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Formulation and development of hesperidin loaded solid lipid nanoparticles for diabetes

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ABSTRACT
Most of the research on Diabetes has been focused on synthetic compound however the disease burden of Diabetes has not been reduced, natural chemistry has provided various safer options in different disease areas. Hesperidin (bioflavonoid) has shown good promise in anti-diabetes efficacy however its bioavailability is low. In this work, SLN of Hesperidin wasprepared, physiochemically evaluated and tested for anti-diabetic potential. Various drug- lipid ratio were tested along with other formulation parameter for fabrication of formulation and optimization. The optimized formulation has been shownuniform particle size with negative surface charge with more than 90% entrapment efficiency and 91.45% drug release. The formulation exhibited Korsmeyer-Peppas release kinetic. The optimize formulation showed 21 % increase in anti-diabetes activity of hesperidin.
Keywords: *Solid Lipid Nanoparticles; Hesperidin; Diabetes; Bioavailability.*

1. INTRODUCTION

Oral administration is the easiest route of administrationwhere a substance is used by the mouth. Many drugs are taken by mouth as they are designed to have a systemic effect, reaching via the bloodstream to distinct areas of the body. DM is a collection of associated illnesses in which the body has been unable to control the quantity of glucose in the blood. The blood provides glucose to cell to provide energy to carry out all the daily operations of a person. The liver transforms a person's food into glucose. The bloodstream then releases the glucose. In a healthy individual person, several hormones, mainly insulin, regulate the blood glucose level. People with diabetes either don't produce enough insulin (type 1 diabetes) or can't use insulin correctly (type 2 DM) or both (diabetes occurring in several types). This not only hungers altogether the cells that need fuel for glucose, but also harms other organs and tissues exposed to the greater glucose levels [1-3]. Type 1 diabetes: Type 1 DM is an autoimmune disease in which the β-cells of the pancreas do not produce sufficient insulin, a hormone thathelps use blood sugar (glucose) for energy. The cells become kept from vitality and there will be overabundance of glucose in the blood. This is then followed by life threatening conditions of hypoglycemia, low blood sugar, and hyperglycemia, high blood sugar. At the point when hypoglycemia creates, cells don't get enough glucose and patients endure perplexity, loss of cognizance, and trance like state. Indeed, even demise can results when the mind is denied of glucose for a really long time.

Prevention of diabetes mellitus: By managing the diabetes, improve the quality of life and productivity of individuals with diabetes, Biguanides: Metformin, Sulfonylureas: Glyclopyramide, Glibenclamide, Meglitinides: Repaglinide, Thiazolidinediones: Rosiglitazone, Alpha-glycosidase inhibitors: Acarbose, Bioflavonoid: Hesperidin These are the same oral hypoglycemic agents, and most newly categories are bioflavonoids. Bioflavonoidflavonoids consist of the long group of polyphenolic compounds with a composition of benzo-ɣ-pyrone and are ubiquitous in plants. these are produced by Phenylpropanoid pathway. Reports available demonstrate that secondary phenolic metabolites, including flavonoids, are responsible for the variety of pharmacological activity [4]. Flavonoids are phenolic hydroxylated substances and are known as plant synthesizers in reaction to microbial infection [5]. Their activities depend on the framework of Flavonoids. Chemical nature depends on their structural class, degree of hydroxylation, other replacements and conjugations, and degree of polymerization [5]. The potential health advantages from the antioxidant activities of these polyphenolic compounds have stimulated growing interest in these drugs. By scavenging free radicals and/or chelating metal ions, functional hydroxyl groups in flavonoids mediate their antioxidant impacts [6].

Because of their increased antioxidant capacity both *in vivo* and *in vitro* systems, flavonoids are claimed to have health-promoting characteristics as a nutritional component [5].Flavonoids are capable of inducing human enzyme protective mechanisms. The body of the research proposed that flavonoids have protective impacts against many infectious bacterial and viral diseases and degenerative diseases such as cardiovascular diseases, cancers and other age-related diseases [5 and 5]. In plant tissues exposed to various abiotic and biotic stresses, flavonoids also function as a secondary antioxidant protection mechanism. In plants such as auxin, they also control growth factors [1-3].

