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Research Article

DEVELOPMENT OF STEVIOSIDE LOADED PELLETS FOR REGULATING THE BLOOD GLUCOSE LEVEL IN DIABETES: *IN VIVO*-*IN VITRO* CHARACTERIZATION

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ABSTRACT

Stevioside is a non-caloric sweetener and used as the alternative to sugar in many countries. It has been reported to have promising antidiabetic activity. In the present investigation, we have developed a multi-particulate system of stevioside consisting of nonpareil pellets for antidiabetic patients. The drug loading on pellets was performed by solvent evaporation and suspension layering method. The loaded pellets were evaluated for yield (%), drug loading efficiency (%), flow properties and *in-vitro* dissolution study. The stevioside loaded pellets (STL 4) showed yields (92.36%), drug loading efficiency (86.60%), friability (0.74%), drug release (98.31%) within 60 min and good flow properties. The *in vivo* study indicates the significant reduction in blood glucose levels in alloxan induced diabetic rats after administration of stevioside loaded formulation (STL 4). The developed formulation of stevioside pellets can find its dual applications as a sugar substitute having the potential anti-hyperglycemic effect which can be given easily and safely to the diabetic patients.

Keywords: Stevioside; pellets; non-caloric sweetener; anti-hyperglycemic; diabetes.

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1. INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder which at present has affected more than 400 million people across the globe. It is the 7th root cause of death universally and is progressing with a constant pace¹. Recent statistics and surveys have forecast to affect 592 million people with diabetes by 2030 globally². Likewise, in India, it is threatened seriously with the highest number (32 million in 2000) of the diabetic population followed by China and USA³. Moreover, it has been proposed that from 2006 to 2025 the diabetic population will grow rapidly from 40 million to 69.9 million⁴. The hyperglycemic state of a diabetic patient is difficult to treat but the blood glucose level can be

managed by administration of a daily dose of the antidiabetic drug⁵. Antidiabetic drug from natural sources is preferred because of their less toxicity and a negligible side effect on to the body⁶.

Stevioside is a sweet tasting glycoside present in large quantity in the leaves of *Stevia rebaudiana* Bertoni (Asteraceae)⁷. It is composed of stevia, a diterpenic carboxylic alcohol with three glucose molecules attached to an aglycone, the steviol moiety⁸. It is about 200-300 times sweeter than sugar; its sweetness potency is alike to that of aspartame⁹. Stevia is finding its worldwide application as a non-caloric sweetener¹⁰. Apart from its sweetness properties, the stevioside has promising contraceptive¹¹, antihypertensive¹² and anti-diabetic potential¹³⁻¹⁵. Stevioside has been used for

many years in the treatment of diabetes in several countries^{16,17}. The antidiabetic potential of stevioside is due to the stimulation of insulin secretion from the beta cells of the pancreas¹⁸.

The present investigation aimed to develop a multi-particulate system consisting of stevioside loaded pellets. Multi-particulate system have many advantages over the single unit dosage form like, better distribution in gastrointestinal tract, less dependent on gastric emptying, avoidance of local irritation, less inter and intra-subject variability¹⁹ and therefore considered for present investigation. Stevioside being a non-calorie sweetener can be given as a substitute for sugar to the diabetic patient. Besides, a sweetener stevioside through its anti-hyperglycemic action helps to regulate the blood glucose level. Therefore, the loaded pellets were evaluated for its antidiabetic potential.

2. MATERIAL AND METHODS

Stevioside powder was procured from Yucca Enterprises, Mumbai, India. Glibenclamide was a kind gift sample received from Alkem Laboratories, Mumbai, India. The non-pareil pellets were obtained from Zim Laboratories, Nagpur. HPMC-K4M was

provided by the Colorcon Asia Pvt. Ltd., Mumbai, India. Polyvinyl pyrrolidone (PVP) K30 was purchased from Himedia Ltd., India. The alcohol and the other chemical used were purchased from Loba Chemie, Mumbai, India.

2.1 Method of loading of stevioside pellets

The loading on pellet was carried out by two methods i.e., 1) Solvent evaporation and 2) Suspension layering method.

