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Niosomal formulation of Quercetin and Resveratrol and *in-vitro* release studies

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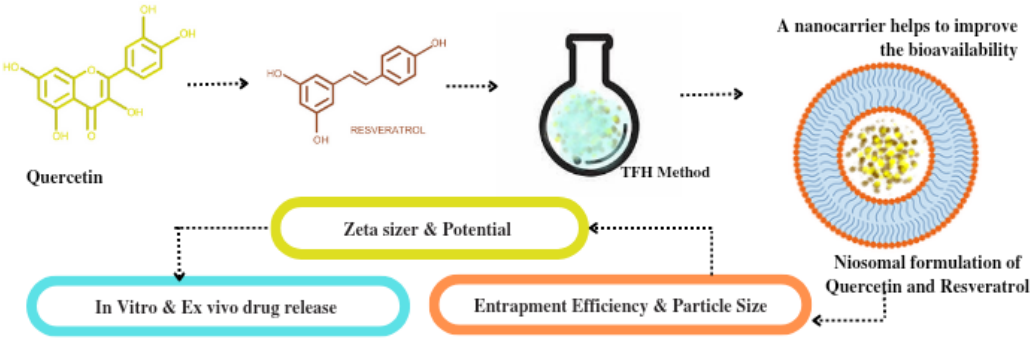
ABSTRACT



Dietary polyphenols from plant origins play a major role in the human diet. They supply efficient antioxidants that reduce or prevent ROS production depending on the concentration. However, these polyphenols are less bio-available in the body due to various parameters, including low intrinsic activity, poor absorption, high metabolism, inactivity of metabolic products, and/or rapid elimination. Quercetin and resveratrol are dietary polyphenols that are often found in the human diet. However, they lag in bioavailability, which makes them less preferred nutraceuticals. This particular study is aimed at increasing the bioavailability of quercetin and resveratrol through the nano vector system, niosomes. In this study, niosomes entrapped with Quercetin and Resveratrol were produced in different concentrations of Span 60 and cholesterol using the thin film hydration method. The best suitable composition, which provides maximum entrapment, was taken for further study. The niosomal formulation of quercetin and resveratrol was evaluated using various methods like solubility and shape. The entrapment efficiency was determined to be 61.55%. The niosomes were then characterized using a zeta sizer and a potential. The average particle size of niosomes was 194 diameter values in nanometers, and their zeta potential was -20 mV, which indicated their good stability. The results of the *in vitro* drug release research, which was conducted using phosphate buffer saline pH 7.4, were that 92.6% in 24 hours was significantly increased compared to quercetin and resveratrol release, 71.30%. The *ex vivo* drug release was 94.5% after 24 hours, which was higher when compared to quercetin and resveratrol release of 75.74%. The results of this study indicate that the niosomes significantly enhanced the bioavailability of quercetin and resveratrol.

Keywords: Quercetin, Resveratrol, Bioavailability, Niosomes, Drug release

INTRODUCTION

Polyphenols are families of naturally present organic compounds characterized by multiples of phenol units. They are abundant in plants and have varied activities.[1] Flavonoids are one class of phenolic compounds that are present in different parts of plants both in their free state and as glycosides. Some biological actions of these flavonoids include antimicrobial, antiulcer, antiarthritic, antiangiogenic, mitochondrial inhibition, and protein kinase inhibition.[2–4]

The basis of dietary sources is food, but dietary supplements offer significant health and disease prevention benefits even at such high levels.[5] Consuming different types of plant compounds in dietary supplement form is a regular practice. Phenolic compounds from plants are highly desired as supplements for their multiple benefits in humans. However, phenolic compounds are limited by many factors, such as low solubility, poor permeability, instability, rapid release, susceptibility to environmental influences, and low bioavailability.[6]

Among a range of plant phenolic compounds, quercetin and resveratrol find major health-related applications. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is linked to a family of flavonols and may help heal the liver, prevent neural cell apoptosis, and prevent cancer chemoprevention.[7,8] Quercetin, as a polyphenol, has an