Chemistry of Flavonoids: is a group of natural compounds that are discovered in plants with variable phenolic structures. A new substance from oranges was isolated in 1930. It was thought that at that moment it was a member of a new vitamin class and was designated as vitamin P. Later on it became clear that this substance was a flavonoid (rutin) and till now more than 4000 varieties of flavonoids have been identified [6-9]. Chemically

flavonoids are based on a 15-carbon skeleton composed of two benzene rings connected through a heterocyclic pyrane ring (C).

They can be divided into a variety of classes such as flavones (e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin). Nanoparticles for drug delivery system: Normal doses face many criteria to penetrate at target site. Most of the drug molecule fail to reach at target site. To overcome these problems, various nanoparticles (NPs) based drug delivery systems have been designed by different researchers NPs are defined as particles sized below 1000 nm [7-9] and can be formulated from different biodegradable materials including natural or synthetic polymers, lipids or phospholipids, even metals. However, some other scientists regard the nanoscale range from 1 to 100 nm and 1 to 200 nm [10-14]. Nanoparticles presented excellent versatility and employing them is one of the best possibilities to deliver drugs at specific sites. Solid lipid nanoparticles (SLNs):Most active pharmaceutical ingredients (APIs) are badly water-soluble and have bad bioavailability. Nanotechnology is an approach to overcoming traditional drug delivery systems difficulties. Solid lipid nanoparticles display interesting therapeutic characteristics. The primary benefit is that they are ready with well tolerated lipids that are physiologically well. SLN as novel lipid-based Nano carriers ranging from 10 to 1000 nm in size. In order to solve polymeric nanoparticles issues, SLNs were implemented. [14-18]. As a novel colloidal medication carrier for intravenous applications, nanoparticles produced from strong lipids attract significant attention as they were suggested as an alternative particulate carrier scheme. SLN iscolloidal sub-micron carriers varying from 50 to 1000 nm, consisting of physiological lipid, dispersed in water or aqueous surfactant. SLN offers unique characteristics such as small size, big surface area, elevated drug loading and interaction of interface stages and is appealing for its ability to enhance pharmaceutical efficiency.

2. MATERIALS AND METHODS

2.1. Materials.

Hesperidin purchased from (Central Drug House (P) Ltd. Corp), Dimethyl Sulphoxide (Central Drug House (P) Ltd. Corp.), Chloroform buy from (Thermo Fisher Scientific India Pvt. Ltd), Methanol (Thermo Fisher Scientific India Pvt. Ltd), n-Octanol Central Drug House (p) Ltd. Corp.), Egg Lecithin (Central Drug House (p) Ltd. Corp.) Tween 80, Poloxamer-188 (Thermo Fisher Scientific India Pvt. Ltd). Glyceryl trimyristate (GMBH), Glyceryl monostearate (Central Drug House (p) Ltd. Corp.) Glyceryl tristearate (GMBH) Benzoic acid (Central Drug House (p) Ltd. Corp.), Propylparaben (Central Drug House (p) Ltd. Corp.), Methylparaben (Central Drug House (p) Ltd. Corp.),Sodium hydroxide pellets (Central Drug House (p) Ltd. Corp.)

