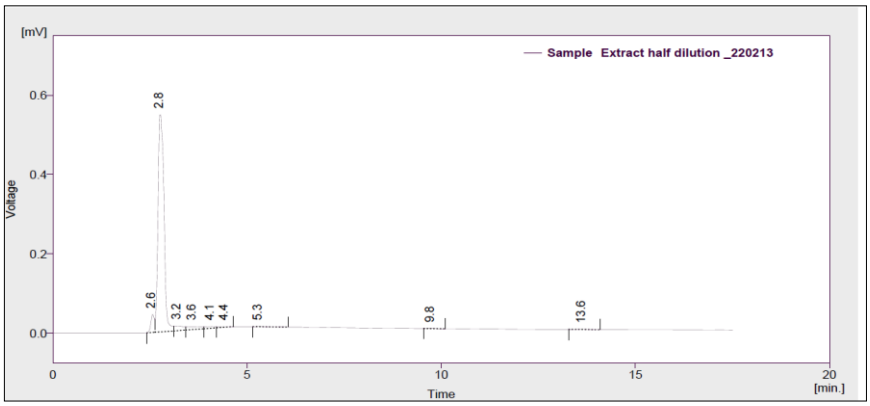

Fig 2: HPLC spectra of isolated glycyrrhizin

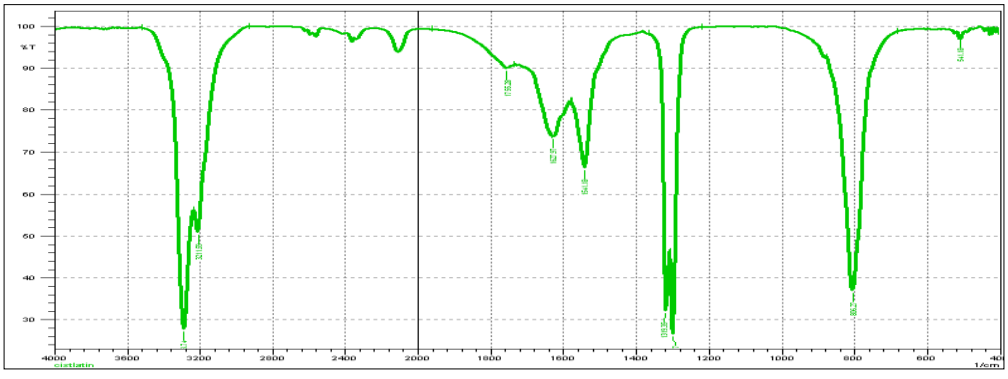

Fig 3: IR Spectra of Isolated glycyrrhizin

Phytochemical study of *Glycyrrhiza glabra*

Isolated rutin from *Aegle marmelose* showed a melting point at 210^{0}C which was in agreement with the standard range of 205-215^{0}C, as reported.

The Rf values of isolated and standard glycyrrhizin in several mobile phases are shown in table below.

Table 2: Comparison between the RF values of isolated and standard glycyrrhizin in different mobile phase (TLC)

Solvent system in TLC	Rf value of isolated rutin	Rf value of standard rutin
Ethyl acetate : formic acid : acetic acid : water	0.31	0.33

Table 3: Comparison between the RF values of isolated and standard glycyrrhizin in different mobile phase (Paper chromatography)

Solvent system in Paper Chromatography	Rf value of isolated glycyrrhizin	Rf value of standard glycyrrhizin
Isopropyl alcohol : water	0.43	0.47

Pharmaceutical screening of active constituent

1. **Acute toxicity study:** The acute toxicity study is use to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD_{50}/ED_{50}). The animals were divided into four groups and each group consisted of five mice. The defined or fixed dose level of aqueous and ethanolic extracts (2000 mg/kg) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Table 4: Result of acute toxicity study of glycyrrhizin

S. No.	Group	No. of animal used	Treatment Dose (mg/kg) body wt.	No. of animals recovered after study		
				24 hrs.	72 hrs.	14 days
1	Group A (Alcoholic Extract)	5	2000	5	4	5
2	Group B (Aqueous Extract)	5	2000	5	5	5

2. **Oral glucose tolerance test:** Animals were divided in nine groups and each group consisted of six rats. Overnight fasted rats were used for study.

