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RESEARCH ARTICLE

Development and Characterization of Charantin Loaded Transferosomes based Transdermal Formulation: An Effective and Patient Friendly Way for Diabetes Management

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Abstract

Transferosomes, marvels of scientific ingenuity, represent exquisitely optimized and ultra- deformable (ultra-flexible) lipid supramolecular aggregates, uniquely capable of traversing the mammalian skin intact. These remarkable carrier systems have harnessed the power of transdermal delivery, enabling the transport of drugs, irrespective of their molecular weight from the tiniest compounds to the most substantial ones. The incorporation of Charantin into transferosomal gel formulation holds immense promise in advancing topical therapeutics. Charantin, a bioactive compound derived from *Momordica charantia*, possesses potent medicinal properties with antidiabetic, anti-inflammatory, antioxidant, and antimicrobial activities. When integrated into the lipid-based transferosomal system, Charantin's efficacy is significantly amplified due to enhanced skin penetration and improved stability against degradation. The versatile nature of transferosomal gels allows for encapsulating both hydrophilic and lipophilic compounds, making it an ideal carrier for Charantin regardless of its solubility characteristics. Driven by the pursuit of excellence, the present study sought to masterfully optimize the Transferosomes to orchestrate an unparalleled symphony of enhanced skin delivery for Charantin.

Keywords: Antidiabetic, Anti-inflammatory, Antioxidant, Charantin, Transferosomes, Transferosomal Gel

1. Introduction

The controlled release capabilities of transferosomal gels ensure sustained and targeted drug delivery, optimizing therapeutic effects and reducing dosing frequency[1]. This innovative approach opens avenues for combination therapy, enabling comprehensive treatment of various skin conditions while maintaining patient compliance through its user-friendly application. Charantin has been reported to have antioxidant, adipogenesis-reducing, antilipolytic, hypoglycemic, antidiabetic, anticancer, antifertility, anthelmintic, antimicrobial, antiviral and hepatoprotective activity[2]. As research progresses, the potential scope of Charantin in transferosomal gel formulations appears promising, promising new horizons in the management of dermatological disorders and improving patient outcomes. However, further investigations and clinical studies are essential to validate its safety, efficacy, and commercial viability.

2. Materials and Methods

2.1 Preparation of Transferosomes

The transferosomes were created using the established technique of combining rotary evaporation and sonication, following the procedure outlined by Gregor Cevc *et al.*, in 1994. Table 1 provides an overview of the materials commonly used for the preparation of Transferosomes. The methods employed for their preparation consist of two main steps. Initially, a thin

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film is created and hydrated, which is subsequently sized according to the desired specifications through sonication. In the second step, the sonicated vesicles undergo homogenization via extrusion using a polycarbonate membrane[3].

In a sterile and dry round-bottom flask, a solution of phospholipid and surfactant in ethanol was mixed with a drug solution in a 20 ml blend of methanol and chloroform, maintaining a 1:1 ratio. The mixture of organic solvents was removed using vacuum-assisted rotary evaporation at a temperature exceeding the lipid transition point. Any residual solvent remnants were eliminated under vacuum conditions for the duration of the night. The resultant lipid layer was rehydrated using a drug solution in a saline phosphate buffer (PBS) with a pH of 6.5. The hydration process involved rotating the flask at 60 rpm for 1 hour at room temperature, leading to the formation of Large Multilamellar Vesicles (LMLVs). To obtain smaller vesicles, the LMLVs were further treated using probe sonication at 40˚C and 40 W for duration of less than 30 minutes[4-6]. This step aimed to reduce the size of the vesicles and achieve the desired characteristics for the transferosomes. Different 9 formulations were prepared by varying Soya Phosphatidyl Choline (SPC) and surfactant quantity as described in Table 2.