2.2. Methods.

2.2.1. Methods employed for hesperidin.

Preparation of Hesperidin loaded SLNs.Hesperidin loaded SLNs were prepared by a slight modification of the previously reported solvent emulsification–diffusion technique [14-18]Lipid (drug-lipid ratio 1:2- 1:4) was dissolved in a mixture of ethanol and chloroform (2.5 %, 1:1) as the internal oil phase. Drug (50 mg) was dispersed in the above mixture solution. This natural stage was then emptied drop by drop into a homogenizer cylinder containing aqueous solution of tween 80 (1-2 % w/v) as the outside fluid stage and homogenized for 30 min at 3000 rpm to prepare emulsion (o/w). After homogenization, the organic solvents were removed by primary vacuum evaporator at 400 mbar, 40 °C, 20 min. The above emulsion was added to 75 ml of super cold water (2-30C) containing surfactant (1-2 % w/v). The blending (2000-3000 rpm) was proceeded for 2 h to get SLN dispersion. The SLN dispersion was sonicated for 5 minutes to get SLN dispersion of uniform size. The dispersion was then centrifuged at 18,000 rpm for 20 min (Remi Instruments Pvt, Ltd, India) to isolate the SLN containing the medication. This was then re-dispersed in aqueous surfactant blend of tween 80 (1-2 % w/v) and sonicated for 5 min to acquire the SLN scattering of uniform size. The SLN dispersion was lyophilized by adding mannitol to get lyophilized SLN.

2.2.2. Physiochemical characterization of formulated SLNs.

Particle size, PDI and zeta potential.All samples were diluted in 1:10 ratio with deionized water to get optimum counts. Average particle size, PDI and zeta potential were measured by photon correlation spectroscopy (PCS; Zetasizer, HAS 3000; Malvern Instruments,). Measurements were carried out with an angle of 90 degrees at 25^0C .

Scanning electron microscopy study. Scanning electron microscopy (SEM) was utilized to explore the morphology of the SLNs. The assistance of gold sputter module, the stub was then covered with gold (200 to 500Å thickness) under an argon environment in a high vacuum evaporator. After coating, the sample was examined under Quanta 200 ESEM scanning electron microscope (FEI, USA) (magnification: 24000x; accelerating voltage: 10 kV) at 25±2 0C.

Entrapment efficiency -A fixed amount of SLNs dispersion (10 ml) was taken and centrifuged at 18,000 rpm for 15 min at 15 ºC, The lipid bit was separated and the absorbance of the hesperidin in the supernatant was determined spectrophotometrically at 285 nm, The entrapment efficacy (%) was determined by utilizing the equation [15-18].

$$\textit{Entrapment efficiency (\%)} = (Wt - Ws) \times 100 / Wt$$

Where Wt. is the total weight of drug used, Ws. weight of drug in the supernatant.

In-vitro drug release study.The dissolution and drug release behavior of formulation wereinvestigated using USP apparatus II at a stirring rate of 50 rpm and a volume of the dissolution medium (phosphate buffer 6.8) of 1000 mL at a temperature of 37°C. The samples ware withdrawn at selected [17-22]. time intervals and volume was replaced with fresh medium. The collected samples were suitably diluted and analyzed by UV-visible spectrophotometer.

2.2.3. Anti-diabetic activity.

Induction.Diabetes was induced by injecting a dose of 120 mg/kg of Streptozotocin intraperitonially [23-28]. The rats ware kept for 7 days with free access to food and water. The rats will fast on the 8th day for 12 hours and their Blood glucose level was determined using Glucometer. Rats with glucose levels above 120 mg/dl were used for the study.

Treatment. The diabetic rats were randomly divided into five groups (n = 6/groups). Group 1st received free hesperidin (400 mg/kg; P.O.), group 2nd received hesperidin loaded SLN (400 mg/kg; P.O), group 3rd received 2 mg/kg glibenclamide, and group 4th received normal saline. Group 5th as negative control. Blood sugar determination: Blood samples were collected from the tail vein after an overnight fast at the intervals of 0, 2, 4, 8, 16, and 32 hrs. The blood glucose level in the sample ware assessed utilizing Glucometer.

