RESEARCH PAPER

Transdermal delivery of Rosuvastatin from proniosomal gels for hyperlipedemia management in Hypercholesterolemic Wister rats

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ABSTRACT

Rosuvastatin (ROS) is a statin derivative, which is primarily used for lowering cholesterol levels in individuals with hyperlipidemia (high levels of fats, such as cholesterol, in the blood). It works via inhibition of HMG-CoA reductase enzyme. In the current investigation, ROS-containing proniosomal gels were prepared and evaluated for transdermal application in the management of hyperlipedemia. At first, ROS-containing proniosomal suspensions were prepared via the coacervation phase separation method, where the ratio of non-ionic surfactant to soy lecithin was 1:2. The ROS-containing proniosomes (R1) prepared using Span 40 exhibited much greater drug (ROS) entrapment efficiency (85.39 ± 0.56%) than other formulations. Four different ROS-containing proniosomal gel formulations (F1-F4) were then successfully prepared using different combination of Carbopol 934 P, guar gum, and HPMC K100 at a concentration of 2% (w/v). After successful preparation, all these ROS-containing proniosomal gel were evaluated. Amongst the formulated ROS-containing proniosomal gels, F1 gel formulation exhibited clear physical appearance and good homogenity, pH- 5.2, and higher drug content (97.66 ± 1.24%). The in vitro drug release studies and ex vivo permeation study of the ROS-containing proniosomal gel of F1 formulation showed (96.45±0.54%) in 9 h and (81.32 ± 1.07 %) in 10 h, respectively. The in vivo study using hypercholesteromic Wister rats showed a significant reduction of total cholesterol, after topical application of ROS-containing proniosomal gel of F1 formulation as comparison to oral suspension containing pure ROS and control group. This ROS-containing proniosomal gel showed a significantly lowered hypercholesterolemia effect in hypercholesteromic Wister rats as compared to oral suspension containing pure ROS. Therefore, the formulated ROS-containing proniosomal gels can be used for transdermal application in the management of hyperlipidaemia.

Keywords: Rosuvastatin, Proniosomes, Lecithin, Hypocholesterolemic effect, Transdermal delivery

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INTRODUCTION

Hyperlipidemia is characterized by irregularities in plasma lipid levels, and is known as a condition where there are abnormally high levels of lipids or lipoproteins in the blood [1]. This condition describes increased level of low-density lipoprotein (LDL), triglyceride levels, total cholesterol, or lipoprotein levels and a reduction in high-density lipoprotein (HDL) concentration. The main causes of hyperlipidaemia include a high-fat diet, a sedentary lifestyle, and inadequate exercise [2]. They categorised by two type. It is based on lipid profile type (hypercholesterolemia and hypertriglyceridemia) causing variables include both inherited and acquired factors [3]. The treatment plan of hyperlipidaemia focuses on diet and lifestyle modification, hygiene, proper food, glycemic management, and lipid-lowering medications, if needed [4].

As lipid lowering medication, statins (for examples: rosuvastatin, atorvastatin, simvastatin, pitavastatin, lovastatin, fluvastatin, *etc*.) are commonly used in the management of hyperlipedimia [5]. Statins inhibit HMG-CoA reductase, which transforms HMG-CoA into mevalonate (a precursor to cholesterol) to decrease the cholesterol level in the blood [5]. Rosuvastatin (ROS) is a member of statin derivative, which is well-known synthetic lipid-lowering drug and primarily used for lowering cholesterol levels in individuals with hyperlipidemia (high levels of fats, such as cholesterol in the blood) [6]. It works *via* inhibition of HMG-CoA reductase enzyme, which plays a crucial role in

cholesterol synthesis and reduces the cholesterol production in the blood [6]. When taken in combination with a healthy diet, ROS helps to raise your high density lipoprotein(HDL) with decreasing triglycerides and low density lipoprotein(LDL) in the blood. ROS is classified as a biopharmaceutical (BCS) class II, indicating that it is weakly water-soluble and has limited oral absorption due to gastrointestinal degradation [7]. It has a low aqueous solubility and substantial first pass metabolism, limiting its absolute bioavailability to around 20% [7]. Therefore, transdermal delivery of ROS offers a promising alternative to conventional oral administration, achieving sustained and controlled drug effects, reducing the chances of side-effects, and bypassing the first-pass metabolism.