2.2 Optimization of Developed Transferosomal Formulations[7,8]

2.2.1 Drug Entrapment Studies (% Entrapment Efficiency Studies)

HPLC analysis was performed to ascertain the Charantin concentration within the formulation post disruption of the vesicles (liposomes and Transferosomes) using Triton X-100 at a 0.1% w/v concentration, maintaining a 1:1 volume ratio. The resulting mixture underwent appropriate dilution with PBS (pH 7.4). Subsequently, the solution containing vesicles and Triton X-100 was subjected to centrifugation at 10,000 rpm and 4°C for a duration of 10 minutes. To ensure transparency, the resulting supernatant was then passed through a 0.45 µm nylon syringe filter. The obtained solution was subjected to HPLC analysis (Shimadzu) with UV detection at 205 nm for quantification of the drug content. The Charantin-loaded formulation's encapsulation efficiencies were determined using the provided formula:

$$\text{Percentage Entrapment} = \frac{\text{Entraped Drug}}{\text{Total Drug}} \text{x}100$$

2.2.2 Vesicle Size Estimation

The prepared Transferosomes sample was placed inside the SEM chamber. The acquired SEM images were analyzed using specialized image analysis software. Individual Transferosomes were selected within the images, and their sizes are measured directly using calibrated measurement tools provided by the software.

2.3 Preparation of Transferosomal Gel

Integration of transferosomes into a gel was achieved by blending 1.5 g of carbopol 940 with 100 ml of water, followed by continuous stirring for a duration of 24 hours. Subsequently, a solution of triethanolamine (0.5% w/w) was introduced to neutralize the gel and attain a clear consistency. The mixture of transferosomes and carbopol 940 gel was combined in a 1:1 w/w proportion,

Table 1. List of materials used in formulation of transferosomes

Class	Material Used	Uses
Phospholipids	Soya phosphatidyl choline	Vesicles forming component
Surfactant	Sod. Cholate	For providing flexibility
Solvent	Methanol and chloroform	As a vehicle
Buffering agent	Saline phosphate buffer (pH 6.5)	As a hydrating medium

Table 2. Composition of prepared formulations

Sr. No.	Name of formulation	Sonication Time (minute)	Drug (mg)	SPC (mg)	Surfactant (mg)
1	KD1	20	50	150	20
2	KD2	20	50	150	25
3	KD3	20	50	150	30
4	KD4	20	50	175	20
5	KD5	20	50	175	25
6	KD6	20	50	175	30
7	KD7	20	50	200	20
8	KD8	20	50	200	25
9	KD9	20	50	200	30

yielding a formulated product with a particle size measuring 62 nm. The gel was prepared using the cold mechanical method with carbopol 940 and Bromopol sprinkled gradually on purified water and stirred until soaked. Cremophor, PEG, and isopropyl alcohol were then added, followed by the complete dispersion of Charantin into the gel through continuous stirring[9]. All the ingredients and their required quantity are summarized in Table 3.

2.4 Assessment of the Gel Containing Transferosomes

2.4.1 pH Analysis

Transferring 50 gm of gel formulation into a 10 ml beaker, we utilized a digital pH meter to measure its pH. For effective penetration of skin, the pH of the topical gel formulation should ideally fall within the range of 3 to 9[10].

2.4.2 Spread-ability

The spread characteristics of the gels were evaluated by observing their slip and drag properties. This evaluation encompassed the utilization of the equation: S = ml/t, wherein 'S' stands for spreadability, 'm' represents the weight applied on the upper slide within the pan, 't' signifies the time taken to cover a defined distance, and 'l' corresponds to the distance covered. To ensure uniformity in the measurements, the weight and length remained unchanged while 't' was determined. The spreadability of each formulation was measured on three occasions, and the averaged outcomes are presented as the final results[11].

2.4.3 Drug Content

To initiate the process, 1 gram of the prepared gel was mixed with 100 ml of ethyl alcohol. Subsequently, various aliquots with different concentrations were generated through suitable dilutions subsequent to the filtration of the initial solution. The absorbance of these solutions was gauged at 205 nm. Conclusively, the drug content was calculated using linear regression analysis, relying on the calibration curve[12,13].

