

Development of Rutin Suspension and Evaluation of Corneal Permeation across the Goat Cornea

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ABSTRACT

Rutin, a bioflavonoid drug of natural origin, has significant antioxidant and anti-inflammatory property and can decrease the intraocular pressure. The present research work is aimed to formulate rutin suspension and to evaluate the corneal permeation of rutin. Rutin suspension has been formulated by using suspending agent and homogenizer. The formulated drug containing suspension were characterized for average particle size, zeta potential, polydispersity index, pH, *in-vitro* drug release and *ex-vivo* corneal permeability in the goat cornea. The rutin suspension has an average particle size of 1.1 μm , zeta potential of -58 mV, polydispersity index of 1 and pH of 7.0. Drug release study of rutin suspension and rutin aqueous dispersion showed 42.32 % & 17.39 % rutin release at 8 h respectively. *Ex-vivo* corneal permeation of rutin from its suspension and aqueous dispersion across the goat cornea was found to be 2889.31 ng & 855.52 ng respectively. It concluded that developed rutin suspension produced enhanced *in-vitro* drug release and *ex-vivo* corneal permeation in comparison to rutin aqueous dispersion.

KEY WORDS: Rutin, Suspension, Particle size, *In-vitro* drug release and Corneal permeation.

1. INTRODUCTION

Topical administration is the most suitable and patient acquiescent route for administration of drug (s) utilized for the treatment of diseases associated with anterior segment. The drugs applied topically will mix with the ocular tear fluid and spread over the corneal surface. However, various pre-corneal factors such as nasolacrimal drainage, noncorneal absorption, and induced tear fluid preserve the ocular drug absorption by reducing the residence time of the applied drug substances on cornea. Drug administrated topically over a corneal surface penetrates across the cornea primarily and through the non-corneal routes (Tirucherai, 2002; Gunda, 2006; Gallarate, 2013; Tirucherai and Mitra, 2003). The diffusion of the administered drug takes place across the conjunctiva and sclera of non corneal route and considered to be important for poorly absorbed drugs (Jaswal, 2016; Vakam, 2008). To overcome the problems associated with ocular drug delivery and to improve the ocular bioavailability, various drug delivery systems were adopted such as solutions, emulsions, ointments, suspension, aqueous gels, nanoparticles, hydrogels, etc., (Patel, 2013). Topical administrations of dosage forms to the eye impose certain difficulties such as reduced drug absorption, pulse-drug entry, and poor intraocular drug delivery (Mainardes, 2005). Only 5% of the installed drug dose reaches the intraocular tissues. Recent advances on drug delivery to the anterior segments to improve the residence time of the drug on the eye surface and corneal permeability are achieved by incorporation of excipients having viscosity and permeation enhancing properties in the conventional formulations (Patel, 2013). Suspensions are widely used non-invasive topically applied drug carrier systems. It is defined as insoluble API dispersed in an aqueous solvent consisting of a suitable suspending and dispersing agent (Lang, 2009; Pitknen, 2007; Salminen, 1985). Drug particles in the suspension improved the residence time by holding the drug particles in pocket of cornea and showed enhanced duration of action over a drug solution (Schoenwald, 1990). Increases in the residence time of drug improve the bioavailability and will reduce the dosage frequency, the amount of drug required and side effects. Particle size of the drug in the carrier system has an impact on the duration of action and drug absorbed into the ocular tissues. Rutin, also called as rutoside or quercetin-3-O-rutinoside (Figure.1), is a bioflavonoid drug of natural origin. It has significant antioxidant, anti-inflammatory, anti thrombolytic and antinociceptive property; ability to inhibit aldose reductase enzyme activity and reduce the intraocular pressure. In many country's rutin used as a medication in multivitamin preparations and several herbal remedies for the protection of blood vessels (Vetruigno, 2012; Rhee, 2001). To reduce the intraocular pressure in eye, rutin needs to permeate across the cornea. Permeation of rutin across the goat cornea is not yet reported. Hence, in the present work an attempt has been made to formulate rutin suspension using polymeric suspending agent and to evaluate the corneal permeation of rutin.

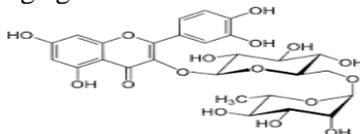


Figure.1. Structure of rutin

2. MATERIALS AND METHODS

Materials: Rutin procured from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India and carboxymethyl cellulose acquired from S D Fine Chemical Ltd., Mumbai, India. All the other chemicals and solvents used were of analytical grade.