Transdermal drug delivery (TDD) across the skin to the systemic circulation facilitates benefits like avoidance of first pass metabolism, minimizations of drug degradation and loss, lesser occurrences of side-effects, and improved patient compliance [8]. However, one of the major concerns in TDD is the low penetrating rate through the outermost layer of epidermis. Recent years, a number of vesicular drug releasing systems (e.g., liposomes, proliposomes, niosomes, proniosomes, *etc*.) have been investigated and designed for their potential of overcoming the skin barrier.These vesicular drug releasing systems are able to increase bioavailability of encapsulated drugs. Amongst these vesicular systems, the proniosomal approach has been reported to minimize/reduce the problems of physical stability associated with niosomes during the storage of dosage [9]. Therefore, ROS-containing proniosomal gels for transdermal application would be beneficial in the management of hyperlipedemia to avoid problems associated with conventional delivery system. In the current investigation, ROS-containing proniosomal gels were prepared and evaluated for transdermal application in the management of hyperlipedemia. Prior preparing the ROS-containing proniosomal gels, ROS-containing proniosomal suspensions were prepared *via* the cocervation phase separation method. In the preparation of ROS-containing proniosomal suspensions, different non-ionic surfactants like Span 40, Tween 20, and Tween 80 were used with soy lecithin in different combinations. In this investigation, ROS-containing proniosomal suspensions, and ROS-containing proniosomal suspensions (which were formulated using ROS-containing proniosomal suspensions) were evaluated, *in vitro*. Finally, the formulated ROS-containing proniosomal gel was evaluated for management of hyperlipedemia in hypercholesteromic Wister rats with transdermal application. Though a few investigations have recently been reported for the designing of ROS-containing proniosomal transdermal gels, the *in vivo* evaluation of ROS-containing proniosomal transdermal gels in hypercholesteromic Wister rats with transdermal application has not been reported till date.

EXPERIMENTAL:

MATERIAL AND METHODS:

ROS calcium (Sun pharma, India), ethanol (Merck chemical Ltd., India), guar gum (Merck chemical Ltd.,), soy lecithin (SRL chemicals), Carbopol 934 P and HPMC K100 Span 40, Tween 20 Tween 80 were obtained from Sisco Research Laboratories Pvt. Ltd, India are used. All of the chemicals and reagents used in research were of analytical grade.

METHODS

Preparation of ROS-containing proniosomal suspension

ROS-containing proniosomal suspensions were produced using the coacervation-phase separation technique, as described in the works of Perrett et al. [10] and Vora et al. [11]. Lecithin and ROS calcium were mixed with ethanol (about 0.5 ml) in glass vials containing precisely measured quantities of surfactants. The vials were then heated in a temperature controlled water bath at 55–60 °C for 5 min, with occasional shaking to ensure the lecithin was completely dissolved. After adding 1.6 ml pH 7.4 phosphate buffer, the mixture was heated in the water bath at 55–60 °C for about 2 min, resulting in the formation of a clear mixture. The prepared mixtures were then left to cool at room temperature for 24 hours, resulting in a two-phase liquid, or a translucent, transparent, or white creamy ROS-containing proniosome suspension. Different non-ionic surfactants like Span 40, Tween 20, and Tween 80 were used with soy lecithin, where the ratio of non-ionic surfactant to soy lecithin was maintained 1:2. The composition of ROS-containing proniosomal suspensions were given in Table 1.

Table 1 Composition of ROS-containing proniosomal suspensions

Ingredients of excipients with drug	**Formulation Code**		
	R1	R2	R3
ROS calcium (mg)	20	20	20
Span 40 (mg)	80	-	-
Tween 20 (mg)	-	80	-

Tween 80 (mg)	-	-	80
Soy lecithin (mg)	160	160	160
Ethanol (ml)	0.5	0.5	0.5
Distilled water (ml)	2	2	2

Evaluation of ROS-containing proniosomal suspension

Measurement of drug entrapment efficiency (EE, %)

To measure EE (%), 1 mL of prepared ROS-containing proniosomal suspension from each formulation was subjected to 30 min of sonication in a bath sonicator. Then the sonicated sample was centrifuged by a centrifuge (REMI 12C Centrifuge, India) at 14,000 rpm for 30 min at room temperature and untrapped drug was separated. After being collected, the supernatant became diluted with pH 7.4 phosphate buffer and evaluated using a UV-VIS spectrophotometer (Shimadzu UV-VIS 1900, Japan). The following formula was employed to measure the drug entrapment [12].

$$EE\ (\%) = [(C_a - C_b)/C_a] \times 100$$

EE is the drug entrapment efficiency (%), C_a and C_b are the concentration of total ROS, and of free ROS respectively.

Determination of vesicle size

Using Zetasizer Nano-ZS (Malvern Instruments Ltd.,), the vesicle size, was measured at 25 ± 0.5°C with a 45 mm focus lens and a 2.4 mm beam length. The samples of prepared ROS-containing proniosomal suspension were transferred to cuvette.