2.4.4 In Vitro Diffusion Study

Utilizing a modified Franz diffusion cell, an *in vitro* study on drug release was carried out. The configuration involved the placement of a dialysis membrane with a molecular weight cutoff of 5000 Daltons between the donor and receptor compartments. The donor compartment contained a Transferosomal gel containing Charantin, while 24 ml of phosphate buffer with a pH of 7.4 was introduced into the receptor compartment. The diffusion cells were consistently maintained at a constant temperature of 37 ± 0.5 °C throughout the experiment, with continuous stirring at 50 rpm. At specific intervals, 5 ml samples were drawn from the receptor compartment using a side tube and subsequently subjected to drug content analysis using a UV-Visible spectrophotometer[14,15].

2.4.5 Stability Studies[15]

Enhanced stability assessment was performed on the finest formulations of transferosomal gel by exposing them to storage conditions at two distinct temperatures: 4 ± 1 °C and room temperature (25 ± 1 °C). These preparations were kept in small glass bottles with amber tint and screw caps at the specified temperatures. At intervals of 7, 14, 21, and 28 days, the samples were examined to assess vesicle size and drug content.

2.4.5.1 Effect of Storage Temperature on Vesicle Size

The modifications in vesicle dimensions of the solutions stored at temperatures of 4 ± 1 °C and 25 ± 1 °C for durations of 7, 14, 21, and 28 days were assessed using the Zetasizer device produced by Malvern Instruments, a company based in the United Kingdom.

Table 3. Composition of charantin transferosomal gel

Sr. No.	Ingredient	Quantity taken
1	Charantin loaded Transferosomes (KD5)	1.4 gm
2	Carbopol 940	1.4 gm
3	Bromopol	12 gm
4	PEG	12 gm
5	IPA	14 gm
6	Triiethanolamine	0.07 gm
7	Cremophor	10 gm
8	Purified water Q.S	100 gm

2.4.5.2 Effect of Storage Temperature on Drug Content

Evaluating the impact of storage temperature on drug content, both formulations were subjected to examination following storage periods of 7, 14, 21, and 28 days. The drug content within the transferosomal gel was measured through a spectrophotometric technique, which enabled an indirect estimation of the quantity of drug encapsulated within the gel.

2.4.6 Oral Glucose Tolerance Test (OGTT Model for Anti-hyperglycemic Study)

Each combination, prepared at a single dose of 500mg/kg body weight, was given orally to the rats using a gastric cannula. After 1 hour of drug administration, all animals were orally administered 2 g/kg of glucose. Blood samples were taken from the retro-orbital plexus of the rats, under mild anesthesia, at four time points: before the test drug was given (baseline), right before glucose administration (0 min), 60 and 120 minutes after glucose intake. The serum glucose levels were measured using the Glucose oxidase-peroxidase method[16].

A set of normal rats that had fasted overnight was divided into five groups, each containing five rats, as outlined Normal control (0.5% CMC solution), Standard, Streptozocin induced (60mg/kg) and Low as well as high dose of Noval Preparation.

2.4.6.1 Collection of Blood Sample

Blood samples were collected on 0, 7, 14, and 21 days of the investigation by gently puncturing the retro-orbital plexus while the animals were under mild anesthesia. The serum was isolated by spinning it at 3000 revolutions per minute (rpm) for 15 minutes using a centrifuge, for analyzing serum glucose levels, body weight and biochemical parameters.

3. Result and Discussion

3.1 Assessment of Vesicle Size and Entrapment Efficiency

Table 4 presents the data concerning vesicle size and encapsulation efficiency for various transferosome formulations. The recorded vesicle sizes spanned from 136.62 to 198.76 nm, while the range of entrapment efficiency was between 45.62% and 83.46%. These percentages for entrapment efficiency and vesicle size are specifically obtained for each distinct formulation, as highlighted in Table 4.

The findings revealed that formulation KD3, characterized by smaller vesicle size and improved entrapment efficiency, was chosen as the optimized formulation for subsequent evaluations.