Formulation of rutin suspension: Carboxymethyl cellulose (CMC), 2% W/V, was dissolved in double distilled water to obtain aqueous solution of CMC. A Known amount of rutin (1%) was added to above solution, and the dispersion was homogenized 30 min using homogenizer (T18 Digital Ultra-Turrax, India) to obtain uniform suspension. Rutin was added to double distilled water and homogenized 30 min to obtain rutin aqueous dispersion.

Characterization of rutin suspension: Average particle size, zeta potential and PDI of rutin suspension and rutin aqueous dispersion were determined by using laser diffraction and photon correlation spectrophotometer (Zetasizer ZS 90, Malvern instruments, UK) (Li, 2011). pH of the suspension and dispersion was determined using Professional Portable Pen Type pH Meter (Swastik Scientific Instruments Private Limited, Mumbai).

In-vitro drug release by using dialysis membrane: The *in-vitro* drug release of formulated rutin suspension and rutin aqueous dispersion were assessed over a time period of 8 h by using dialysis membrane bag method consisting of a membrane (cut-off: 3500 Da) loaded with 1 mL of rutin suspension and rutin aqueous dispersion respectively were loaded in the dialysis membrane (cut-off: 3500 Da) bag and suspended in 50 mL of receptor medium of phosphate buffer pH 7.4 at 34°C (Ye, 2008). The receptor medium was stirred at 600 rpm by using the magnetic stirrer. Aliquots, each one mL of aliquots were withdrawn in predetermined intervals and replenished with an equal volume of the fresh receptor medium, and aliquots were analyzed by HPLC to quantify the total amount of rutin released from the dialysis bag.

Ex-vivo corneal permeation by using goat cornea: *Ex-vivo* corneal permeation of formulated rutin suspension and rutin aqueous dispersion is evaluated across the goat cornea by using side-by-side corneal cell, both donor and receptor cell had an internal jacket and side arms (Tirucherai, 2002). The side arm of cells was used for loading and sampling of solution respectively. The goat cornea was obtained from the slaughter house. At most care was taken to remove the cornea along with adjacent scleral tissue (2 - 4 mm) and thoroughly washed with ice cold saline. Corneas were stored in freshly prepared phosphate buffer solution (pH 7.4). The cornea was placed between donor and receptor cell and clamped. Epidermal surface of the cornea was focused on the donor side of the cell. The receptor cell was filled with 4.2 mL of phosphate buffer (pH 7.4) and rutin suspension and rutin aqueous dispersion were dispersed in the phosphate buffer (pH 7.4) was placed in donor cell (4mL), and both the cells were stirred with teflon coated magnetic stirr bar at a speed of 150 rpm. The temperature of the entire corneal cell assembly was kept at 34°C by using sub-zero water circulating constant temperature bath. Each 1 mL of samples was withdrawn from receptor cell at 15, 30, 45, 60, 90 and 120 min, replacing with fresh buffer solution and samples were analyzed by HPLC.

3. RESULTS AND DISCUSSIONS

Formulation and characterization of rutin suspension: The rutin suspension and rutin aqueous dispersion made by using homogenizer is shown Figure.2. The CMC was used as suspending agent to get uniform drug dispersion and to decrease the particle size of drug particles (Patel, 2010).

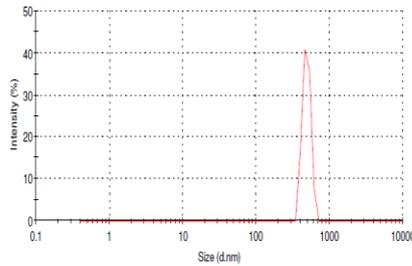


Figure.2. Rutin suspension and rutin aqueous dispersion

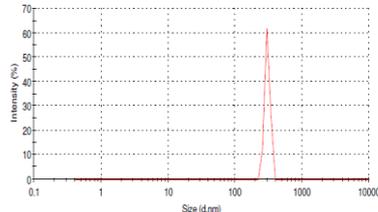
The average particle size, zeta potential, PDI and pH of rutin suspension and rutin aqueous dispersion are shown in Table 1. Rutin suspension showed the particle size of 1.1 μm and zeta potential of -58 mV. Rutin aqueous dispersion showed particle size of 3.3 μm and zeta potential of -9.82 mV (Figure.3). The particle size of the rutin aqueous dispersion was observed to be higher compared with rutin suspension and decrease in the particle size of the suspension might be due to the incorporation of CMC. Zeta potential indicates the degree of particle-particle repulsion forces necessary to avoid agglomeration and aggregation and used as an indicator to determine the stability of the formulations. Zeta potential value of rutin suspension is high compared with rutin aqueous dispersion and it indicates the physical stability of the developed rutin suspension formulation.