Group I: Normal control rats administered saline (0.9% w/v);

Group II: Diabetic rats administered standard drug Glibenclamide (2.5 mg / kg) daily

Group III: Diabetic rats administered test sample (50 mg/kg);

Group IV: Diabetic rats administered test sample (100 mg/kg);

Table 5: Effect of Rutin from *Aegle marmelos* on oral glucose tolerance test in rats

S. No.	Treatment n=6	Fasting blood glucose level (mg / dl)			
		0 min	30 min	60 min	120 min
1	Normal	92.42± 0.12	132.33± 1.12	117.29± 1.11	111.03± 1.17
2	Standard (Glibenclamide, 2.5mg/kg)	93.01± 0.43	110.33±0.56*	83.09 ± 0.97*	79.39± 0.05*
3	Glycyrrhizin (50mg/kg)	96.01± 1.62	123.33±1.48*	104.67±0.92*	91.01± 0.37*
4	Glycyrrhizin (100mg/kg)	104.09±1.57	129.04±1.46	108.31±1.87*	93.83± 2.11*

Normal Control- Vehicle 10 ml/kg, Reading are values ± S.E.M,
n = Numbers of animals in each group
* $P < 0.05$ v/s Normal control; One-way ANOVA followed by Dunnett test

3. **FBS (Fasting blood glucose level):** Fasting blood sugar level was determined by using glucose oxidase peroxidase reactive strips.

Table 6: Effect of glycyrrhizin on fasting blood glucose levels in rats.

S. No.	Treatment N=6	Fasting blood glucose level (mg / dl)			
		Day 0	Day 5	Day 10	Day 15
1	Normal	97.14±1.53*	94.17±1.25 *	91.83± 1.01*	88.67±1.15 *
2	Diabetic control	181.67±1.12	189.11± 0.88	196.83± 1.08	199.18± 1.31
3	Standard (Glibenclamide, 2.5mg/kg)	184.33±1.45	127.55±0.76*	116.51±1.01*	107.67±1.14*
4	Glycyrrhizin (50mg/kg)	180.11±1.83	137.33±1.33	125.83±1.34*	119.18±0.97*
5	Glycyrrhizin (100mg/kg)	179.67±0.65	135.65±1.50*	126.33±1.03*	116.52±1.08*

Values expressed as mean ±S. E. M.; n = no. of animals in each group. * $p < 0.05$ significant Vs diabetic control. One-way ANOVA followed by Dunnett test

4. Effect of glycyrrhizin on serum lipid profile

Table 7: Effect of glycyrrhizin on serum lipid profile in rats

S. No.	Treatment n=6	TG	TC	HDL	LDL	VLDL
1	Normal	88.86±1.04*	55.52±0.94*	23.88±0.73	13.45±0.87*	18.72±0.20
2	Diabetic control	136.52±1.54	96.56± 1.03	15.55±0.83	53.73± 0.41	27.29± 0.33
3	Standard (Glibenclamide, 2.5mg/kg)	101.56±1.07*	57.21±1.08*	19.20±0.52	17.35±0.44*	20.85±0.21
4	Glycyrrhizin (50mg/kg)	115.87±1.14*	75.27±1.20*	14.26± 0.39	37.84±0.86*	23.17±0.23
5	Glycyrrhizin (100mg/kg)	107.06±1.04*	65.19±1.24*	18.59±0.38	25.23±1.40*	21.37±0.30

Values expressed as mean ± S. E. M.; n = no. of animals in each group. * $p < 0.05$ significant Vs diabetic control. One-way ANOVA followed by Dunnett test

Preparation of solid lipid nanoparticles

Microparticles were optimized on the basis of % entrapment, drug content, stirring time and no. of particles formed. Optimized formula used for further work. Solvent evaporation method has been used in which Glyceryl monostearate was dissolved in acetone and ethanol (1:1 v/v) in water bath at 60ºC and this was added to aqueous phase (distilled water) under mechanical agitation for 45 minutes. This was subjected to centrifugation at 4000 rpm for 10 minutes. Resuspended in water which results in formation of solid particles. Finally the microparticles were collected by filtration and are washed with demineralized water.

Table 8: Optimization of Drug: polymer ratio

Formulation code	Ratio (Drug: Polymer)	Average size(µm)	No. of particles per mm^3x1000	% Entrapment
F-1	9:1	2.23±0.35	27±2.5	64.4±1.2
F-2	8:2	2.34±0.54	28±2.2	67.8±0.98
F-3*	7:3	2.69±0.57	36±1.9	73.5±1.10
F-4	6:4	2.75±0.15	28±1.6	68.4±1.43
F-5	5:5	2.77±0.24	23±1.5	64.8±0.85

*Data are shown as mean ± SD (n= 3)

Characterization of liposomes

1. **Particle size and shape:** Nanoparticles were visualized under Philips Morgani 268 Scanning Electron Microscope. A drop of the different formulations was placed on different carbon coated copper grids to leave a thin film on the grids. Then, the film was negatively stained with 1% phosphotungastic acid (PTA) by placing a drop of the staining solution on to the film and the excess of the solution was drained off with a filter paper. The grid was allowed to dry thoroughly and formulations were viewed under a scanning electron microscope and photographs were taken at suitable magnification. (Fig No.: 5).