***In vitro* drug release study of ROS-containing proniosomal suspension**

The dialysis bag technique was used under sink conditions to calculate the cumulative % drug released from ROS-containing proniosomal suspension for all three batches. Before being used, the dialysis bag was initially submerged for 24 h in the pH 7.4 phosphate buffer. The dialysis bag was filled with the prepared ROS-containing proniosomal suspension sample (2 ml), closed at both ends, tied to the shaft of the dissolution test apparatus and submerged in 900 mL pH 7.4 phosphate buffer. The rotation of the shaft was 50 rpm and the temperature was maintained at 37 ± 0.5 °C. Samples (5 ml) were taken out at regular intervals and an equivalent volume of freshly prepared release medium was replenished. Each sample was filtered using 0.2 µm Millipore membrane filters, and analysed at 241 nm (λmax) for ROS content using a UV-VIS spectrophotometer (Shimadzu UV-VIS 1900, Japan). The % CDR was measured and plotted against time [13, 14].

Preparation of ROS-containing proniosomal gels using ROS-containing proniosomal suspension

The selected ROS-containing proniosomal suspension was taken to prepare proniosomal gels by using three different gelling agents like Carbopol 934 (P), guar gum, and HPMC K100. The polymer solutions were prepared with proper concentration and allowed to swell for 24 h. During this time, the polymers absorbed water and swelled to form polymeric gels. Then, the selected ROS-containing proniosomal suspension (2 ml) was added to 2% polymeric gels with the help of mechanical stirrer (Remi Motors, India) at 200 rpm. After complete mixing, the mixture containing ROS-containing proniosomal gels was kept overnight to ensure uniform distribution and to remove any air bubbles that may have been introduced during mixing [15]. The different formulations of ROS-containing proniosomal gels are presented in Table 2.

Table 2 Composition of gels

	Formulation Code			
Formulation Ingredients	**F1**	**F2**	**F3**	**F4**
Carbopol 934(P)	2% (w/v)	-	-	1% (w/v)
HPMC K100	-	2% (w/v)	1% (w/v)	-
Guar gum	-	-	1% (w/v)	1% (w/v)

Characterization of ROS-containing proniosomal gels

Visual observation

The prepared ROS-containing proniosomal gels were viewed with the naked eye to observe the gel's colour and appearance [16].

pH measurement

A digital pH meter (Systronics pH Meter 335) was used to measure the pH of the formulated four ROS-containing proniosomal gels. After dissolving a sample of 0.1 g gel in 10 ml of distilled water, the electrode of pH meter was dipped into the solution, and a continuous reading was recorded [17]. Three times on average were the readings taken.

Estimation of drug content

A glass tube containing 0.1 g of ROS-containing proniosomal gels from each formulation was filled with 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was subjected to sonication in a sonicator bath (Rolex, India) and then, centrifugation was carried out using a centrifuge (Ultra centrifuge) at 9000 rpm for 30 min at room temperature. The supernatant was obtained and its unentrapped ROS content was measured at 241 nm (λmax) using a ultraviolet (UV) spectrophotometer (Shimadzu, Japan) [18]. The drug content was calculated using the following formula:

Drug content (%) = Practical drug content / Theoretical drug content ×100

***In vitro* drug release studies of ROS-containing proniosomal gels**

The cumulative % drug released from different ROS-containing proniosomal gels was determined by dialysis bag method. Before being used, the dialysis bag was initially submerged for 24 h in the pH 7.4 phosphate buffer. The formulated ROS-containing proniosomal gel sample (containing 20 mg ROS) was taken in the dialysis bag, which was then closed at both ends and tied to the shaft of dissolution test apparatus type II. Afterwards, it was immersed in 900 mL of pH 7.4 phosphate buffer. The shaft rotated at 50 rpm and the temperature remained at 37 ± 0.5 °C. Samples (5 ml) were taken at regular intervals and supplemented with an equal volume of freshly prepared dissolution media.Each sample was filtered using 0.2 µm Millipore membrane filters, and analysed at 241 nm (λmax) for ROS content using a UV-VIS spectrophotometer (Shimadzu UV-VIS 1900, Japan). The % CDR was measured and plotted against time [19, 20].

