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# Formulation and Development of Microparticles containing Herbal Plant Extract

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## ABSTRACT

Diabetes can not be cured completely. 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. "Polymeric microparticles" considered as novel carrier technique to control the release of herbal plant extracts from vesicular system. Extraction of crude drug (*Glycyrrhiza glabra*) 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 but glycyrrhizin which is active constituent of roots, which have anti-hyperglycemic effect. Polymeric microparticles formulated with emulsification method. After characterization the microparticles 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. *Glycyrrhiza glabra* decrease the blood glucose level in albino rats.

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## INTRODUCTION:

Yastimadhu (*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.

**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:

Roots of Yastimadhu (*Glycyrrhiza glabra*) were collected from agriculture college, Indore. Their identification and authentification was confirmed by Department of Botany, Holkar Science College, Indore. 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 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 vacuum.

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.

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.

Table1: Successive extractive values of the powdered roots of *Glycyrrhiza glabra*

| S. No. | Extracts | Yield (% W/W) |
|---|---|---|
| 1. | Petroleum Ether Extract | 3.12 |
| 2. | Chloroform Extract | 2.43 |
| 3. | Ethyl acetate Extract | 3.01 |
| 4. | Methanol Extract | 3.37 |
| 5. | Ethanol Extract | 4.11 |

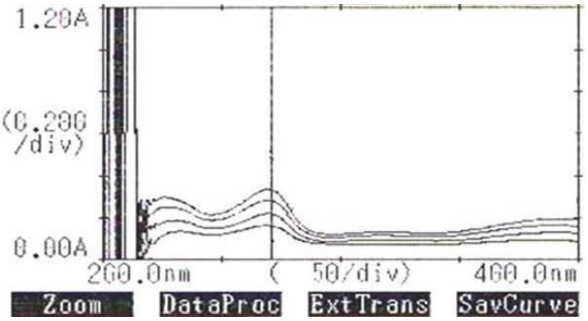


Figure 1: UV spectra of isolated Glycyrrhizin

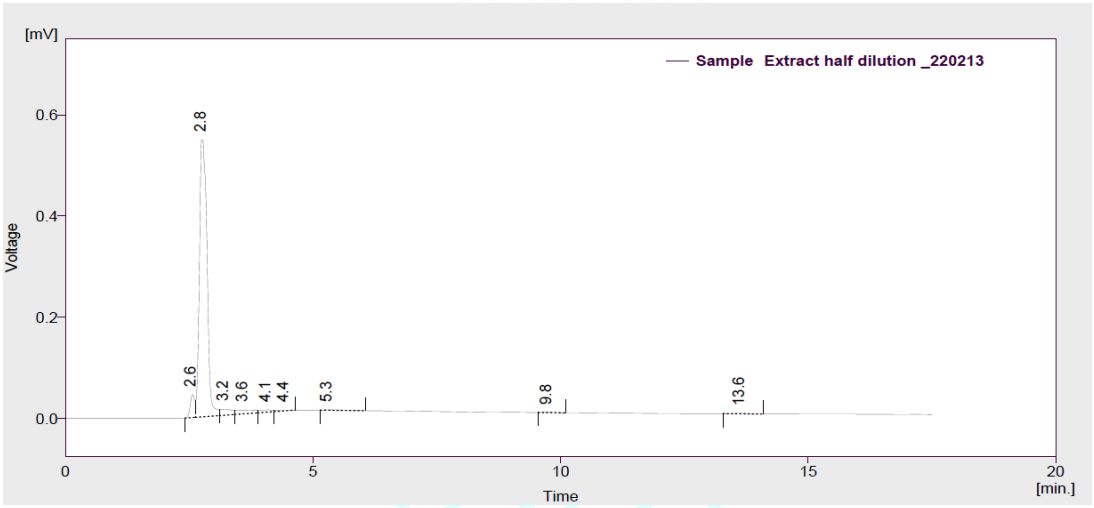


Figure 2: HPLC spectra of isolated Glycyrrhizin

**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 2: 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 | 5 | 4 |
| 2 | Group B (Aqueous Extract) | 5 | 2000 | 5 | 5 | 4 |

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 3: Effect of Glycyrrhizin from *Glycyrrhya glabra* 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 | Glycyrrhizin (50mg/kg,) | 98.01± 1.32 | 113.33±1.48* | 102.67±0.92* | 90.01± 0.37* |
| 4 | Glycyrrhizin (100mg/kg) | 104.09±1.67* | 125.04±1.46 | 104.31±1.87* | 91.83± 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 4: Effect of Glycyrrhizin on fasting blood glucose levels in rats.

| Sl. 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 | 212.67±1.12 | 219.11± 0.88 | 227.83± 1.08 | 234.18± 1.31 |
| 3 | Standard (Glibenclamide, 2.5mg/kg) | 210.33±1.45 | 180.55±0.76* | 162.51±1.01* | 133.67±1.14* |
| 4 | Glycyrrhizin (50mg/kg) | 211.14±1.83 | 197.33±1.33 | 175.83±1.34* | 144.18±0.97* |
| 5 | Glycyrrhizin (100mg/kg) | 215.67±0.65 | 185.65±1.50* | 176.33±1.03* | 146.52±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

**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.

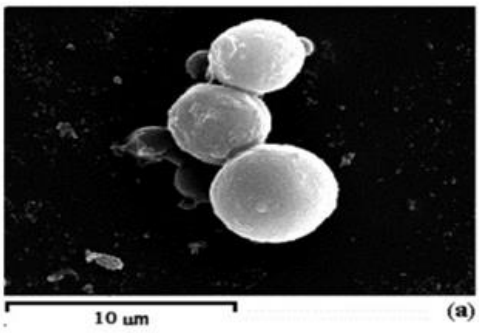


Figure 3: SEM

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).

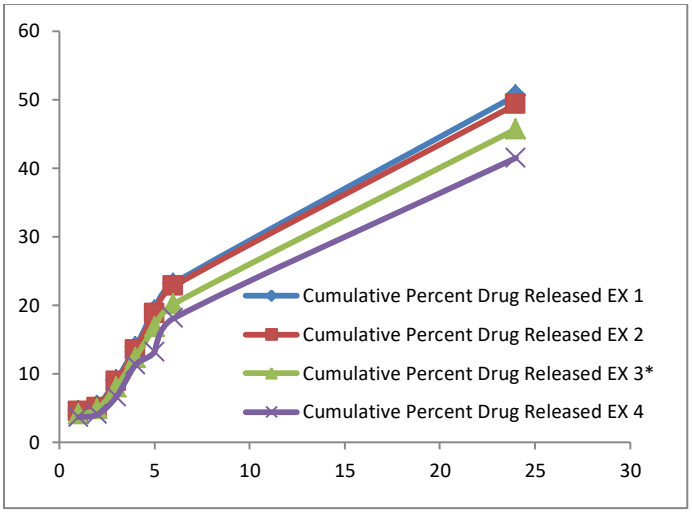


Figure 4: Cumulative % Drug release

## 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. Because of herbal origin thy side effects associated with synthetic drug also will less.

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