Stability testing. Stability testing gives an indication about variation in nature of pharmaceuticals due to ecological conditions. As per ICH guidelines, the formulations can be assessed at accelerated conditions (40ºC ± 2ºC/75% RH ± 5% RH) for 6 months for tropical countries i.e. India

3. RESULTS

3.1. Preparation of Hesperidin loaded SLNs.

Hesperidin loaded SLNs were prepared by a slight modification of the previously reported solvent emulsification–diffusion technique. Lipid (drug-lipid ratio 1:2- 1:4) was dissolved in a mixture of ethanol and chloroform (2.5 %, 1:1) as the internal oil phase. Drug (50 mg) was dispersed in the above mixture solution. This natural stage was then emptied drop by drop into a homogenizer cylinder containing aqueous solution of tween 80 (1-2 % w/v) as the outside fluid stage and homogenized for 30 min at 3000 rpm to prepare emulsion (o/w). After homogenization, the organic solvents were removed by the primary vacuum evaporator at 400 mbar, 40 °C, 20 min. The above emulsion was added to 75 ml of super cold water (2-30C) containing surfactant (1-2 % w/v). The blending (2000-3000 rpm) was proceeded for 2 h to get SLN dispersion. The SLN dispersion was sonicated for 5 minutes to get SLN dispersion of uniform size. The dispersion was then centrifuged at 18,000 rpm for 20 min (Remi Instruments Pvt, Ltd, India) to isolate the SLN containing the medication. This was then re-dispersed in aqueous surfactant blend of tween 80 (1-2 % w/v) and sonicated for 5 min to acquire the SLN scattering of uniform size. The SLN dispersion was lyophilized by adding mannitol to get lyophilized SLN.

3.2. Determination of particle size, poly-disparity index (PDI), Zeta potential.

All samples were diluted in 1:10 ratio with deionized water to get optimum counts. Average particle size (Figure 1), PDI was measured.

Table 1. Physiochemical chemical parameter of all the formulation

FC	D:L	S. conc.%	SS	PS
F1	1:2	2	3000	178.5 ±3.48
F2	1:3	2	3000	336.1 ±4.71
F3	1:4	2	3000	433.5 ±2.64
F4	1:5	2	3000	276.2 ±5.56
F5	1:6	2	3000	294.3 ±4.96
F6	1:7	2	3000	314.8 ±2.45
F7	1:8	2	3000	236.1 ±2.44
F8	1:9	2	3000	266.3 ±4,16
F9	1:10	2	3000	301.3 ±0.57

Table 1 represents the physiochemical chemical parameter of all the formulation. It is clearly evident that formulation F1, prepared with 1:2 drug lipid ratio, having 2% surfactant concentration, when formulated at 3000 rpm stirring speed resulting in optimum particle size 178.5nm and 90.01% drug entrapment.

The formulation was prepared using 1:2 – 1:9 drug lipid ratio. The entrapment efficiency was observed to be between 71.01- 90.01% which corresponding to the drug loading of 11.99% to 31.1%Table 2. As the drug lipid ratio increase, drug entrapment was decreased.

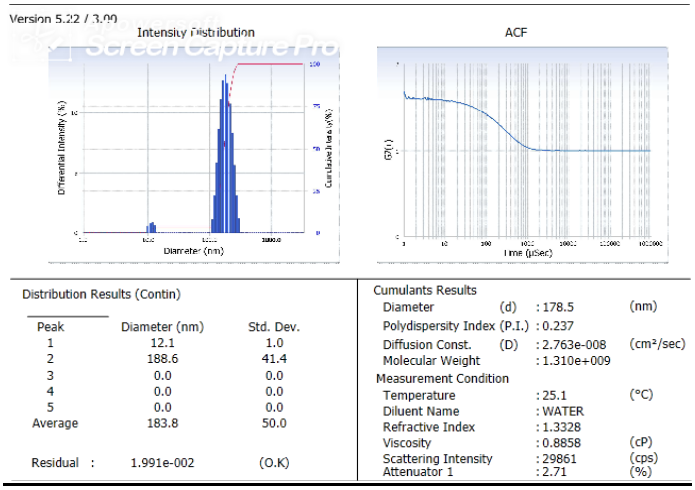

Figure 1. Particle Size & PDI Optimize Formulation (F1) Entrapment efficiency.