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extensive range of biological activities, including metal ion chelating, anti-oxidant (mainly anti-radical), anti-inflammatory, and anticancer properties. Oral administration of quercetin enhances human health through the prevention and treatment of various diseases and disorders. Quercetin has low bioavailability due to its small dietary intake, extensive metabolism, and rapid elimination.[9]

Resveratrol (3, 5, 4′-Trihydroxystilbene) is found in grapes and wine. This stilbene-structured polyphenol has anti-inflammatory, antioxidant, anticancer, and immunomodulatory properties.[10] Resveratrol has lipophilic properties, resulting in high absorption in humans but low bioavailability when taken orally.[11,12]

To overcome this restriction on bioavailability, polyphenols are frequently loaded into different carriers and delivered. This carrier-mediated delivery increases biocompatibility prevents humiliation caused by the external environment and prevents reactions with other components in the human body.[13,14] Nanocarriers are an effective material for encapsulating phenolic compounds and increasing their bioavailability.[6]

Niosomes can perform as nanocarriers that allow the maintenance of a constant plasma concentration of the encapsulated compounds for an extended time.[15,16] Niosomes have the advantages of low production cost and better chemical stability. These niosomes have become promising polyphenol delivery systems that have been used for continuous, controlled, and targeted drug delivery of polyphenols and other drugs.[17–20]

The purpose of this research was to determine the ability of niosomes to increase the bioavailability of quercetin and resveratrol combinations through *in vitro* and *ex vivo* methods. Further in this study the solubility, size, and shape, entrapment efficiency, of the niosomes were studied.

EXPERIMENTAL SECTION

Materials: Quercetin, Resveratrol, and Cholesterol were purchased from Sigma-Aldrich. All the other chemicals and reagents used in the study were purchased from HiMedia and Fisher Scientific, India.

Determination of Solubility: The solubility of Quercetin and Resveratrol was determined in various solvents (with slight modifications by Gyati Shilakari Asthana *et al*.)[21] such as Phosphate Buffer Saline (PBS-pH 7.4), ethanol, chloroform, and deionized water. For this, a minimum amount of 1 g of samples was taken into different test tubes and added with 5 ml of solvents, and mixed well. The test tubes were capped and placed in the mechanical shaking water bath at 50°C for 30 minutes and then sonicated for 10 minutes, and thus sufficient time was provided for contact to produce saturated solutions.[21]

Preparation of Niosomes: Thin film hydration (TFH) method is employed in this study for the niosome preparation as reported earlier with slight modifications from M K Bhalaria *et al.* Niosomes were prepared using the major components span 60 and cholesterol in different ratios. In a round-bottom flask, 250 mg of span 60 and 250 mg of cholesterol were taken and added with 20 ml of chloroform. The flask was shaken well for complete dissolving. 50 mg of Resveratrol and 50 mg of Quercetin were taken and dissolved in 10 ml of ethanol. This mixture was then added to the round-bottom flask and mixed well. The flask was then fitted to a rotary vacuum evaporator and evaporated for thin film formation at 22 rpm. The water bath of the rotovap was maintained at a temperature of 50–55ºC. The evaporation process was terminated with the evaporation of the solvent. The thin film was completely hydrated by adding 15 ml of deionized water drop-by-drop to the flask using a clean syringe.[22]

Entrapment efficiency of Quercetin & Resveratrol Niosomal Formulation: Entrapment efficiency (EE %) was determined following the procedure of Bonepally *et al.* with slight modifications. Quercetin and Resveratrol niosomal formulations were transferred into a centrifuge tube and centrifuged at 4500 rpm for 25–30 minutes. The niosomal dispersion and the pellet were collected. The pellet was quickly washed using 10 ml of ethanol by repeated centrifugation. For further experiments, the niosomal pellets were collected and suspended the pellet in deionized water and stored under refrigerated conditions. The supernatant was collected separately and subjected to a quantitative test (Folin-ciocalteu test for polyphenols) to determine the unentrapped drug. The amount of entrapped drugs was calculated by deducting the concentration of unentrapped drugs from the total drug concentration.[23] The entrapped efficiency (EE %) of resveratrol and quercetin niosomal formulation is calculated by the following formula.