Table.1. Average particle size, zeta potential, PDI and pH of rutin suspension and rutin aqueous dispersion

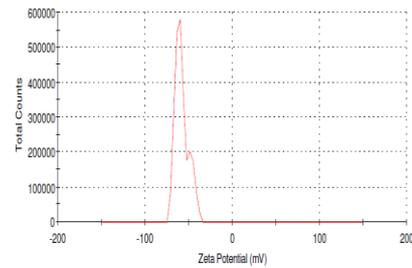
| Formulations | Average particle size (μm) | Zeta potential (mV) | PDI | pH |
|--------------------------|---|---------------------|-------|-----|
| Rutin suspension | 1.1 \pm 0.8 | -58 \pm 3.0 | 1 | 7.0 |
| Rutin aqueous dispersion | 3.3 \pm 0.1 | -9.82 \pm 2.1 | 0.846 | 7.0 |



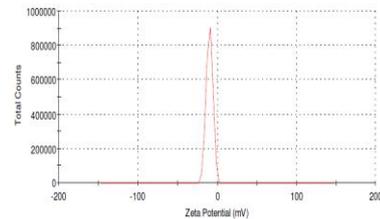
Average particle size: 1.1 μm



Average particle size: 3.3 μm



Zeta potential: -58 mV



Zeta potential: -9.82 mV

Figure.3. Average particle size & zeta potential of rutin suspension and rutin aqueous dispersion

In-vitro drug release by using dialysis membrane: Dialysis membrane bag method was utilized to study the *in-vitro* drug release of formulated rutin suspension and rutin aqueous dispersion. The quantity of rutin released from the rutin suspension was quantified by HPLC method. The representative chromatogram for the estimation of rutin release from the suspension is shown in Figure.4. The *in-vitro* drug release profile for the rutin suspensions and rutin aqueous dispersions are shown in Figure.5. Within 8 h of the study, rutin suspension showed 42.32 % of release of rutin where as rutin aqueous dispersion showed 17.39 % of rutin release. The increased drug release from the rutin suspension may be due to the diffusion of drug in the presence of suspending agent.

Ex-vivo corneal permeation by using goat cornea: The *ex-vivo* corneal permeation profiles of the rutin suspension and rutin aqueous dispersion across the goat cornea for the duration 2 h are shown in Figure 6. The amount of rutin permeated across the goat cornea is compared with rutin aqueous dispersion. *Ex-vivo* corneal permeation of the rutin cornea within 120 min rutin suspension and rutin aqueous dispersion was found to be 2889.31 ng & 855.52 ng respectively. The higher corneal permeability of rutin suspension may be due to the presence of suspending agent of carboxymethyl cellulose and lower particle size.

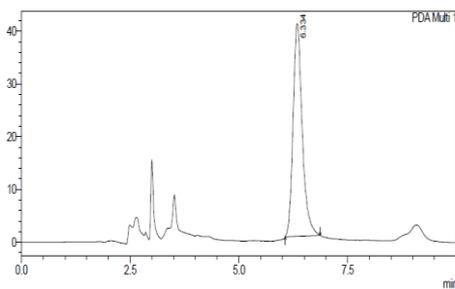


Figure.4. HPLC chromatogram of estimation of rutin released from the suspension at 45 min.

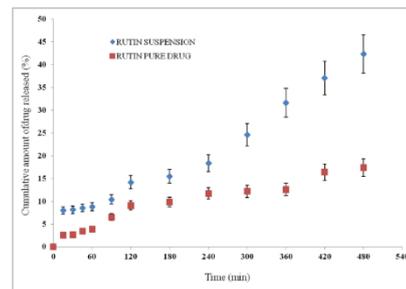


Figure.5. *In vitro* drug release of rutin suspension and rutin aqueous dispersion by dialysis bag method

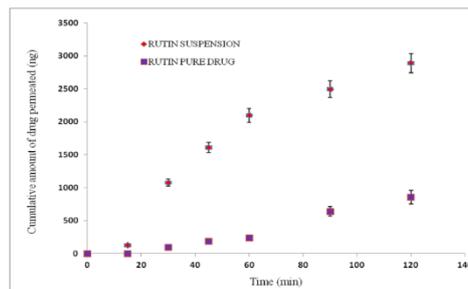


Figure.6. *Ex-vivo* corneal permeation of rutin from the suspension and rutin aqueous dispersion across goat cornea

4. CONCLUSION

In the present study, uniform rutin suspension was developed, and it showed better/acceptable particle size, zeta potential, PDI and pH. The incorporation of suspending agent in the formulation of rutin dispersion showed an increase in the *in-vitro* drug release and corneal permeation of rutin across the goat cornea. It was concluded that the development of novel formulations of rutin with optimized size and excipient's concentration might produce enhanced *ex-vivo* corneal permeation of rutin on topical administration to eye.

5. ACKNOWLEDGEMENT

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