2.1.1 Solvent evaporation method

In this method, the stevioside powder in different weight ratios (1 to 4 % w/v) (Table 1) was dissolved in ethanol (50mL). The mixture was sonicated for 15 min to ensure complete solubilization of the compound. The suitable quantity of pellets (100mg/10mL) was incorporated into the mixture. The mixture was then kept at 80°C in hot air oven for about 45 min. After a specific time interval, the loaded pellets were collected and stored properly at ambient temperature until further use.

Table 1: Method of formulation stevioside loaded pellets

Bath code	Solvent evaporation method	Suspension layering method
	Stevioside (% w/v) in ethanol	Stevioside (% w/v) in coating solution
STE1	1%	-
STE2	2%	-
STE3	3%	-
STE4	4%	-
STL1	-	1%
STL2	-	2%
STL3	-	3%
STL4	-	4%

2.1.2 Suspension layering method

This method involves wetting of the core material with a coating solution containing the drug. The process was continued with intermittent drying till the drug was completely loaded onto the core. Stevioside powder in different weight ratios (1 to 4 % w/v) was homogeneously dispersed in coating solution (Table 1). The coating solution composed of carrier HPMC-K4M (2% w/w) and binder PVP-K30 (15% w/w) dissolved in ethanol. Basic core non-pareil pellets (18#20) were transferred into coating pan (R & D coater) and the prepared coating solution was sprayed on it. The spray rate and inlet air temperature were adjusted in such a way that the drug coated pellets reached a temperature of about 37°C-42°C. The process parameters of a coating are given in Table 2. The talc was frequently added to the coating pan in order to avoid over wetting of the

drug coated pellets as it may cause agglomeration. The dried pellets were sized on a sifter to remove agglomerates, broken pellets, and fine powder. The weight of the pellets was checked and the yield was noted.

2.2 Evaluation of drug loaded pellets

2.2.1 Percentage yield

The yield of the pellets was determined as a percentage of the ratio of the initial weight of the pellets before the loading process to the final weight gain after loading. The percentage yield was calculated according to the following formula.

$$\text{Percentage yield (\% w/w)} =$$

$$\frac{\text{Weight of pellets before loading/Total weight after loading of the drug}}{\times 100}$$

Table 2: The process parameters for stevioside loaded pellets

Sr. No.	Process Parameter	Specification
1.	Pan size	4 inch
2.	Batch size	10 g
3.	Spray rate	0.5-2 g/min
4.	Air flow	0.3-1.5 bar
5.	Atomizing air pressure	2lb/in ²
6.	Nozzle diameter	0.8 mm
7.	Inlet air temperature	60-70°C
8.	Product temperature	40-50°C
9.	Pan speed	26 rpm
10.	Spray gun type	Cone type

2.2.2 Drug loading efficiency (%)

Accurately weighed quantities of 100 mg stevioside loaded pellets were crushed in a mortar using a pestle and dissolved in 100 ml of 0.1 M HCl (pH 1.2). The solution was sonicated for 15 min and undissolved particles were removed by filtration through membrane

filter (0.45µm). The stevioside content in the filtrate was determined by measuring the absorbance at λ_{max} 331nm using UV spectrophotometer (Shimadzu UV-1800, Japan).

The drug loading efficiency (%) was calculated according to the following relationship;

$$\text{Drug loading efficiency (\% w/w)} = \frac{\text{Actual amount of stevioside found in pellets}}{\text{Theoretical amount stevioside in pellets}} \times 100$$

2.2.3 Flow properties of pellets

Stevioside loaded pellets were evaluated for flow properties such as angle of repose, Hausner's ratio, bulk density, tapped density and Carr's index. The angle of repose was determined by funnel method²⁰. The accurately weighed amount of pellets was taken in a funnel. The height of the funnel (h) was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the pellets. The pellet was allowed to flow through the funnel on to the surface. The diameter of the formed cone of the pellets was measured and angle of repose was calculated using the following formula;

$$\tan \theta = h/r \text{ or } \theta = \tan h/r$$

Where, θ = angle of repose,

h = height of pile,

r = radius of pile base

Bulk density and tapped density was determined by cylinder method²¹. The pellet free of any agglomerates was introduced into a 10 mL measuring cylinder. After the initial volume was noted, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2 sec intervals the tapping was continued until no further change in the volume was noted. The bulk volume and tapped volume was noted and density was calculated.