EE (%) = (Drug content entrapped)/ (Total amount of drug) X 100

Characterization of Niosomal formulation: The niosomal formulation was characterized by its size and morphology, zeta potential, polydispersity index, and encapsulation efficiency.

Morphology: On a clean glass slide, a drop of niosomal suspension was placed and covered with a cover slip. A compound light microscope was used to examine the vesicle's size and shape (by Firthouse *et al.* with slight modifications).[24]

Zeta Potential and Sizer: Quercetin and Resveratrol niosomal formulations were further characterized by zeta potential and zeta sizer. The measurements were performed by using the Zeta sizer Nano (Malvern). The Zeta potential was determined using the following Smoluchowski equation:

m =¼ ez /h

Where z is the zeta potential, m is the mobility, e is the dielectric constant, and h is the absolute viscosity of the electrolyte solution.[25]

Drug release analysis:

In vitro release studies: *In vitro* release studies were carried out using a modified Franz diffusion cell chamber involving an artificial dialysis membrane. The membrane was activated using the standard procedure and positioned between the donor and receptor compartments of the diffusion chamber. In the donor compartment, the niosomal formulation was added, while in the receptor compartment, phosphate-buffered saline was added. The entire system was mounted on a magnetic stirrer, and magnetic pellets were used to continuously swirl the solvent in the receptor compartment at 37°C for a period of 24 hours. 1 ml samples were taken at intervals of 0, 1, and 2,3,4,5,6,12 and 24 hours, they were tested for drug content using Folin Ciocalteu assay.[26]

Ex vivo Release studies in animal skin: The *ex vivo* release studies were performed by (with slight modifications by Madishetti

et al.) using a modified Franz diffusion cell chamber involving goat intestine. Deionized water and phosphate-buffered saline were used to clean domestic goat intestine obtained from the local market. The intestine was then positioned between the donor and receptor compartments of the diffusion chamber. In the donor compartment, the niosomal formulation was added, while in the receptor compartment, phosphate-buffered saline was added. The entire system was mounted on a magnetic stirrer, and magnetic pellets were used to continuously swirl the solvent in the receptor compartment at 37°C for a period of 24 hours. 1 ml samples were taken at intervals of 0, 1, and 2,3,4,5,6,12 and 24 hours, they were tested for drug content using Folin Ciocalteu assay.[26]

RESULTS AND DISCUSSION

This research has been carried out on quercetin and resveratrol combo to enhance the bioavailability using a niosomal nano formulation system. The solubility of Quercetin and Resveratrol was determined by dissolving the samples in different solvents such as Phosphate Buffer Saline (PBS-pH 7.4), ethanol, chloroform, and deionized water. The results as shown in Table 1 revealed that the solubility of ethanol and chloroform was highest, whereas the lowest solubility was recorded in PBS 7.4 and poor solubility in deionized water.

Table 1: Solubility analysis of quercetin and resveratrol

S.No	Solvents	Quercetin	Resveratrol
1	PBS - pH 7.4	++	++
2	Ethanol	+++	+++
3	Chloroform	+++	+++
4	Deionized water	-	-

+++ indicates high solubility, ++ indicates medium solubility and - indicates poor solubility.

Niosomes were prepared in the different ratios of Cholesterol and Span 60 in 1:1, 1:2, and 2:1. Cholesterol is an important component that aids in the stability of the niosome membrane. Nonionic surfactants, such as Span 60, are non-immunogenic, biocompatible, and biodegradable, and vesicles made from them can be exploited as an effective drug delivery system.[27] The most stable niosomes were those made from cholesterol and Span 60. This is because these surfactants can exist as solids due to their high melting points.[28] The niosome preparation process has a direct impact on vesicle size and distribution, entrapment efficiency, the number of double layers, and vesicle membrane permeability. It is critical to thoroughly understand the techniques before deciding on the best niosome preparation method. Thin film hydration (TFH) is one of the most common and simple methods for producing niosomes. In this method, the membrane-forming materials such as cholesterol and Span 60 are dissolved in an organic solvent in a flask. This is a widely used, repeatable, and researched method for producing multilayer vesicles (MLV).[29] The entrapment efficiency of the quercetin and resveratrol niosomal formulation was analyzed for all the three formulation variants: 1:1, 1:2, and 2:1. From the above findings, 1:1 niosomal formulation was the highest and it was determined to be 61.55% (Table 2). For further research experiments, a 1:1 ratio of niosomal formulation was taken. Furthermore, when comparing the various niosomal formulations containing different ratios of Span 60 and cholesterol, at 1: 1, the Span 60 and cholesterol ratio-containing niosomal formulation demonstrated the most efficient entrapment when compared to the other formulations.[21]

