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Research Article

DEVELOPMENT AND CHARACTERIZATION OF MICROPARTICLES CONTAINING HERBAL PLANT EXTRACT FOR TREATMENT OF DIABETES

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ABSTRACT

Diabetes is the disease which is mainly associated with increased dosing frequency which is a major drawback, to overcome by this we have chosen novel carrier to modify the drug release mechanism from the dosage form. Incidence of diabetes mellitus increasing day by day. Synthetic drugs which are used for the treatment of diabetes have many side effects and frequency of dosing is more. To overcome such problems novel carrier system has been chose. Herbal extracts have been widely accepted as the potential medicines with less side effects as compared to synthetic drug molecules. Biodegradable polymers are having wide use for the preparation of vesicular system to control the drug release pattern of drugs. Extraction of crude drug (Aegle marmelos) done with successive solvent extraction method by using different solvents like Petroleum ether, ethyl acetate, chloroform, methanol, and ethanol. In phytochemical screening we found different constituents, which decreases blood glucose level. Polymeric microparticles formulated with hot melt method and emulsification method. After characterization the microparticles which are made from hot melt method shows good results of drug release and entrapment efficiency. In the current research work micoparticles has been developed of chitosan employed to enhance the drug release. Polymeric micopartcles were characterized and evaluated for antidiabetic activity. Aegle marmelos decrease the blood glucose level in albino rats.

KEYWORDS: *Microparticles, Aegle marmelos, Hot Melt method, Double Emulsification, Diabetes Mellitus.*

INTRODUCTION

Bael (*Aegle marmelos Corr.*) is another Indian medicinal plant, which has enormous traditional values against various diseases and many bioactive compound shave been isolated from this plant [12].

Objective:

The objective of present research work was to control the drug release from carrier system and to reduce the side effects related with the use of synthetic drug molecules. Dosing frequency was the another drawback of conventional drug delivery system, to overcome by this we used sustained release dosage form and extracted anti-diabetic agents from herbal origin then encapsulated in polymeric microparticles.

MATERIAL AND METHODS

Leaves of Bael (*Aegle marmelos Corr.*) were collected from agriculture college, Indore. Their identification and authentification was confirmed by Department of Botany, Holkar Science College, Indore. The rhizomes were collected, washed well to remove all the dirt and were shade dried and then powdered transferred into airtight containers with proper labeling for future use.

Preparation of plant extract:

Petroleum ether Extract:

The coarsely powdered, dried plant parts (50 g) were extracted with 300 ml -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

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completion of extraction the solvent was removed by distillation and concentrated in vacuo.

Methanol Extract:

The marc left after the ethyl acetate extraction was dried and extracted with 300 ml -500 ml methanol by hot extraction process (soxhlet) for 4 hours. After completion of the extraction the solvent was removed by distillation and concentrated in vacuo.

Ethanol Extract:

The marc left after the methanol extraction was dried and extracted with 300 ml -500 ml ethanol by hot extraction process (soxhlet) for 4 hours. After completion of the extraction the solvent was removed by distillation and concentrated in vacuo.

The above extracts were used for phytochemical studies. The extractive values for each extract were calculated and recorded.

Extraction method for Phytochemical study of Bael:

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 No. 1: Successive extractive values of the powdered leaves of *Aegle marmelos*

S. No.	Extracts	Yield (% W/W)
1.	Petroleum Ether Extract	2.30
2.	Chloroform Extract	3.15
3.	Ethyl acetate Extract	3.65
4.	Methanol Extract	5.21
5.	Ethanol Extract	4.10