Skin permeation analysis *ex vivo*

Ex vivo permeation analysis of ROS-containing proniosomal gels was performed using chicken skin as a model membrane [21]. The chicken dorsal skin was obtained from freshly killed chicken from the local slaughter house. Then, the skin was dipped with physiological solution at room temperature for 2 h to keep it fresh. The skin was cleaned with pH 7.4 phosphate buffer to remove adhering fat and again, washed with distilled water to ensure cleanliness. The cleaned skin was washed thoroughly with isotonic phosphate buffer (pH 7.4) before use. The *ex vivo* permeation evaluation was performed employing the method used by El-Alim *et al.* with slight modifications [22]. A circular piece of skin (approximately 4 cm in diameter) was carefully cut and attached to the dialysis tube by thread. Then, the formulated ROS-containing proniosomal gel sample (containing 20 mg ROS) was placed inside the tube. The whole assembly was attached to the shaft of dissolution test apparatus II so that the membrane was just below the surface of the release media, pH 7.4 phosphate buffer, rotated at 50 rpm speed and the temperature was maintained at 37 ± 0.5 °C. Samples (5 ml) were taken out at regular time intervals and an equivalent volume of freshly prepared release medium was replenished. Each sample was filtered using 0.2 µm Millipore membrane filters, and analysed at 241 nm (λ_{max}) for ROS content using a UV-VIS spectrophotometer (Shimadzu UV-VIS 1900, Japan). The cumulative % percent drug release was measured and plotted against time [19, 20].

***In vivo* evaluation study**

Ethics approval

The *in vivo* study was carried out according to ARIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for animal experiment and the protocol (IAEC/SPS/SOA/143/2023) was previously approved by the Institutional Animal Ethics Committee (IAEC) of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, 751003, Odisha, India.

Assessment of hypocholesterolemic effect

The purpose of the *in vivo* study was to compare the hypocholesterolemic effect of oral suspension containing pure ROS to the transdermal application of ROS-loaded proniosomal gel formulation F1 in hypercholesterolemic rats. Three groups of six male Wistar rats each were clarified weighing 130 ± 20 g. Each rat was kept in a chamber with controlled lighting and temperature. Before causing hypercholesterolaemia, blood samples were taken through the retro-orbital sinus of each rat through heparinised glass capillary tubes onto a glass slide. To provide a constant blood volume, 15 µl blood capillary collectors (CardioChek®) were used to transport blood to the cholesterol test strips. The CardioChek® Analyser (Polymer Technology Systems Inc., USA) was used to measure total cholesterol (TC) [23].

For 30 days, the rats were given an atherogenic diet to induce hypercholesterolaemia. Hydrogenated fat (5%), butter (5%), cholesterol powder (1%), and cholic acid (0.5%) from coconut oil were the ingredients of the atherogenic cholesterol diet, which was combined with powdered chow [24]. The TC of rats was measured after one month. Three sets of six rats each were grouped from the hypercholesterolemic rats. One group (negative control group) was not offered any medication. The second group of rats' dorsal skin was trimmed, and ROS-loaded proniosomal gel formulation F1 was applied for transdermal administration to the skin in an amount equal to 20 mg/kg. Rats in the third group, however, were given an identical dosage of oral suspension containing pure ROS *via* feeding tube. Every day for seven days, both therapies were administered. Each rat in

the three groups had its TC level was tested on the eighth day [23].

Statistical analysis

Means ± standard deviation (SD) are used to report data.

RESULT AND DISCUSSIONS

Preparation of ROS-containing proniosomal suspensions

ROS-containing proniosomal suspensions were prepared by coacervation-phase separation technique, where different non-ionic surfactants like Span 40, Tween 20, and Tween 80 were used with soy lecithin. The ratio of non-ionic surfactant to soy lecithin was maintained 1:2. The compositions of ROS-containing proniosomal suspensions were presented in Table 1.

Evaluation of ROS-containing proniosomal suspension

EE (%)

EE (%) values of ROS-containing proniosomal suspensions prepared using three different surfactants (namely Span 40, Tween 20, and Tween 80) were calculated (Fig. 1). The EE (%) values were found in following order: R1 > R3 > R2. According to the results, ROS-containing proniosomal suspensions prepared using Span 40 exhibited much greater EE (%) value (85.39 ± 0.56%) than other formulations.