Table 2: Entrapment efficiency of different niosomal formulations

Drug entrapment analysis	Encapsulation efficiency (%)*
Cholesterol: Span 60; 1:1	61.55 % ± 0.30551
Cholesterol: Span 60; 1:2	55.15 % ± 0.70238
Cholesterol: Span 60; 2:1	58.55 % ± 0.41633

The morphology of niosomal formulations was observed under a compound light research microscope at 100x. The prepared niosomes were found to be spherical (Figure 1). Previous observations made by Karim Masud Kazi *et al* and V. C. Okore *et al* recorded that the niosome vesicles are spherical in shape under microscopical observation.[30,31]

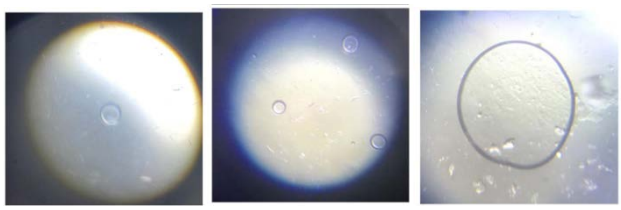

Figure 1. Size of Niosomal formulation

Quercetin and Resveratrol niosomal formulations were selected for further studies, and zeta potential was further confirmed by using Zetasizer. It was apparent from Figure 2 & 3 that the highest zeta potential and zeta potential were observed. Incipient and good stability were indicated by a Zeta potential value of -20 mV, which indicated incipient and good stability. Particle sizes of 194 nm were observed. The negative zeta-potential value revealed that the vesicles had a relatively high stable dispersion into the dispersion medium.[32] Because of the non-ionic surfactants used, the colloidal particles had a negative zeta potential. This may be due to the dipole nature of the ethoxy group of the nonionic surfactant.[33]

The *in vitro* drug release of quercetin and resveratrol from niosomal formulations was investigated by the dialysis method. Figure 4 shows that only 53.8% of Quercetin and Resveratrol were released within the first 12 hours, whereas 77.6% of niosomal Quercetin and Resveratrol were released within the same 12 hours.92.6% of Quercetin and resveratrol niosomal formulations were released in a time period of 24 hours, whereas Quercetin and resveratrol were released at 71.30% in a time period of 24 hours, respectively.