The ideal composition of Charantin-loaded Transferosome systems was chosen by considering the conditions that yielded the highest percentage of entrapment efficiency and a minimized vesicle size. The KD3 formulation emerged as the most optimal, meeting the criteria for an ideal Transferosomal formulation. The impact of sonication on vesicle size and the assessment of entrapment efficiency for the Transferosomal dispersions of the KD3 formulation were also observed and presented in Table 5.

3.2 Evaluation of Transferosomal Gel

3.2.1 Drug Content

The drug content plays a crucial role in transfersome formulation, and the collected data is exceptionally pleasing. The drug content ranged from 96.65% to 98.98%, indicating the excellent capacity of the formulation to retain the drug. Among all the formulations, KD3 exhibited the highest drug content, reaching 97.98%.

3.2.2 pH

The importance of pH in transdermal drug delivery systems is undeniable, and the results from the transfersome formulation validate the suitability of all formulations for effective skin delivery. The pH values of the prepared transfersome gels ranged from 6.87 to 6.99, aligning with the predetermined criteria. Particularly, the optimized formulation KD3 demonstrated a pH

Table 4. Percentage entrap efficiency and vesicle's size of prepared formulations

Sr. No.	Name of formulation	Vesicle Size (nm)	Entrapment Efficiency (%)
1	KD1	141.35	45.62
2	KD2	146.92	73.85
3	KD3	144.51	83.46
4	KD4	180.35	60.26
5	KD5	172.64	80.48

value of 6.99, signifying its excellent skin compatibility and potential for effective transdermal drug delivery.

3.2.3 Spreadability

The evaluation of gel spreadability was based on its slip and drag characteristics, resulting in values ranging from 11.65 to 13.25 gms. cm./sec. An ideal spreadability range is crucial to ensure the gel's easy application at the intended site, avoiding difficulties caused by excessively high or low spreadability values. Remarkably, the optimized formulation KD3 exhibited a spreadability of 12.95, indicating its excellent suitability for convenient and effective application.

3.2.4 Viscosity Measurements

Utilizing a Brookfield viscometer DV-II model, the viscosity of the gels was meticulously determined. To ensure accuracy, a T-Bar spindle combined with a helipath stand was employed during the viscosity measurements. Averaging five readings taken over 60 seconds yielded the final viscosity value as in Table 6. Remarkably, the optimized formulation KD3 exhibited a viscosity of 2257 cps, highlighting its specific flow characteristics.

3.2.5 In Vitro Drug Release Study

A modified Franz diffusion cell was employed for an *in vitro* diffusion study on the transfersome gel (KD3). This cell incorporated a dialysis membrane immersed in phosphate buffer with a pH of 7.4, and the experiment ran for 10 hours. The findings obtained from these diffusion tests have been summarized in Table 7 and a graph has been plotted between cumulative drug release and time (Figure 1), between drug release and square root of time (Figure 2), between drug release and log time (Figure 3). Also, the relation of percentage drug remaining with time and log time was observed by plotting the graph as in Figure 4 and Figure 5. Notably, the rate of drug release from the transfersomal formulation across the dialysis membrane exceeded its penetration through the skin, highlighting the skin's function as a hindrance to drug transportation.

3.2.6 OGTT (Oral Glucose Tolerance Test)

The control group, consisting of normal (vehicle-treated) animals, exhibited a 39.56% increase in serum glucose levels 60 minutes after glucose administration in

Table 5. Effect of sonication

Sr. No.	Name of formulation	Sonication Time(minute)	Drug (mg)	Surfactant(mg)	SPC (mg)	Vesicle size (nm)	% Entrapment Efficiency)
1	KD3	15	50	25	175	190.72	72.3
2		20	50	25	175	162.59	80.6
3		25	50	25	175	150.3	30.2

Table 6. Results of transferosomal gel formulation

Sample Code	Percentage Drug content	pH	Spread ability (Gm.cm/sec.)	Viscosity (cps)
KD3	98.29 ± 0.25	6.9 ± 0.2	12.8 ± 1.2	2397 ± 24