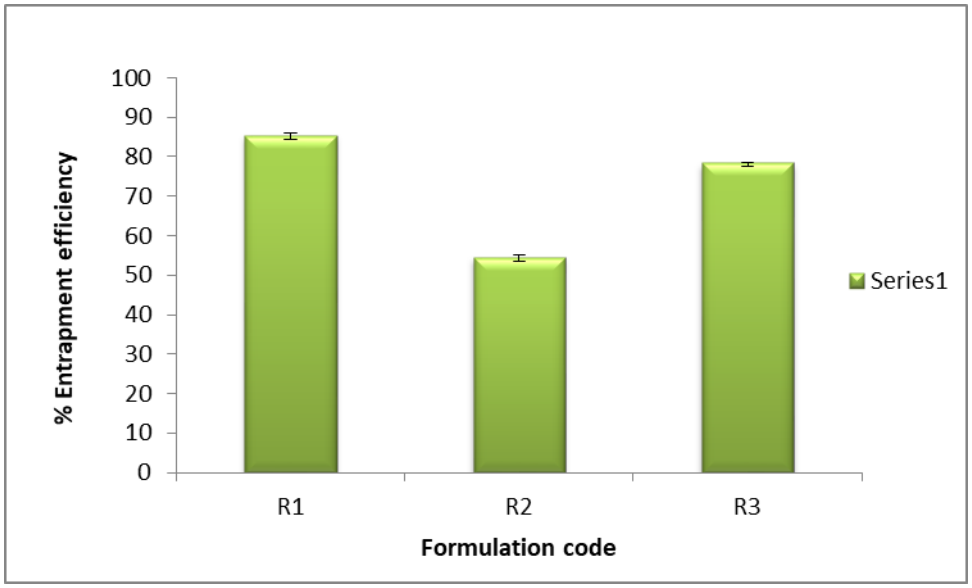

Fig. 1 EE (%) of ROS-containing proniosomal suspensions (Mean ± S.D.; n = 3)

Vescicle size

The Vesicle size distribution of ROS-containing proniosomal suspension of formulation R1 was recorded and represented in Fig. 2. According to the findings, the formulation R1 presented an average vesicle sizing of 288 nm, indicating a reduced vesicle sizing that might facilitate higher drug entrapment, epidermal penetration, and target site specific delivery.

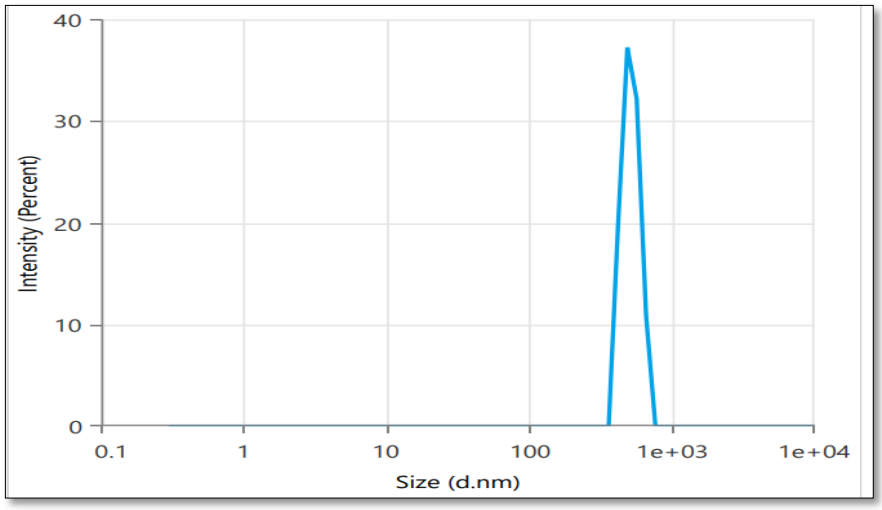

Fig. 2. Vesicle size distribution of ROS-containing proniosomal suspension of formulation R1

***In vitro* release study of ROS-containing proniosomal suspension**

The *in vitro* release behaviour of ROS-containing proniosomal suspension (R1, R2, and R3) was examined in pH 7.4 phosphate saline buffer. Fig. 3 showed the cumulative % drug released from these ROS-containing proniosomal suspensions. There was a quick burst release at the initial period, and then, it followed by a 9 h steady release. The quick release of loaded drug (ROS) within initial phase might be attributed to the leached drug in the dispersion medium, but the succeeding slower release might be due to slower diffusion of drug through the bilayer. Out of all the proniosomal suspensions incorporating surfactants, formulation R1 (ROS-containing proniosomal suspensions prepared using Span 40), exhibited the highest *in vitro* drug release at 9 h (91.31 ± 1.12 %) as compared to others. With this composition, the maximum entrapment efficiency was also noted.

