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FORMULATION AND CHARACTERIZATION OF NARINGIN LOADED ZEIN NANOPARTICLES

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ABSTRACT

The object of this work was to prepared zein nanoparticles and load them with naringin. The zein nanoparticles were optimized by nanoprecipitation method. The stirring time, stirring speed and the pH of nanoprecipitation was also optimized. The optimized conditions included 1:1 zein/gum acacia ratio, stirring speed 400 rpm for 30 min and nanoprecipitation pH of 4.0. Naringin was loaded at three mass ratios with respect to zein (20:1, 10:1 and 5:1) for nanoprecipitation. The average particle size of the blank zein nanoparticles was obtained by DLS to be 167.3 nm with a zeta potential of -25.9 mV. The yield of the naringin loaded nanoparticles was found to be 78-84% whereas the encapsulation ranged from 39.1 to 78.4%. The particle size was found to be 191.5 nm to 327.7 nm with polydispersity index ranging from 0.516 to 0.891. The highest entrapment and smallest particle size was obtained when 1:20 ratio of naringin-zein was used for release and antioxidant study. A maximum of 46.49% naringin was released in 4 hours. The decline in cumulative drug percentage may be due to the degradation of naringin in solution after 4 hours. On the other hand, when encapsulated in zein nanoparticles 82.41% naringin was released steadily over 24 hours. The IC_{50} for DPPH inhibition was calculated from the plot and was found to be 142.24 µg/mL and 99.58 µg/mL for naringin and the naringin loaded zein nanoparticle respectively.

Keywords: Naringin, DPPH, Zein, Nanoprecipitation, antioxidant

INTRODUCTION

Nanoparticle mediated drug delivery has tremendous applications than the conventional methods because its property of controlled release of drug at specific sites.

Nanoparticle can also protect the drug from rapid degradation and improves intracellular penetration of drugs **[1]**. Drug delivery using nanoparticles have a lot of advantages like longer retention of drug in the blood stream, less toxicity and side effects, site specific targeting etc. **[2]**. The encapsulation of biologically active constituent to these nanoparticles will enhance its properties like bio availability and therapeutic index **[3]**.

Zein is the main storage protein found in maize. It is a versatile biopolymer because of its hydrophobic nature. Zein is a prolamine rich protein found in the endosperm of corn kernel. It is insoluble in water unless certain specific condition is applied. The hydrophobic nature of zein is mainly due to the presence of non-polar proteins such as leucine, alanine and proline. Zein nanoparticles were successfully applied as a carrier for controlled release of drug and dietary supplements because of its inherent biodegradability and biocompatibility. For these days there is an increasing interest using zein nanoparticles to incorporate flavonoid and other polyphenolic compounds to increase its water dispersibility, chemical stability and bioavailability **[4]**. Above 50 % of the aminoacids in the zein are hydrophobic and readily soluble in aqueous ethanol but not in water. This property of zein makes it a suitable material for the development of nanoparticle delivery system to incorporate active component **[5]**. The zein based nanoparticle drug delivery prefers oral route due to the slow digestive nature. The hydrophobicity, unique solubility and resistance to the digestive enzymes in the gastrointestinal tract are the most important features of zein which makes them very efficient in controlled drug delivery **[6]**. Zein nanoparticles possess poor stability on exposure to environmental conditions like pH, temperature etc. To overcome this instability, zein particles are coated with polysaccharide which can modulate the electrostatic and steric repulsion between the particles **[7]**.

Naringin is a disaccharide derivative that is (S)-naringenin substituted by a 2-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyranosyl moiety at position 7 via a glycosidic linkage. It has low half-life and low bioavailability **[8]**. The objective of the present investigation is to formulate zein nanoparticles and encapsulate it with phytoconstitutent sesamol and characterize the nanoparticles for various parameters.

MATERIAL AND METHODS

Preformulation Studies

In order to perform the preformulation evaluation of the drug tests of identification such as physical appearance, melting point and FTIR spectroscopy were carried out. The

solubility profile of drug in various solvent systems, incompatibility study by FTIR, partition coefficient and quantitative estimation of drug was also studied **[9]**.