Table 7. Drug release data (*in vitro*)

time (h)	square root of time (h)1/2	log time	cumulative % drug release	Log Cumulative drug release	Cumulative drug remaing	log cumulative % drug remaining
0.5	0.71	-0.301	19.25	1.28	80.75	1.91
1	1.00	0	41.32	1.62	58.68	1.77
2	1.41	0.30103	49.35	1.69	50.65	1.70
4	2.00	0.60206	68.27	1.83	31.73	1.50
6	2.45	0.77815	76.34	1.88	23.66	1.37
8	2.83	0.90309	88.94	1.95	11.06	1.04
10	3.16	1	96.38	1.98	3.62	0.56

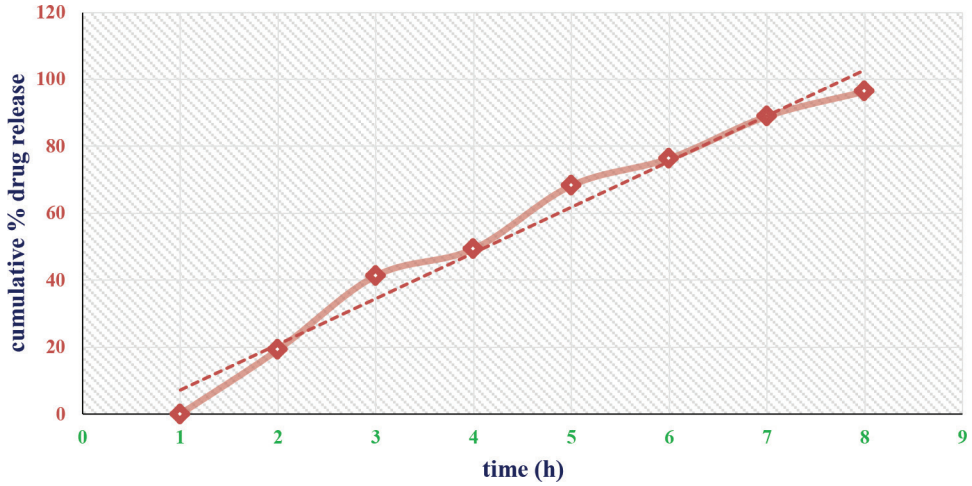

Figure 1. Cumulative Percent (%) drug release vs. time.

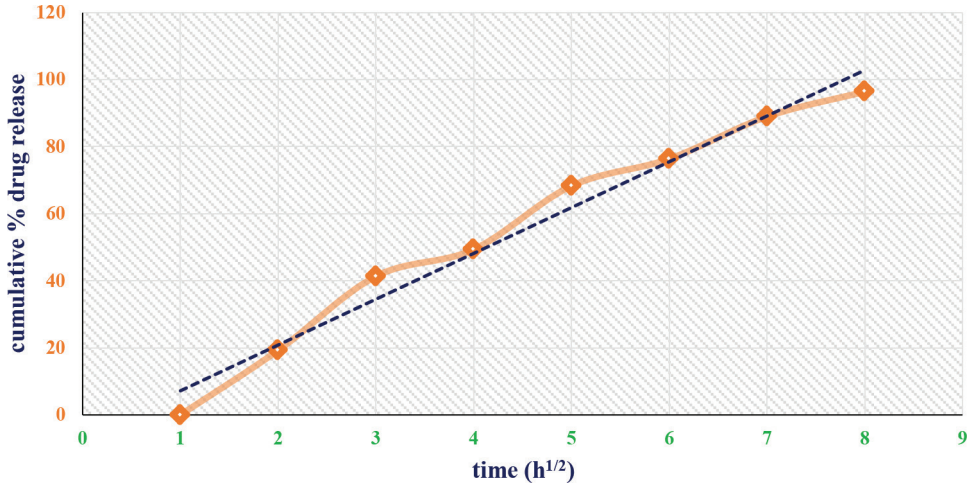

Figure 2. Cumulative percent (%) drug release vs. square root of time.