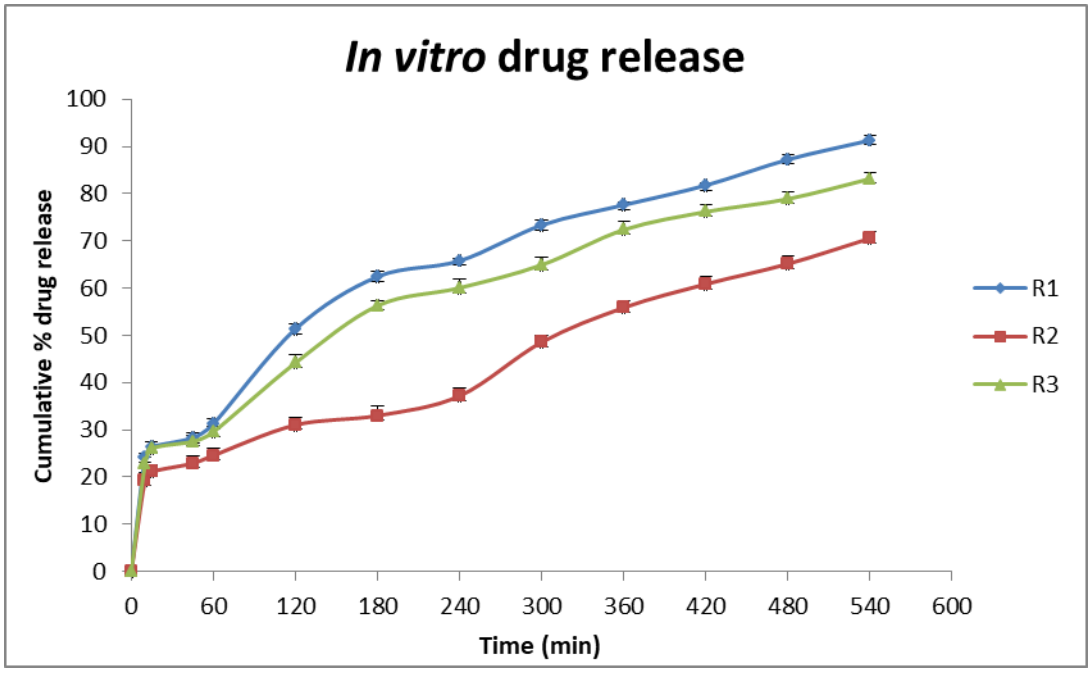

Fig. 3. *In vitro* cumulative % drug release from ROS-containing proniosomal suspensions (R1-R3) (Mean ± S.D.; n = 3)

Preparation of ROS-containing proniosomal gels

Four different ROS-containing proniosomal gel formulations (F1-F4, Table 2) were successfully prepared using selected ROS-containing proniosomal suspension (Formulation R1; prepared prepared using Span 40, where Carbopol 934 (P), guar gum, and HPMC K100 were used as different gelling agents to prepare 2% polymeric gel base. After successful preparation, all these ROS-containing proniosomal gel formulations were evaluated for transdermal drug delivery application.

Evaluation of ROS-containing proniosomal gels

Visual observation

Visual observations of these prepared ROS-containing proniosomal gels were carried out with the naked eye to observe the gel's colour and appearance. Table 3 presents the results of visual observations of these proniosomal formulations. The prepared F1 and F2 gel formulations showed white creamy coloured gels in observation while F3 and F4 gel formulations showed pale yellow coloured gels. The colours of these ROS-containing proniosomal gel formulations were found homogenous, indicating uniform distribution of excipients and drug (ROS) [25].

pH measurement

pH values of each prepared ROS-containing proniosomal gels were determined. The pH was assessed to examine the probability of discomfort upon application. More acidic pH or alkaline pH might cause discomfort after application onto the skin. The measured pH values of these ROS-containing proniosomal gels was found to be ranged from 5.2 to 6.2, indicating the physiological range of skin's surface (Table 3). The pH of the prepared topical gel within the physiological range of skin's surface is helpful to protect against infections and to maintain balance [26, 27].

Drug content (%)

Drug contents of prepared ROS-containing proniosomal gels were measured and presented in Table 3. The result indicated that all the drug contents of all these prepared ROS-containing proniosomal gels were within 87.43 ± 1.99% to 97.66 ± 1.24%. The proniosomal gel formulation F4 containing Carbopol 934 (P) and guar gum was found with highest drug content (97.66 ± 1.24%) as comparison to other gel formulations. The overall drug content results indicated a homogenous distribution of drug (ROS) within these proniosomal gel formulations [28, 29].

Table 3 Results of visual observation (Physical appearance as well as homogeneity), drug content (%) and pH of proniosomal gel formulation (F1-F4)

Code	Physical appearance	Homogeneity	Drug content (%)[a]	pH
F1	White creamy gels	good	95.37 ± 1.09	5.2
F2	White creamy gel	good	91.64 ± 0.98	5.6
F3	Pale yellow gels	good	87.43 ± 1.99	6.2
F4	Pale yellow gel	good	97.66 ± 1.24	6.1

[a]Mean ± S.D.; n = 3

***In vitro* drug release**

The *in vitro* drug release behaviour of these prepared ROS-containing proniosomal gels was studied. The *in vitro* ROS release of from prepared ROS-containing proniosomal gels was found between 86.22 ±1.18 % to 96.45±0.54% (Fig. 4). It was pointed out that the release of ROS from proniosomal gel formulation F1 was nearly 75% within 6 h as comparison to other formulations. The highest percentage of ROS release was found (96.45±0.54%) from proniosomal gel formulation F1 within 9 h. A typical biphasic release model showed up for all these ROS-containing proniosomal gels.