Table 2. Drug entrapment efficacy.

S.N	Drug lipid ratio (Hesperidin:GMS)	Drug loading (%)	Entrapment efficacy (%)
1.	**1:2**	**31.1**	**90.01**
2.	1:3	23.021	89.72
3.	1:4	18.14	88.68
4.	1:5	17.32	86.32
5.	1:6	15.04	82.67
6.	1:7	14.726	81.94
7.	1:8	12.81	79.69
8.	1:9	12.41	79.40
9.	1:10	11.99	76.01

3.3. Scanning electron microscopy.

SEM was used to investigate the morphology of the optimized SLNs, The morphology of optimized SLN was spherical in shape (Figure 2).

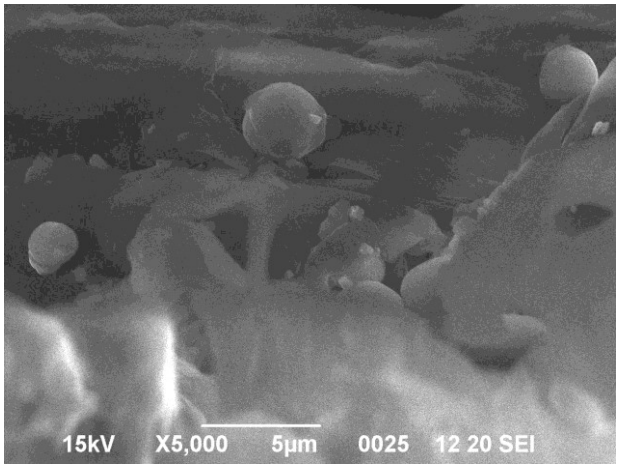

Figure 2. SEM Image Of Optimize Formulation.

3.4. *In-vitro* drug release study.

In-vitro drug release study was performed to ensure the delivery of drug in biological system. Figure 3 shows the percentage cumulative drug release over a period of 24 hr. it can

be observed that formulation F1 shows highest drug release at 91.45 % after 24 hours.

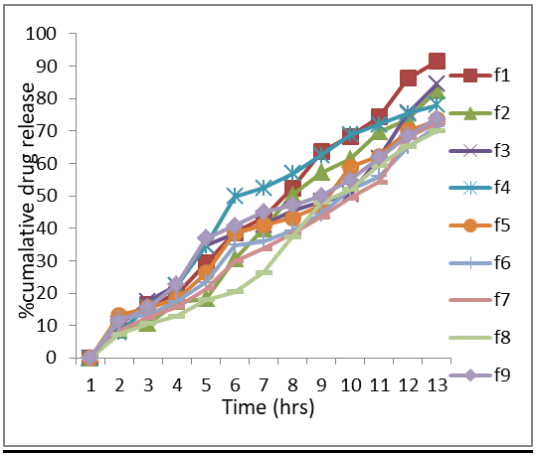

Figure 3.% cumulative drug release over a 24 hour time period.

The drug release kinetic was studiedfor selected formulation F1, the in- vitro drug release data was plotted according to various kinetic model. The value of R^2 was 0.9785 for *Korsmeyer-Peppas model kinetic, which seems to be suiting the in-vitro release data*(Figure 4 a to d)

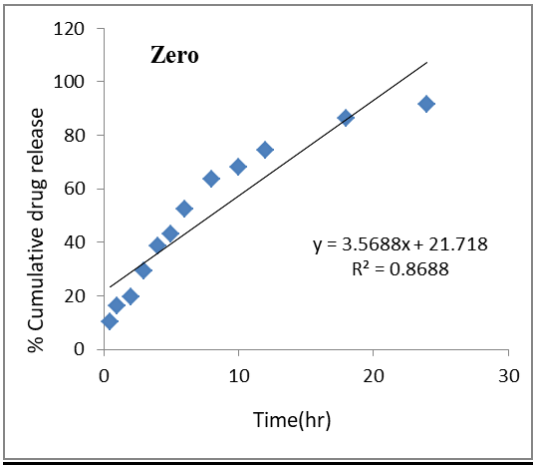

Figure 4a. Zero order kinetic of optimize formulation.