Calibration curve of naringin

The maximum absorption of Naringin in ethanol was observed at 295 nm. The calibration curve was obtained using different concentrations of the drug at the above wave length. The stock solution was freshly prepared by dissolving 5 mg of Naringin in 50 ml of ethanol in a 10 ml volumetric flask and then made up the solution upto the mark using the same buffer for obtaining the solution of strength 100 µg/mL (stock I). 5 mL stock solution was taken and volume made up to 50 mL by using ethanol to obtain 10 µg/ml. From this solution with draw 2, 4, 6, 8, 10 ml of solution in to the 10 ml volumetric flask and volume made up to 10 ml by using ethanol to get the solutions of 2, 4, 6, 8, 10 µg/ml. The absorbance of each dilution was observed at 295 nm using UV spectrophotometer employing ethanol as the reference blank and a calibration curve was plotted.

Preparation of zein nanoparticles

In order to prepare the zein nanoparticles, gum acacia was used as the stabilizing agent. The nanoprecipitation method was used to form zein/gum acacia nanoparticles in suspension **[10]**. Briefly, zein (2.5% wt) was dissolved in an ethanol aqueous solution at 80% by weight in ethanol. Gum acacia (2.5%) was prepared in distilled water by stirring using magnetic stirrer for 5 h and stocked overnight at ambient temperature. 10 mL of zein solution was added drop-wise to 30 mL of gum acacia solution under continuous stirring (400 rpm) for 30 min. Samples were subjected to ethanol elimination under reduced pressure at 30°C using a rotary evaporator. The eliminated volume (about 75% of the initial volume) was replaced by water. The different suspensions were adjusted to pH 4 and stocked at 4°C.

Optimization of zein nanoparticles

The effect of zein to gum acacia ratio was studied by preparing nanoparticles using 2.5% zein and 0.25%, 0.5%, 2.5%, 5% and 7.5% gum acacia. The effect of stirring time was studied by using 2.5% zein and 2.5% gum acacia and stirring for 15, 30 or 45 min. The effect of stirring speed was studied by using 2.5% zein and 2.5% gum acacia, stirring for 30 min at 200, 400 and 1000 rpm. Effect of pH on nanoprecipitation was studied at pH values, from 2 to 8.

Preparation of naringin loaded zein nanoparticles

The drug (naringin) was dissolved in 10 mL of the zein (2.5%) stock solution in ethanol to give mass ratios of zein:naringin of 20:1, 10:1, and 5:1. The solution was sonicated for 5 min

and then added dropwise to 30 mL of pectin aqueous solution (2.5%) under magnetic stirring (400 rpm) at room temperature for 30 min **[11]**.

Evaluation of the naringin loaded zein nanoparticles

Yield

The nanoparticles were dried by lyophilization and the mass of obtained by weighing the dried particles. The yield was determined by dividing the mass used for preparation of particles by the dry weight of particles obtained.

Encapsulation Efficiency

The encapsulation efficiency of the different formulations was assessed by quantifying the free naringin in the aqueous phase and deducting the amount of the encapsulated naringin. Samples were centrifuged at 14000 rpm for 20 min. The supernatant obtained was filtered through 0.2 µm filter. The free naringin were then determined by diluting with methanol and measuring the absorbance at 295 nm by UV spectrophotometer **[12]**.

$$\%\ Encapsulaton = \frac{Total\ sesamol - Free\ sesamol}{Total\ sesamol} \times 100$$

Particle size and polydispersity

The particle size and distribution of the powder was measured by dynamic light scattering spectroscopy (DLS) using a Malvern laser particle size analyzer.

Zeta Potential

The zeta potential testing of the aqueous dispersion of the zein nanoparticles was performed using a Malvern Zetasizer Nano ZS instrument equipped with a HeNe laser operating at 632.8 nm and a scattering detector at 173 degrees.

***In vitro* drug release**

In vitro release of naringin from the formulation in comparison to naringin suspension were performed using USP II dissolution apparatus at 50 rpm and a temperature of 37 ± 0.5 °C, where an accurate amount of the nanoparticle formulation, equivalent to 10 mg naringin, and naringin suspension (10 mg naringin in phosphate buffer) were placed in dialysis membrane bag and immersed in the dissolution medium **[13]**. Drug release studies were conducted in 500 ml phosphate buffer pH 7.4 **[14]**. Samples of 2 ml were withdrawn from the release medium at (1, 2, 3, 4, 5, 6, 8, and 24 h) and replaced with an equal volume of fresh medium and naringin concentration in the withdrawn samples was determined spectrophotometrically at λmax (295 nm). The study was performed in triplicate and the average naringin released at each time interval were calculated.