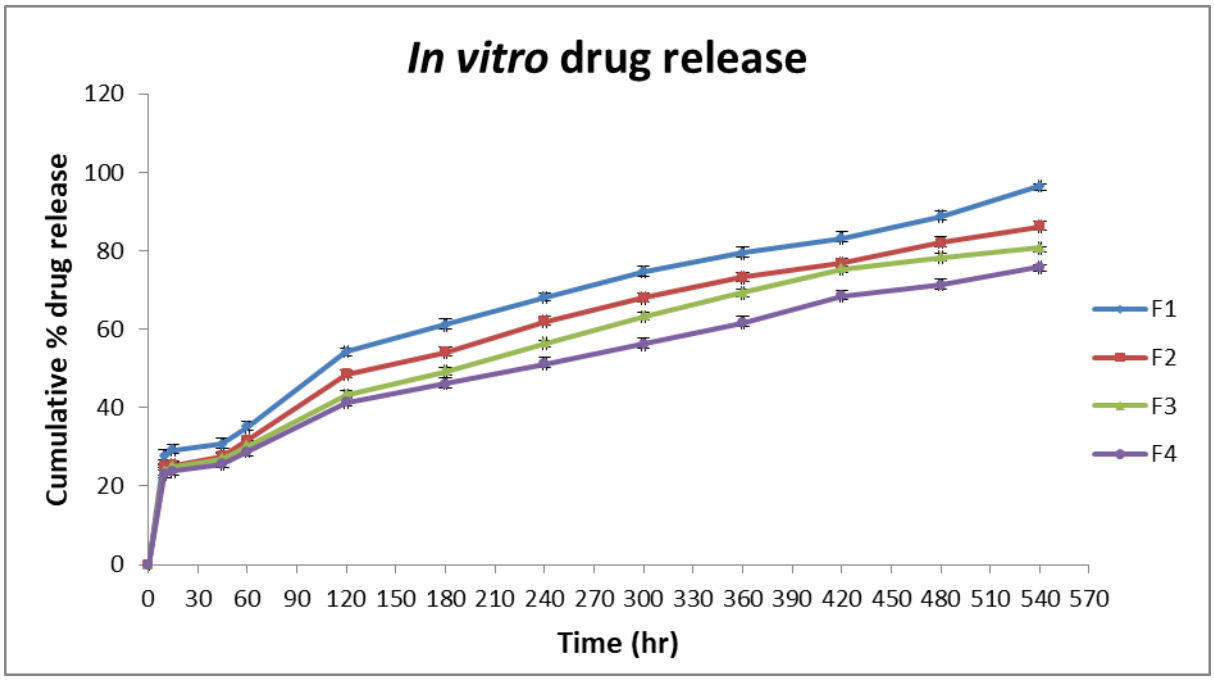

Fig. 4 *In vitro* cumulative % drug release from ROS-containing proniosomal gels (F1-F4) (Mean ± S.D.; n = 3)

***Ex vivo* permeation analysis**

In present study, *ex vivo* drug (ROS) permeation experiment of ROS-containing proniosomal gels was carried out and the drug permeated (%) from these proniosomal gels was plotted against time (Fig. 5). The obtained results clearly showed that the percentage of drug permeation through skin after 10 h was the highest for proniosomal gel formulation F1 (81.32 ±1.07%), followed by proniosomal gel formulation F2 (72.58 ±0.62%), proniosomal gel formulation F3 (54.80 ±0.19%) and finally proniosomal gel formulation F4 (46.49 ±0.63 %). This might be due to highest EE% observed in case of proniosomal gel formulation F1 compared to other formulations.