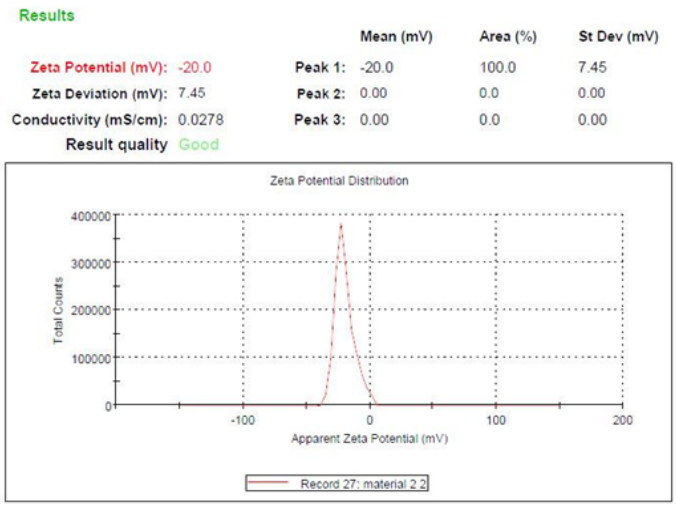

Figure 2. Zeta Potential

Figure 3. Zeta Sizer

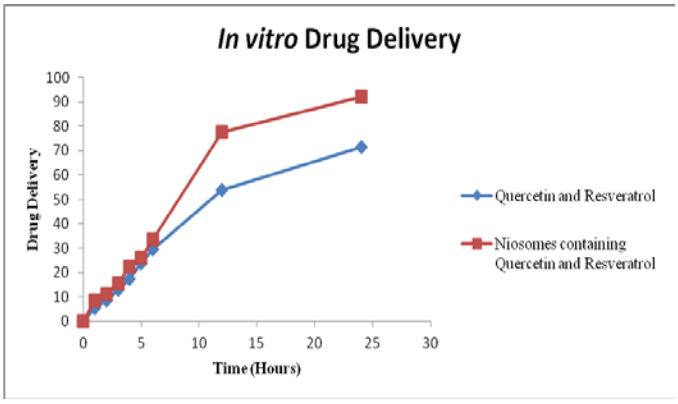

Figure 4. *In vitro* drug delivery

The *ex vivo* drug release of quercetin and resveratrol from niosomal formulations was conducted by the Franz diffusion cell method. Figure 5 showed that only 67.5% of Quercetin and Resveratrol were released within the first 12 hours, while 87.6% of niosomal Quercetin and Resveratrol were released in the same 12 hours. In a time of 24 hours, 94.5 % of Quercetin and resveratrol niosomal formulations were released, whereas Quercetin and resveratrol were released 75.7 % in a time of 24 hours, respectively. Both the experiments have shown a prompt increase in the bioavailability of quercetin and resveratrol. Nonionic surfactants are reported to enhance the half-life of niosomes. With a longer half-life, niosomes could increase the bioavailability in vivo.[34] A further characteristic feature of the niosomes crosses the GI tract through transcytosis of Peyer's patches in the intestinal lymphatics, increasing the feature of bioavailability. This study finds niosomes as a better carrier for improving the oral bioavailability of poor absorption drugs and also improves the therapeutic performance of the molecules by being osmotically active and stable, indirectly.[30,32–34]

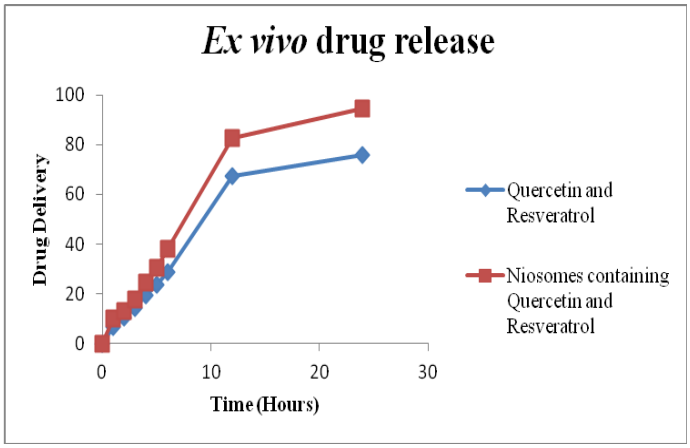

Figure 5. *Ex vivo* drug delivery

Conclusion

The present research studies were conducted to develop a novel carrier to improve the bioavailability of bioactive compounds, namely quercetin and resveratrol, using the nano-sized vector system niosomes. The niosomal formulations were made through the TFH technique with cholesterol and Span 60 (1:1) as key ingredients producing spherical niosomes with the highest entrapment efficiency with the desired particle size of 194 nm. *In vitro* and *ex vivo* release studies of the niosomes also show results that indicated the marked increase in bioavailability of quercetin and resveratrol complex. This current study, with its positive results, indicates the chance to develop and improve the bioavailability of quercetin and resveratrol combo that can be used further in the nutraceuticals and food industry for varied applications.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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