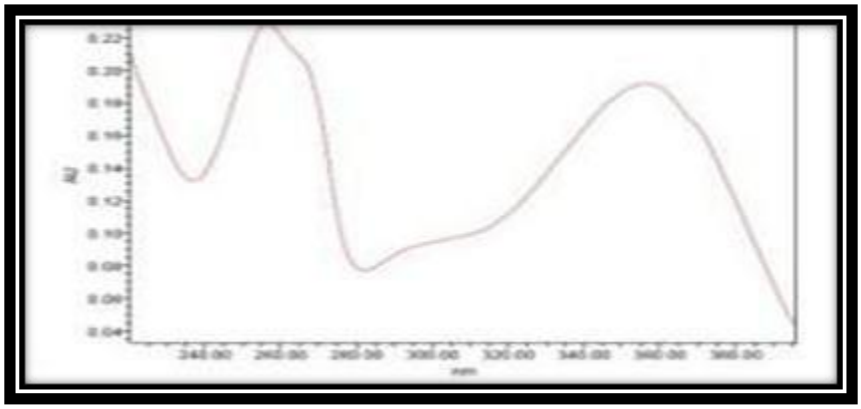

Fig. 1: UV Spectra of standard rutin

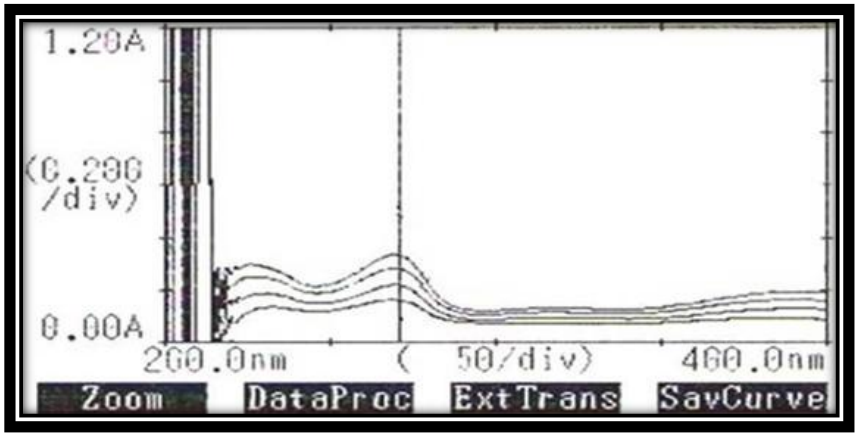

Fig No. 2: UV spectra of isolated Rutin

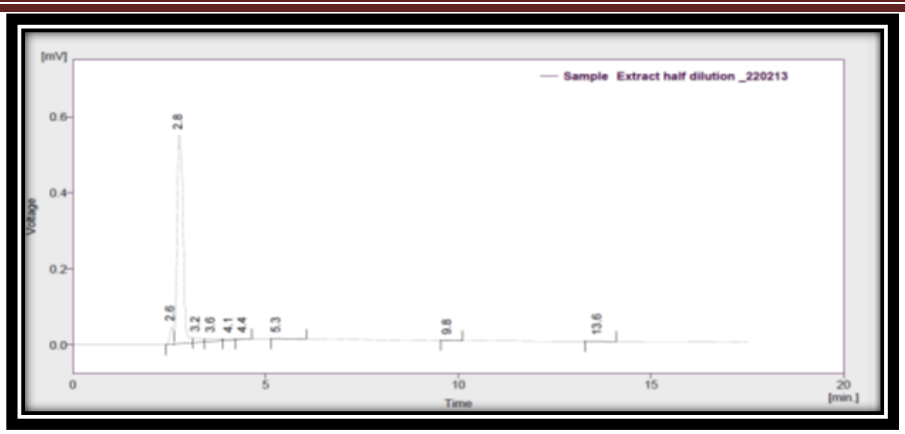

Fig No. 3: HPLC spectra of isolated Rutin

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.

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)

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

Table 2: Result of acute toxicity study of Rutin

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	4
2	Group B (Aqueous Extract)	5	2000	5	5	4

Table 3: 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	91.42± 0.92	132.33± 1.12	117.29± 1.11	111.03± 1.17
2	Standard (Glibenclamide, 2.5mg/kg)	94.01± 0.73	110.33±0.56*	83.09 ± 0.97*	79.39± 0.05*
3	Rutin (50mg/kg)	95.01± 1.32	123.33±1.48*	104.67±0.92*	92.01± 0.37*
4	Rutin (100mg/kg)	103.09±1.67	129.04±1.46	108.31±1.87*	94.83± 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

Table 4: Effect of Rutin on Fasting Blood Glucose (FBS) 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	Rutin (50mg/kg)	180.11±1.83	137.33±1.33	125.83±1.34*	119.18±0.97*
5	Rutin (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 rutin on serum lipid profile:

Table 5: Effect of rutin on serum lipid profile in rats

Sl. 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	Rutin (50mg/kg)	115.87±1.14*	75.27±1.20*	14.26± 0.39	37.84±0.86*	23.17±0.23
5	Rutin (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 polymeric microparticles:

Microparticles were optimized on the basis of % entrapment, drug content and no. of particles formed. Optimized formula used for further work. Double emulsion method has been used in which polymer (chitosan) was dissolved in DMSO and emulsified into plant extract to form a emulsion. Primary emulsion then was subjected to the homogenization which results in formation of dispersion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. Finally the microparticles were collected by filtration and are washed with demineralized water.