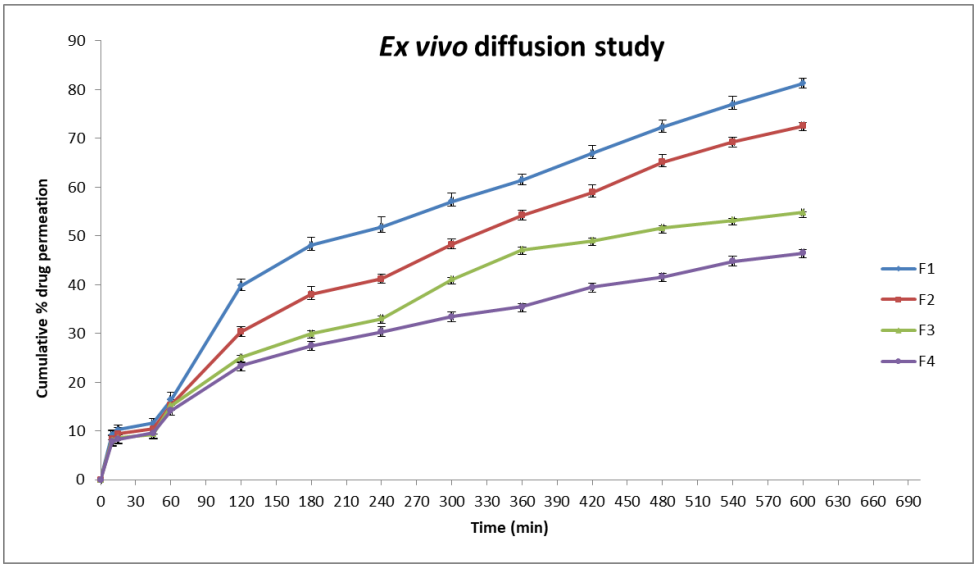

Fig. 5 *Ex vivo* drug permeation from ROS-containing proniosomal gels (F1-F4) (Mean ± S.D.; n = 3)

Monitoring of hypocholesterolemic effect

Following 30 days on a normal atherogenic diet, it was observed that, the weight of rats in all groups were increased, which indicated the successful induction of hypercholesterolemia among three groups. Table 4 displayed the mean plasma TC before treatment and after

7 days of therapy with freshly prepared ROS-loaded proniosomal gel formulation F1 and oral ROS suspension in hypercholesterolemic rats. Before treatment, the mean plasma TC of group 1 (control), group 2 (ROS-loaded proniosomal gel formulation F1), group 3 (oral suspension) was estimated as 73.10 ± 1.08 mg/dl, 72.76 ± 1.02 mg/dl and 73.23 ± 1.12 mg/dl, respectively. After consecutive 7 days therapy, the mean plasma total cholesterol of group 2 and group 3 was reduced. The photography images of rat skin before and after application of ROS-loaded proniosomal gel formulation F1 have been given in Fig. 6. However, in case of group 2 (ROS-loaded proniosomal gel formulation F1), the mean plasma TC was found significantly ($p < 0.05$, ANOVA followed by post-hoc Tukey HSD test) reduced as comparison to group 3 (oral suspension), suggesting the efficacy of the formulated ROS-containing proniosomal gel for transdermal application in the management of hyperlipedemia.

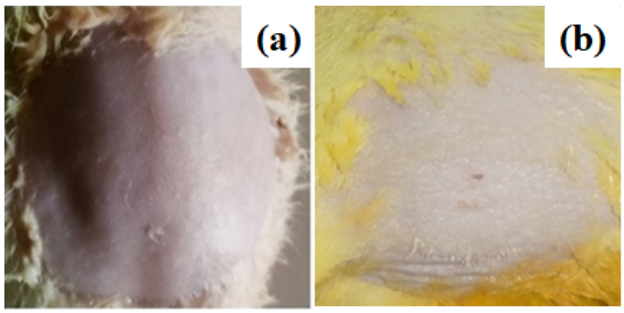

Fig. 6 The photography images of rat skin before application (a) and after application (b) of ROS-loaded proniosomal gel formulation F1.

Table 4. Mean plasma TC (mg/dl ± SD, n = 6) immediately prior to and following 7 days of therapy with ROS-loaded proniosomal gel formulation F1 on epidermis or orally ROS suspension in hypercholesterolemic rats.

Groups	**Mean plasma TC (mg/dl)[a]**	
	Before medication	**After medication**
Group 1 (Control)	73.10 ± 1.08 (mg/dl)	73.10 ± 1.08 (mg/dl)
Group 2 (ROS loaded proniosomal gel formulation F1)	72.76 ± 1.02 (mg/dl)	55.31 ± 1.09(mg/dl)
Group 3 (Oral suspension)	73.23 ± 1.12 (mg/dl)	63.76 ± 1.34(mg/dl)

[a]Mean ± SD; n = 6

CONCLUSION

A transdermal proniosomal delivery method for the poorly soluble drug, ROS, was designed and evaluated (*in vitro, ex vivo* and *in vivo)*. The topical administration of ROS-loaded proniosomal gel (designed of lecithin: Span 40, 2: 1) onto skin, greatly increased the hypocholesterolemic impact in the treatment of hypercholesterolemic rats. All the results obtained from different parameters have given satisfied results. To sum up these, ROS proniosomal gel presents can be employed as an option for transdermal delivery of ROS.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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