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RESEARCH ARTICLE

Design and evaluation of sesamol loaded hyaluronic acid functionalized phospholipid nanovesicles: DPPH radical scavenging potential assay

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ABSTRACT

Objective(s): The unfavorable physicochemical properties of well recognized antioxidant phytoactive sesamol limits its oral bioavailability as well as potential application as an antioxidant drug. The aim of the study is to design and evaluate sesamol encapsulated hyaluronic acid anchored phospholipid nanovesicles to enhance its antioxidant potential.

Methods: Drug encapsulated hyalurosomes were prepared using thin film hydration method and evaluated for particle diameter, physical stability, drug encapsulation efficiency, sesamol release behavior in vitro and DPPH radical scavenging assay.

Results: The selected method was found to be effective for fabrication of phospholipid nanovesicles with particle diameter 200 ± 10.173 nm and zeta potential -29.8 ± 4.16 mV. The drug loaded hyalurosomes revealed significantly better radical scavenging potential compared to free sesamol and unloaded hyalurosomes.

Conclusions: Hyaluronic acid functionalized phospholipid nanovesicles is novel phospholipid based carrier for delivery of phytoactives. Thus formulated phospholipid based system could be acceptable system for delivery sesamol with improved antioxidant potential.

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INTRODUCTION

The poor gastrointestinal bioavailability and significant metabolism via conjugation are major hurdles in oral delivery of plant based antioxidant phytoactive sesamol (SM). SM is phenolic chemical constituent isolated from sesame oil [1]. Chemically SM is [3, 4-methylenedioxyphenol]. Various landmark studies have proved liver protective and antioxidant activities of SM [2]. The solubility of SM in polar solvents is good. In addition to this, the lipophilicity of SM is also good [3]. To solve drawbacks of SM and to enhance oral bioavailability, it need to formulate novel drug delivery system, which can release encapsulated drug in controlled manner and possibly improve drug circulation in the body.

Colloidal nanocarriers are promising nanosized particles for delivery of phytoactive [4]. novel nanovesicles like liposomes [5, 6], ethosomes [7, 8], transfersomes [9, 10], glycerosomes [11], glycethosomes [12] and hyalurosomes [13] have been investigated phytoactive delivery [14].

Hyalurosomes are phospholipid novel nanovesicles designed using phospholipid and natural polymer i.e. hyaluronic acid [15]. These

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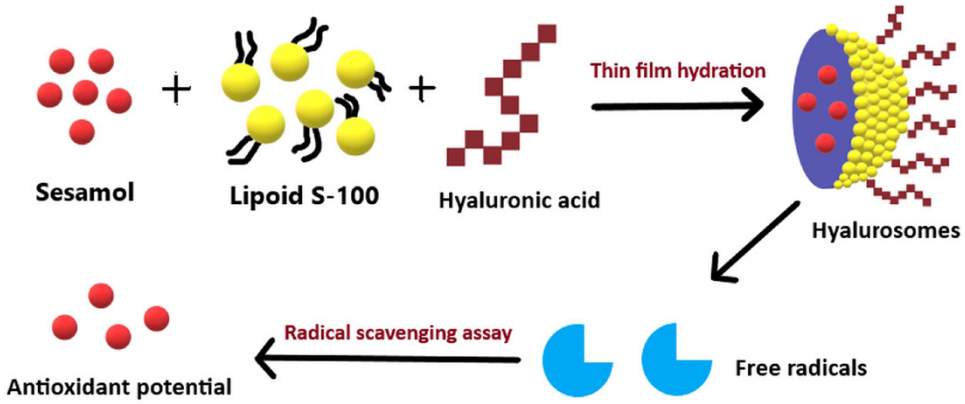

Fig. 1. Schematic illustration of the formation, stability and antioxidant potential of SM hyalurosomes.

are phospholipid nanovesicles surface coated with hyaluronic acid. Hyaluronic acid have better stability and radical scavenging potential [13]. Recently use of hyalurosomes for phytoactive delivery has investigated by many scientific experts.

Manca et al.[13] have proved improved antirheumatic potentials of curcumin on its loading in hyalurosomes with significant reduction of anti-apoptotic protein production in synovial cells. Castangia et al.[16] have showed greater protective effect against H_2O_2 induce human keratinocytes damage on treatment with phycocyanin loaded hyalurosomes. Castangia et al.[17] have showed improved antioxidant activity of liquorice extract loaded hyalurosomes. Thus hyalurosomes is acceptable for skin delivery of antioxidant natural chemical constituent.

Thus present study initiated with aim to encapsulate SM in hyalurosomes to improve its antioxidant efficacy. The hyalurosomes revealed acceptable physicochemical properties, *in vitro* drug release behavior and significantly better ($p < 0.05$) radical scavenging activity compared to free SM. Thus hyalurosomes is promising alternative to enhance antioxidant potential of SM.