2.2.3 Friability of pellets

Friability of pellets was determined using Roche friabilator testing apparatus. Previously weighed pellets

were put into the friabilator. The friabilator was revolved at a speed of 25 rpm for 4min. After the revolution the fines and broken pellets were separated and intact pellets were weighed. The percentage friability was calculated as total weight loss of pellets during the revolution.

2.2.4 In vitro dissolution studies

The *In vitro* drug dissolution study was performed on stevioside loaded pellets filled in capsule using USP dissolution test apparatus II (Electrolab TD7-08L, Mumbai, India). The capsule containing loaded pellets equivalents to 15 mg of stevioside was placed in 900 mL of 0.1M HCl (pH 1.2) dissolution medium maintained at 37±0.5°C and 50 rpm of paddle speed. At definite time intervals, 5 mL aliquots were withdrawn, filtered, suitably diluted and analyzed for drug release for 60 min at 331 nm using UV-spectrophotometer (Shimadzu UV-1800, Japan).

2.2.5 Fourier transmission infrared (FTIR) spectroscopy

FTIR spectrum of pure stevioside powder, nonperial pellets and stevioside loaded pellets were recorded by potassium bromide dispersion techniques. About 2-3 mg of each sample were mixed well to form a fine powder with KBr and analyzed using FTIR spectrophotometer (IRAffinity-1, Kyoto, Japan) in the scanning wavelength region between 4000 and 600 cm⁻¹. The spectrums recorded were carefully examined for any interaction between drug and excipients.

2.2.6 Stability studies

The optimum formulation was subjected stability testing as per the International Conference on Harmonization (ICH) guidelines²². The stevioside loaded pellets were filled manually in hard gelatin capsule. The capsules were sealed in aluminum packaging and kept in a polyethylene bottle. The packed container was subjected to accelerated stability testing at $40\pm 2^{\circ}\text{C}$ and $75\pm 5\%$ RH in the humidity chamber (Bio-Technics, India, Ltd.). After the specific time intervals (0, 45 and 90 days) the capsules were withdrawn and observed visually for change in appearance, and drug release (%).

2.3 In vivo anti-diabetic study

2.3.1 Experimental subjects

All experiments and protocols performed in the study were approved by the Institutional Animal Ethical committee (Reg. No. KNCOP/ R&D/ AN-PROT/2012-13/02), Kamla Nehru College of Pharmacy, Butibori, Nagpur, India. The healthy Swiss albino rats of either sex weighing 150-200 g were employed for the study and were procured from Shree Farm House, Bhandara, India. The animals were housed under standard conditions of temperature ($25\pm 2^{\circ}\text{C}$) and (12:12 hr light/dark cycle) with free access to water.

2.3.2 Study protocol

The Swiss albino rats were divided into five groups each containing six rats ($n=6$) and were placed in separate metabolic cages. The blood glucose levels of each animal were determined in fasting state with free access to water. Group I (control group), the animals received normal saline solution through the oral route. Group II (diabetic untreated control), diabetes was induced in animals by giving alloxan monohydrate intraperitoneally. Group III (standard control), the animals received the standard drug. Group IV and Group V were treated with the optimized formulation of stevioside pellets containing 15 mg and 30 mg of stevioside respectively and given orally in the form of suspension (prepared with 0.5% CMC) by gastric gavage. Diabetes was induced in animals by the intraperitoneal injection of alloxan monohydrate at a dose 150mg/kg body weight. After 48 hr of injection, blood glucose level was measured every day up to seven days. On the seventh

day, the animals with blood glucose levels above 250 mg/dL were considered as diabetic and used for the study. Group III animals were treated with standard glibenclamide at a dose of 10 mg/kg body weight and served as reference. The standard drug and test formulation were given daily for a period of 21 days. The blood samples were withdrawn at 0hr, 1hr, 2hr, 3hr, 4hr and 24hr, after treatment by tail tipping method. Blood samples were analyzed for the blood glucose levels, using glucometer (Glucometer One Touch, Horizon, Life Scan, Johnson and Johnson Ltd.). A drop of blood obtained by tipping tail was placed on customized test strips inserted on glucometer and blood glucose level was read from the digital display²³.