Table 5: Optimization of Drug: polymer ratio

Formulation code	Ratio (Drug:Polymer)	Average size(µm)	No. of particles	% Entrapment
Ex-1	9:1	2.23±0.35	27±2.5	64.4±1.2
Ex-2	8:2	2.34±0.54	28±2.2	68.8±0.98
Ex-3*	7:3	2.29±0.57	36±1.9	70.5±1.10
Ex-4	6:4	2.36±0.69	28±1.6	65.4±1.43
Ex-5	5:5	2.15±0.14	23±1.5	64.8±0.85

**Data are shown as mean ±SD (n= 3)*

Evaluation of polymeric microparticles:

1. Particle Shape: Microparticles were visualized under Philips Morgani 268 Transmission 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 transmission electron microscope and photographs were taken at suitable magnification.

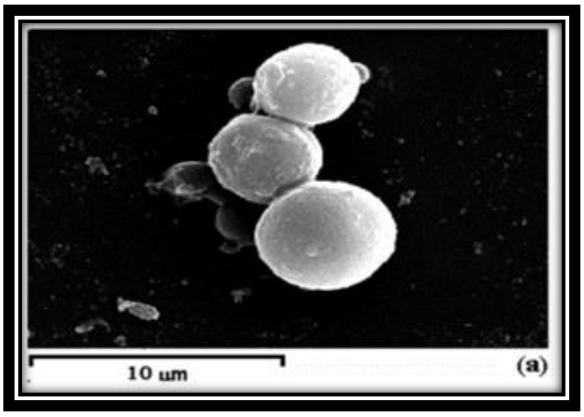

Fig. 4: TEM of Microparticles

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 microparticles 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.

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 microparticles 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 vesicles followed by filtration and subsequent determination of the drug content using spectrophotometric method (Table 5).

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 (Japan).

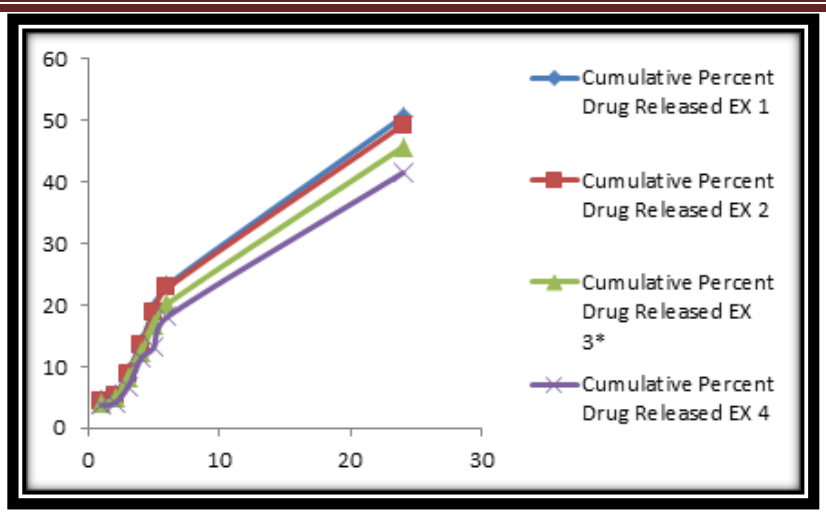

Fig No. 5: In vitro drug release profile

RESULTS AND DISCUSSION

Extraction of leaves of aegle marmelos was successfully done and rutin isolated, identified by HPLC technique. Polymeric microparticles was prepared by double emulsion technique which gave highest entrapment efficiency of rutin. Animal Study shows that the extracted rutin decreases the blood glucose level which is then encapsulated in miroparticles.

CONCLUSION

Microparticles prepared by double emulsion technique to improve the drug release profile. In-vitro drug release studies shows that drug release controlled over prolong period of time, this will also decrease the dosing frequency of active constituent.

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