MATERIAL AND METHODS

Material

Sesamol and hyaluronic acid were obtained from Sigma-Aldrich Co. LLC (USA). Lipoid S-100 was gifted by Lipoid (Germany). Oleic acid and Tween 20 were obtained from S.D. Fine Chemicals Ltd. (India). All other reagents, solvents and chemicals were purchased from locally available sources.

Methods

Fabrication and characterization of SM loaded hyalurosomes

SM loaded hyalurosomes were designed using thin film hydration method [18]. Briefly solution of Lipoid S-100 and stabilizer (Tween 80) were prepared using chloroform as solvent. SM was solubilize in acetone and added in phospholipid solution.

The thin film of resulting phospholipid solution was casted using rotary evaporator at 58^{0}C. To hydrate casted dried film of phospholipid, the aqueous dispersion (1% w/w) of hyaluronic acid was used (Fig. 1). The concentration of SM in the formulation was 0.1% w/w. The hydrated film was subjected heat-cool cycle by heating in water bath up to 58^{0}C and cooling to room temperature with vortexing. The fabricated SM loaded vesicles were pass through polycarbonate membranes (200 nm) and evaluated with respect to particle diameter, physical stability and drug encapsulation efficiency. The physical stability of formulated system was measured by assessing zeta potential value.

The particle diameter and zeta potential of the formulated SM loaded hyalurosomes were measured using Malvern Zetasizer (UK) at 25°C. The encapsulation efficiency of drug loaded hyalurosomes was measured through UV spectroscopic estimation.

Drug release behavior In vitro

The dialysis diffusion technique was used to assess drug release behavior *In vitro* [18]. THe hyalurosomes dispersion containing 8 mg of SM was taken filled in dialysis membrane (molecular

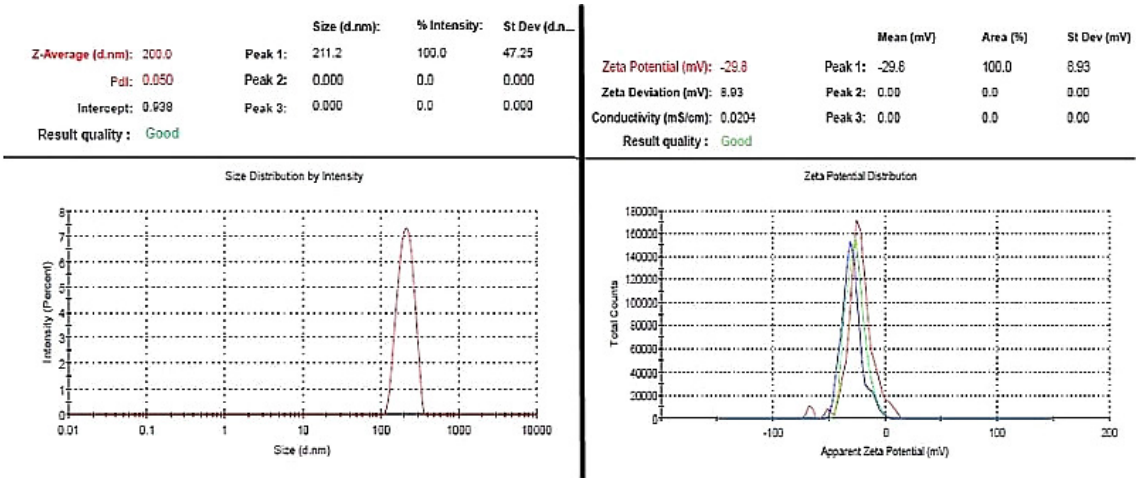

Fig. 2. Intensity plots showing particle size and zeta potential of SM hyalurosomes.

cut-off 12–14 kDa). For first 2 hours drug release behavior was assessed in 350 ml 0.1 N HCl at 37 °C and in 500 ml phosphate buffer (pH 7.4) for next 12 hours. Throughout study, 2 mL of release medium was withdrew, filtered and analyzed by spectrophotometrically using respective medium as a blank.