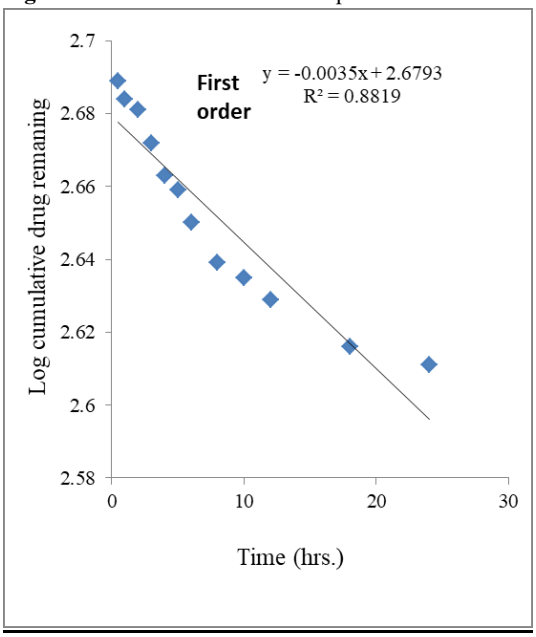

Figure 4b. First order kinetic of optimizingformulation.

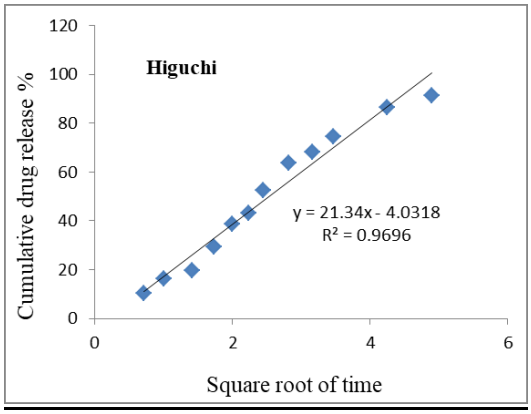

Figure 4c. Higuchi kinetic of optimizingformulation.

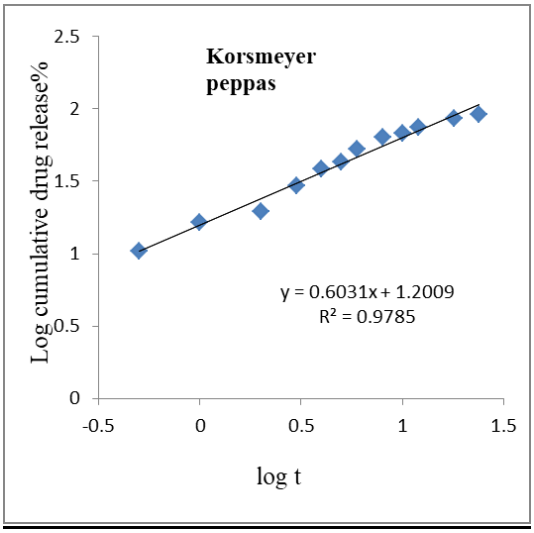

Figure 4d. Korsmeyer peppas kinetic of optimizingformulation.

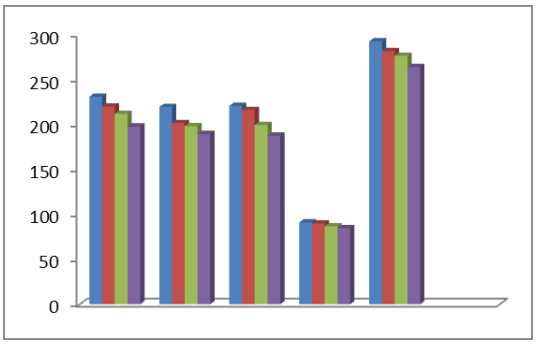

Figure 5. Effect of Hesperidin loaded SLN on blood glucose levelagainst streptozotocin induced diabetes.