2.3.3 Statistical analysis

Data represented with mean and standard deviation subjected to statistical analysis using one-way analysis of variance. The statistical probability (p) value less than 0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

The stevioside loaded pellets were found to be whitish spherical shape, uniform in size and had the smooth appearance. The process parameters for coating were critically monitored and adjusted for optimum performance.

3.1 Percentage yield and Drug loading efficiency (%)

The physical evaluation of stevioside loaded pellets showed good practical yield (75-92%), excellent drug loading efficiency (69-86%) (Table 3). The yield of loaded pellets prepared with solvent evaporation method was found to be low as compared to suspension layering method. The low yield of pellets with solvent evaporation method might be due to the dissolution or leaching of the drug in a solvent that remains stick to the wall of the container after evaporation of the solvent. The good loading efficiency was noted in loaded pellets prepared with suspension layering method. Therefore, suspension layering method can be exploited as the better method for loading of stevioside on pellets. The results indicated that with the increase in drug concentration in the appropriate solvent the loading efficiency also increases.

Table 3: The evaluation parameters of stevioside loaded pellets

Batch code	Yields of Pellets (%)	Drug loading efficiency (%)	Friability (%)
STE1	75.24±2.34	69.92±3.49	0.76±0.12
STE2	76.36±1.87	77.33±1.15	0.66±0.13
STE3	78.32±2.36	78.47±0.78	0.57±0.08
STE4	79.23±1.98	79.61±1.26	0.76±0.16
STL1	84.65±1.54	80.35± 1.18	0.84±0.11
STL2	86.32±0.97	81.68±1.42	0.79±0.09
STL3	89.56±1.38	83.61±1.25	0.62±0.16
STL4	92.36±1.47	86.60±1.34	0.74±0.07

The values are expressed as a mean ± standard deviation, $n=3$.

Table 4: Flow properties of stevioside loaded pellets

Batch code	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Angle of repose (θ)
STE1	0.516	0.598	26.5
STE2	0.513	0.587	26.9
STE3	0.518	0.585	26.2
STE4	0.522	0.591	26.3
STL1	0.519	0.611	25.6
STL2	0.526	0.604	25.9
STL3	0.541	0.608	25.8
STL4	0.532	0.602	24.5

3.2 Flow Properties

All the batches showed good flow properties (Table 4) which are desired for proper filling of pellets into a capsule.

3.3 Friability of pellets

The friability was found to be less than 1% for all the batches of stevioside loaded pellets (Table 3) suggesting the formulation having required strength and resistibility.

3.4 *In vitro* dissolution studies

The *in-vitro* drug release studies were performed for all the batches of stevioside loaded pellets in 0.1M HCl (pH

1.2). The dissolution profile of all the formulation batches is presented in Fig 1. The formulations STE1-STE4 shows drug release 82.18-88.12%, whereas the formulation STL1-STL4 indicate the drug release 92.81-98.31% at the end of 60 min. The higher percentages of drug release were obtained with the pellets loaded with drug by suspension layering method. Among all the formulations, batch STL4 shows higher drug release (98.31%). The *in-vitro* dissolution data were dealt statistically with one-way ANOVA and the results show the difference in drug release rate are statistically significant.