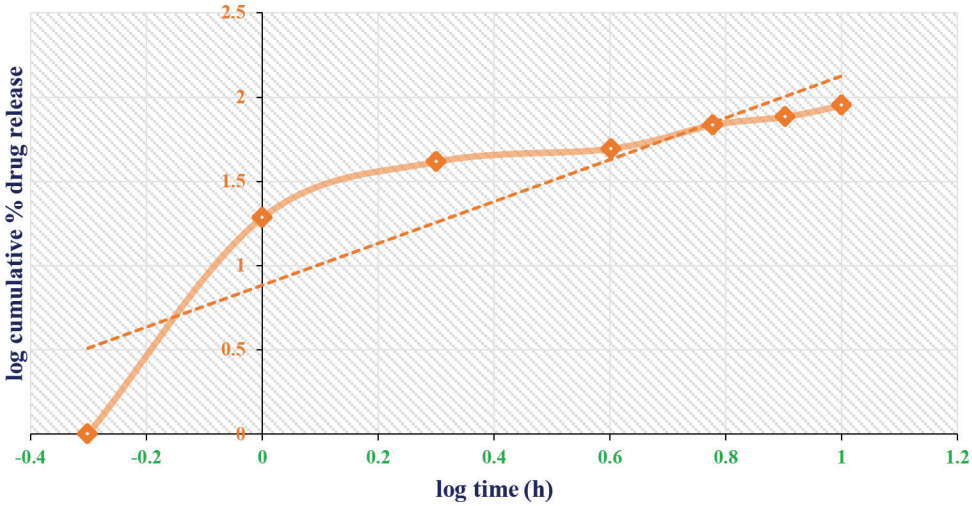

Figure 3. Log cumulative Percent (%) drug release vs. log time.

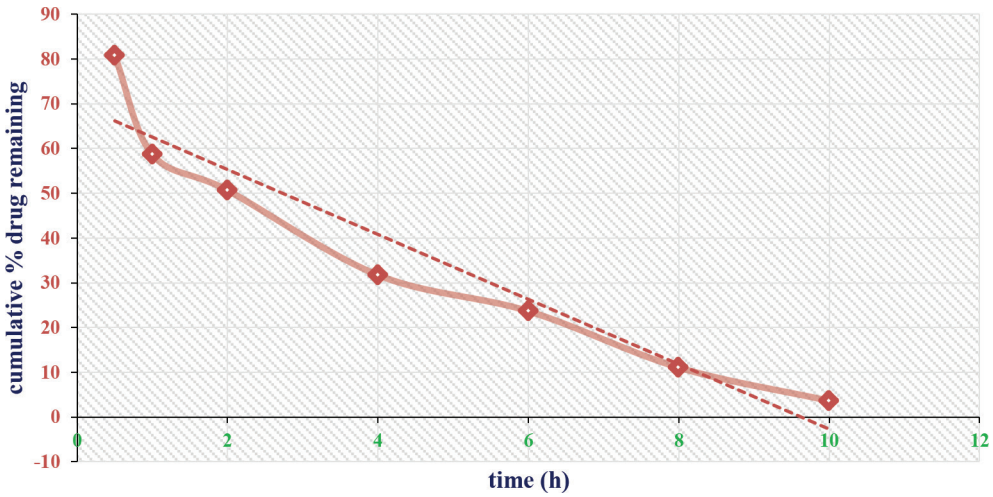

Figure 4. Cumulative % drug remaining vs. time.