DPPH radical scavenging activity assay

Radical scavenging activity of developed SM loaded hyalurosomes was assessed and compared with free SM as well as empty hyalurosomes according to the technique reported earlier [18]. Briefly DPPH was dissolve in ethanol to produce 2 mg/100 ml solution. A serial concentrations (5 to 20 µg/ml) of all three samples were prepared in ethanol. At last, 1500 µl of DPPH solution mixed with 1500 µl of samples separately and allow to stand at room temperature. At last, percent inhibition of radical assessed by UV-spectroscopic estimation at 517 nm. The percent inhibition of radical was calculated using following equation.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_1} \times 100$$

Where A_0 is the absorbance of the control (blank) and A_1 is the absorbance in the presence of the sample formulations. All experimental data was analyzed through one way analysis of variance (ANOVA), followed by the Tukey test. Differences between the means were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Preparation and evaluation of drug loaded hyalurosomes

Drug loaded hyalurosomes revealed 200 ± 10.173 nm particle diameter with PDI 0.05 ± 0.027. Whereas particle size of empty hyalurosomes was found to be 194.71 ± 6.391 nm with PDI 0.061 ± 0.0138 (Fig. 2). Vesicle diameter of the both formulations was found to nearly close, thus loading of SM did not affect the vesicle diameter of hyalurosomes. The zeta potential of both drug loaded hyalurosomes and empty hyalurosomes were found -29.8 ± 4.16 mV and -37.19 ± 6.182 mV respectively (Fig. 2). The negative charge of HA confers higher negative value of zeta potential to hyalurosomes vesicles. The negative charged surface contribute to long term stability to vesicular system. The entrapment efficiency of SM in hyalurosomes was found to be 61.37 ± 2.147%. Thus selected technique was effective for preparation of hyalurosomes vesicles with better physicochemical properties.

In vitro drug release behavior

The *in vitro* release profiles of SM from hyalurosomes was discovered to be lower release character in both media than the SM dispersion. The SM loaded hyalurosomes showed 89.19 ± 2.14 % drug release at the end of 16 hours whereas SM dispersion releases maximum extent (99 ± 0.817) of SM in 4 hours (Fig. 3). The comparative results of drug release from dispersion and hyalurosomes vesicles revealed that, the hyalurosomes improved

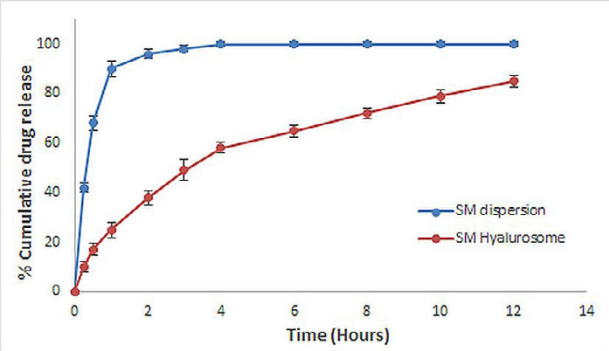

Fig. 3. Percentage of SM released from hyalurosomes and dispersion (n = 3).

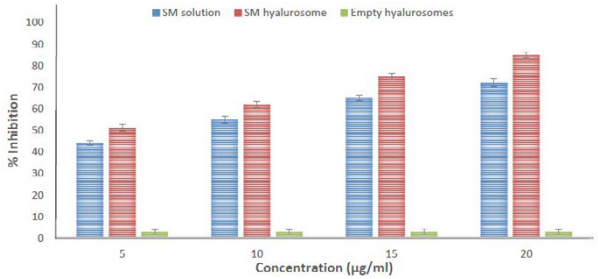

Fig. 4. Scavenging effects of free SM, SM hyalurosomes and unloaded hyalurosomes at different concentrations on the DPPH free radical (n = 3).

stability of SM in gastrointestinal fluid with imparting sustained release character. However better sustained release of SM from hyalurosomes can be achieved by increasing the HA content in formulation. In addition to this, *in vivo* bioavailability study is necessary to confirm improved bioavailability of SM on its encapsulation in hyalurosomes.

DPPH radical scavenging activity assay

DPPH is a stable and extensively used free radical in measuring the antioxidant capability of phytoactives. Free SM, SM hyalurosomes and unloaded/empty hyalurosomes were used as samples to test radical scavenging potential. The % inhibition of DPPH radical was directly related to the concentration of the SM. It was interesting that the loading of SM in hyalurosomes resulted in significantly better ($p < 0.05$) DPPH radical scavenging potential compared to free SM at all concentrations (Fig. 4). The improved antioxidant potential could be due to presence of stronger DPPH radical protector and more stable HA in vesicular assembly. The encapsulation of SM in the new phospholipid vesicles manifested the hopeful properties for the new effective gastrointestinal formulation, which could be used in treatment of oxidative stress induced diseases with administering the lower amount of SM and the higher radical scavenging activity.

CONCLUSION

Results of the study revealed suitability of HA modified phospholipid vesicles i.e. hyalurosomes to encapsulate plant based antioxidant SM. The SM loaded hyalurosomes showed acceptable physicochemical properties which confirm its suitability to load SM. Encapsulation of SM in hyalurosomes resulted in improvement in its stability with significantly better radical scavenging potential. Thus formulated hyalurosomes vesicles can be utilize to control release of encapsulated drug in gastrointestinal tract and to improve its oral bioavailability.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

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