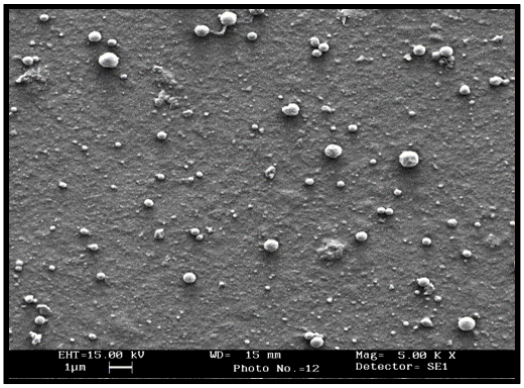

Fig 4: Photomicrograph of Nanoparticles

2. **Particle size and distribution:** The size and size distribution of vesicles was determined using laser diffraction particle size analyzer (Cilas, 1064 L, France). The liposomal suspension was dispersed in distilled water and then it was put into the sample chamber of particle size analyzer and measurement of vesicular size was carried out (Table No. 8).

3. **Entrapment Efficiency:** 1 g sephadex G-75 was allowed to swell in 10 ml of 0.9% NaCl solution in distilled water in a glass screw capped bottle for 5 hours at room temperature. The hydrated gel was filled to the top in the barrel of 1ml disposable syringe plugged with whatman filter pad. The barrel was then placed in the centrifuge tubes. The tubes were centrifuged at 2000 rpm for 3 minutes to remove excess saline solution. Eluted saline was removed from the centrifuge tubes and exactly 0.2 ml of suspension (undiluted) was applied dropwise on the top of the gel bed in the center. Columns were again centrifuged at 2000 rpm for 3 minutes to expel and remove void volume containing nanoparticles in to the centrifuge tubes. Elute was removed and 0.25 ml saline was applied to each column, and centrifuged as previously. The amount of drug entrapped in the vesicles was then determined by disrupting the particles followed by filtration and subsequent determination of the drug content using spectrophotometric method at 283 nm (Table No. 8).

4. ***In-vitro* drug release:** 1 ml of pure suspension was placed in dialysis tube, which in turn was placed in a beaker containing 20 ml of PBS (7.4 pH). The solution containing the dialysis tube was stirred on a magnetic stirrer while keeping the temperature constant at 37±1°C throughout the study. Samples were withdrawn at different time intervals with subsequent analyzed for drug using Shimadzu 1601 UV spectrophotometer at 283 nm.

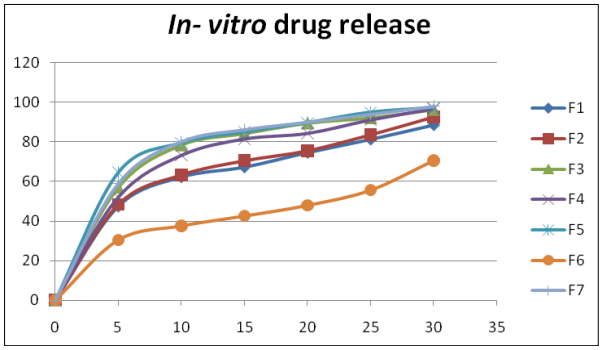

Fig 5: *In-vitro* drug release profile

5. Vesicle count

To characterize formulation for particle count, the dispersion was diluted (5 times) with PBS (pH 7.4) and particles /mm^3 were counted by optical microscopy. The nanoparticles in 80 small squares were counted and calculated using the following formula: (Table 8).

Total numbers of particles/mm^3:

Total number of particles counted x Dilution x 4000

Results and Discussion

Isolated Glycyrrhizin shows effective decrease in blood glucose level as compared the standard glibenclamide drug which is tested by animal studies. Results of OGTT of glycyrrhizin 100 mg/kg after 2 hrs. gives 93 mg/dl. Drug release pattern performed and after different time interval drug release pattern continuously increased in sustained manner. Release also altered with the nanoparticles prepared by solvent evaporation method shows improved drug release profile. In-vitro drug release studies shows that drug release controlled over prolong period of time i.e. after 24hrs. 49.7% drug was released; this will also decrease the dosing frequency of active constituent.

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