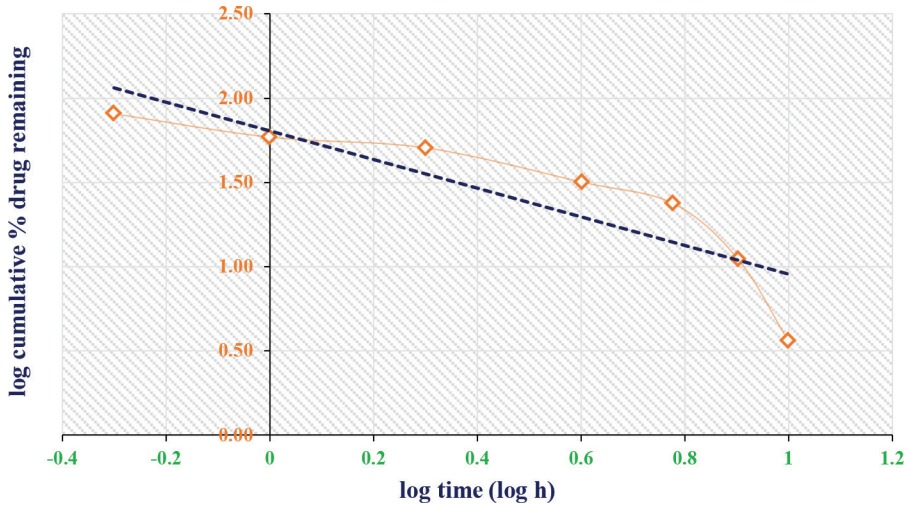

Figure 5. Cumulative % drug remaining vs. time.

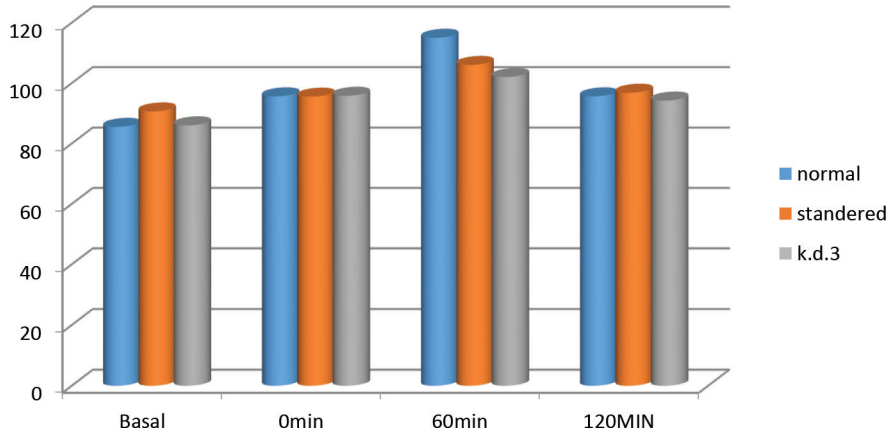

Figure 6. Graphical representation of effect of different formulation on SGL in witsar rats.

comparison to the 0 hr as depicted in Figure 6. Animals pretreated with the developed formulations, at a dose of 250mg/kg,), KD-3 and Standard demonstrated a rise in serum glucose levels by 15.45%(KD-3) and 17.26%, respectively. Based on these findings, KD-3 was chosen for further development into a novel drug delivery system. However, within a span of two hours, all animal groups displayed a return to nearly normal serum glucose levels, indicating the healthy functionality of the animals' pancreas in efficiently managing and clearing the glucose load from the body.

3.2.7 Stability Studies

Stability assessments for the fine-tuned formulations were carried out over a duration of 90 days under controlled temperatures of 4.0 ± 0.5 °C and 28 ± 0.5 °C. Throughout this timeframe, there were no discernible alterations noted in the visual characteristics, mean particle size, or percentage of drug content within the transferosomal gel. The results indicate the formulations' robustness and ability to maintain their integrity under the specified storage conditions.

4. Conclusion

Charantin loaded nonvascular formulation (transferosomes) has been successfully developed with the desirable performance characteristics and optimum penetration ability. The formulation can be scaled up and processed further after the relevant *in vivo* and *ex vivo* experimentations and proof of concept. With successful development, the formulation is poised for scalability, paving the way for further processing after thorough *in vivo* and *ex vivo* experimentations, ultimately establishing a strong proof of concept for its potential applications.

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