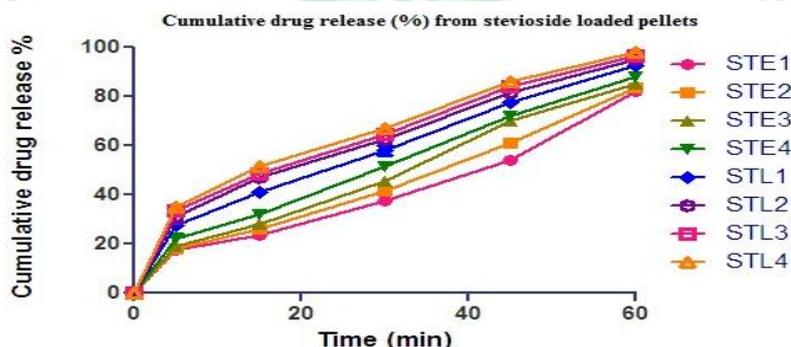


Figure 1: Cumulative drug release (%) from stevioside loaded pellets. (SD ± Mean, n=3)

3.4 Fourier transmission infrared (FTIR) spectroscopy

FTIR spectrum of pure stevioside and stevioside loaded pellets were presented in Figures 2 and 3. FTIR spectrums of stevioside indicate the presence of major functional groups in the structure which supports its

identity. The principle peaks of stevioside observed were 2751 (C-H), 2812 (C=C-H), 1384 (O-H), 1655.48 (>C=O) and 1957 (lactone ring). All the functional peaks were found to be retained in stevioside loaded pellets suggesting no any interaction of drug-excipients.

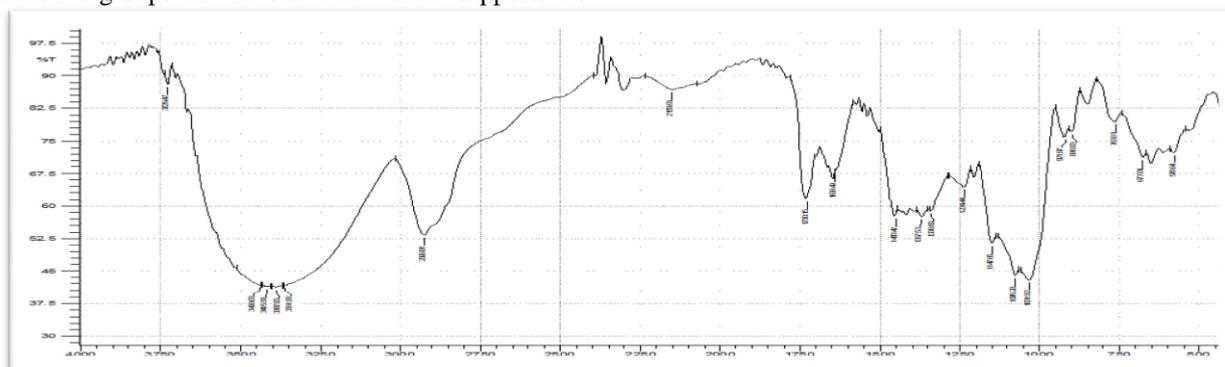


Figure 2: Fourier transform-Infrared spectrum of stevioside powder

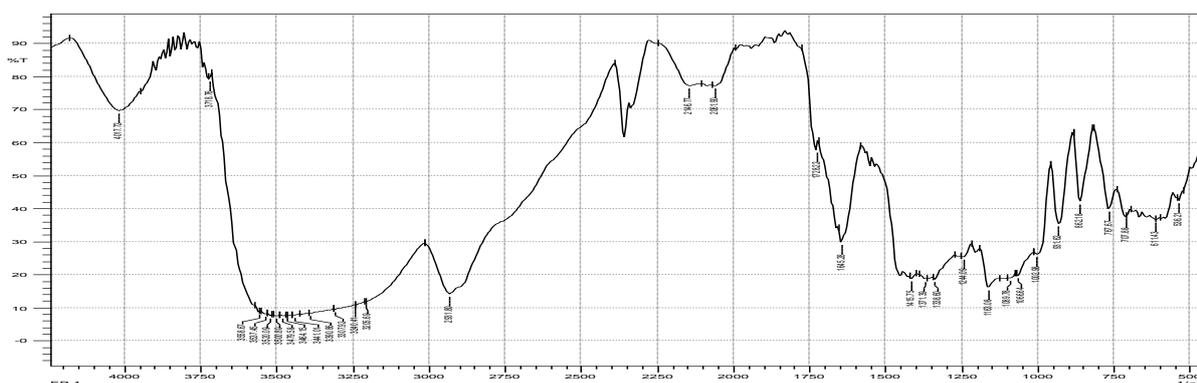


Figure 3: Fourier transform- Infrared spectrum of stevioside loaded pellets

3.6 Stability studies

The stability testing was performed on selected formulation SLT 4. The results indicate that there were no notable changes in physical appearance and drug release after subjecting the formulation at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ & $75\% \pm 5\%$ RH for 90 days (Table 4). Therefore, the

formulation was found to be stable and retain its stability for 90 days.