Series 1- Free hesperidin(Group 1)
Series 2- Hesperidin + streptozotocin (Group 2)
Series 3-Glibenclamide + streptozotocin (Group 3)
Series 4- Normal saline (Group 4)
Series 5- Normal saline + streptozotocin (group 5)

3.5. Stability study of the optimized formulation.

The optimum formulation has shown slight modification in physiochemical parameter over the entire duration of stability testing (Table No.3). The particle size, zeta potential entrapment efficacy and *In vitro* drug release were not affected by more the 5% indicating the stability of optimizingformulation

3.6. Pharmacological activity of hesperidin loaded SLN.

Hesperidin loaded SLN has shown good tolerability at identical dose, Hesperidin loaded SLN should 98% reduction in blood glucose level whereas free hesperidin has shown 87% reduction in glucose level (Figure No. 5). It has shown that SLN loading increase anti- diabetic activity of hesperidin (Table No.4).

Table 3. Stability study at 40ºC ± 2ºC/75% RH ± 5% RH.

Parameter	Initial	1month	2month	3month	4month	5 month	6 month
Particle size	178.5	178.6	178.9	179.5	179.6	179.9	180.1
In- vitro release	91.45	91.43	91.41	91.38	91.36	91.2	91.03
Color	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Zeta potential	-17. 58	-17.50	-17.45	-17.40	-17.37	-17.26	-17.14
Entrapment efficacy	90.01	90.00	89.90	89.70	89.50	89.43	89.20

Table 4. Effect of Hesperidin loaded SLN on blood glucose level in Streptozotocin- induced diabetic rats.

S.NO.	Group	Treatment (mg/kg b.w.)	Blood glucose (mg/dl)			
			0 day	7day	14day	21day
1	I	Free hespiridin	231±12.4	220±9.8	212±2.4	198.9±12.2
2	II	Hrsperidin+ Streptozotocin (500mg/kg)	219.5±8.1	201.7±5.4	198.2±8.2	189.7±7.5
3	III	Glibenclamide + Streptozotocin (500mg/kg)	220.8±10.1	216.2±9.3	199.6±12.4	187.7±9.8
4	IV	Normal saline (0.5ml/kg)	91.16±5.7	89.96±3.9	86.88±4.5	84.78±4.8
5	V	Normal saline +Streptozotocin (0.5ml/kg+60mg/kg)	292.7±9.5	281.8±8.3	276.7±9.6	264.0±14.6

All values were expressed as mean ± SD (n=6). p<0.001 when compared to control group p<0.002 when compared to standard.

4. CONCLUSIONS

To fulfill the objectives of present research project, one diabetes acting drugs Hesperidin were selected and procured. On the basis of physicochemical characterization and identification studies, it could be concluded that the procured drug samples were pure and authentic. SLNs were prepared by modified emulsification diffusion technique. For preliminary studies, drug lipid ratio, surfactant concentration and stirring speed ware modified for optimum formulation Optimized formulation was further evaluated for *in vitro* drugrelease and stability. All the parameters were found to be in acceptable range. *In vitro* release studies that SLN formulations exhibited sustained release. Korsmeyer peppas model was found to be the best fitted model indicating release of drug from SLNs as a log cumulative release dependent process. Moreover; drug incorporated in SLNs was rapidly transferred and produced effect, without any significant side-effect. Stability studies revealed no significant change in the particle size, zeta potential and entrapment efficiency at 40ºC ± 2ºC/75% RH ± 5% RH up to six months.

Based on the above finding, it could be stated that SLN formulation for hesperidin was successfully developed and administered in rat, Oral administration of hesperidin loaded SLN showed good result in comparison to the marketed formulation when compared with free hesperidin the bioavailability was increased upto (1.5) fold.

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