From the observations, the batch STL 4 showed yields (92.36%), drug loading efficiency (86.60%), friability (0.74%), drug release (98.31%) within 60 min and good flow properties and therefore selected as the optimum formulation for carrying *in-vivo* antidiabetic study.

Table 5: Stability testing data of optimized formulation STL 4

Days	Physical appearance	Cumulative drug release (%)
	Before storage	
0	White texture, smooth appearance	98.31±0.43
	After storage	
45	No changes observed	97.43±0.64
90	No changes observed	97.03±0.98

*All values are expressed as mean \pm SD, n=3.

3.7 *In vivo* anti-diabetic study

The results of the *in-vivo* antidiabetic study in diabetes-induced rats showed a significant decrease in blood glucose levels after oral administration of optimized formulations of stevioside. In order to determine the acute effects on blood glucose levels, the stevioside loaded pellets in two dose levels were administered

orally. The variation in the blood glucose levels of individual rats was determined at 0h, 1h, 2h, 3h, 4h and after 24h. Consequently, the significant reduction in blood glucose level was noted in diabetic rats treated with the SLT4 (15mg) and SLT4 (30mg) formulations (Table 6).

Table 6: Acute hypoglycemic effect of stevioside loaded pellets on alloxan induced diabetic rats

Groups	Time (h)					
	0h	1h	2h	3h	4h	24h
Control	84.21±1.53	91.45±2.34	95.84±1.95	88.23±1.17	86.41±1.05	80.26±2.31
Standard	340.23±2.97*	272.42±3.59**	205.64±4.12***	154.29±2.14*	121.33±3.47*	119.25±1.82**
SLT 4 15mg	338.24±4.52*	335.46±4.28**	329.87±3.54*	342.54±5.24*	337.45±4.21**	310.25±5.46*
SLT 4 30mg	341.28±3.87*	333.78±3.44**	327.46±4.68***	349.65±3.20***	330.25±2.98**	287.24±5.18**

Each value represents mean \pm SEM, n=6, * $p < 0.05$ denote value significantly different from control, ** $p < 0.01$, *** $p < 0.001$ denote value significantly different from diabetic control. SEM: Standard error of the mean, p : Probability value.

Table 7: Sub-acute hypoglycemic effect of stevioside loaded pellets on alloxan induced diabetic rats

Groups	Days			
	1-day	7day	14day	21day
Control	91.23±4.71	87.66±5.12	84.34±3.28	89.48±2.8
Diabetic control	341.74±4.42*	355.64±6.47*	369.15±8.67*	388.57±9.71*
Standard	340.27±8.28*	244.25±7.34***	194.56±7.26***	127.71±8.64***
SLT 4 15mg	335.52±6.74*	287.29±5.21**	217.36±4.63**	176.34±5.57**
SLT 4 30mg	333.38±5.84*	268.47±4.87***	204.59±6.14***	158.73±4.45***

Each value represents mean ± SEM, n=6, * $p < 0.05$ denote value significantly different from control, ** $p < 0.01$, *** $p < 0.001$ denote value significantly different from diabetic control. SEM: Standard error of the mean, p : Probability value.

CONCLUSION

The pellets were loaded successfully with stevioside using solvent evaporation and suspension layering methods. The loaded pellets were evaluated critically for various parameters. The *in-vivo* antidiabetic study in alloxan induced rats shows the significant reduction in blood glucose level after administration of selected formulation. Therefore, the developed pellets can be exploited as a potential antidiabetic formulation to the diabetic patient. The stevioside being sweet in nature could satisfy the desire of sweet in diabetic patients along with its promising anti-hyperglycemic effect.

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