***In vitro* anti-oxidant activity**

The free radical scavenging activity of the test solution was measured in terms of hydrogen

donating or radical scavenging ability using the stable free radical DPPH.

Determination of DPPH radicals scavenging activity was performed by the previously reported method5, 16 **[1]**. Separately, 1mM solution of DPPH and test solution (25-125 µg/mL) were prepared in ethanol. 1.5ml of the test solution was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm against the corresponding blank solution which was prepared using 3 mL ethanol. The control sample used was 3 mL of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(A_{con} - A_{test})}{A_{con}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the test solution.

RESULTS AND DISCUSSION

The pure drug (active pharmaceutical ingredient) naringin was purchased from Yucca enterprises, Mumbai and the sample was observed for its organoleptic characters. The drug was pale yellow amorphous powder with no odor and melted at 231-240°C and had $K_{o/w}$ of 0.75. The drug was soluble in water and alcohol. The calibration curve was prepared for a range of 2-10 µg/mL **(Figure 1)**.

The FT-IR spectrum of the procured sample of naringin was obtained using Bruker alpha spectrophotometer **(Figure 2)** and the spectrum was observed for the characteristic peaks of the functional groups present in the compound depicting stretching at 3340.56 (hydroxy), 1699.89 (carbonyl) among others.

The FTIR spectra of physical mixture of zein, gum acacia and naringin revealed a slight migration in wave number of the stretching vibrations (3342.99, hydroxyl; 1690.78, carbonyl) of drug suggesting compatibility among the ingredients **(Figure 3)**.

Preparation of zein nanoparticles

The zein nanoparticles were stabilized with gum acacia and the particles were found to be around 160-183 nm in size with a spherical morphological character. The particles as seen in the photomicrograph by scanning electron microscopy revealed structurally dense spheres having smooth exterior and almost undeviating sizes **(Figure 4)**. The average particle size of the blank zein nanoparticles was obtained by DLS to be 167.3 nm with a zeta potential of -25.9 mV.

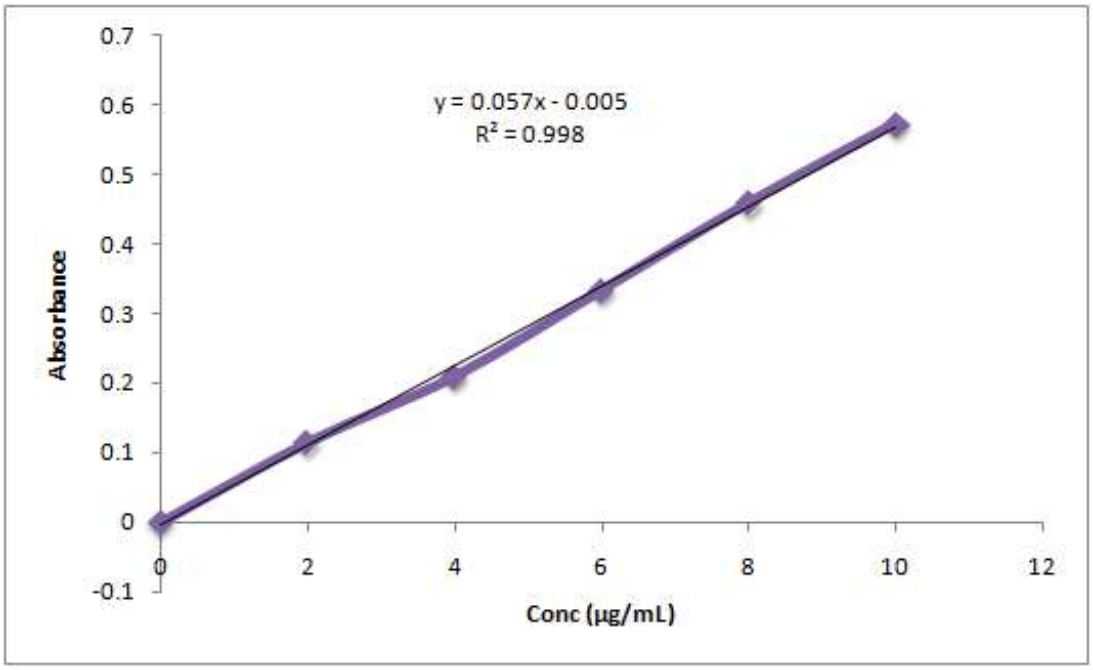

Figure 1: Standard curve of naringin

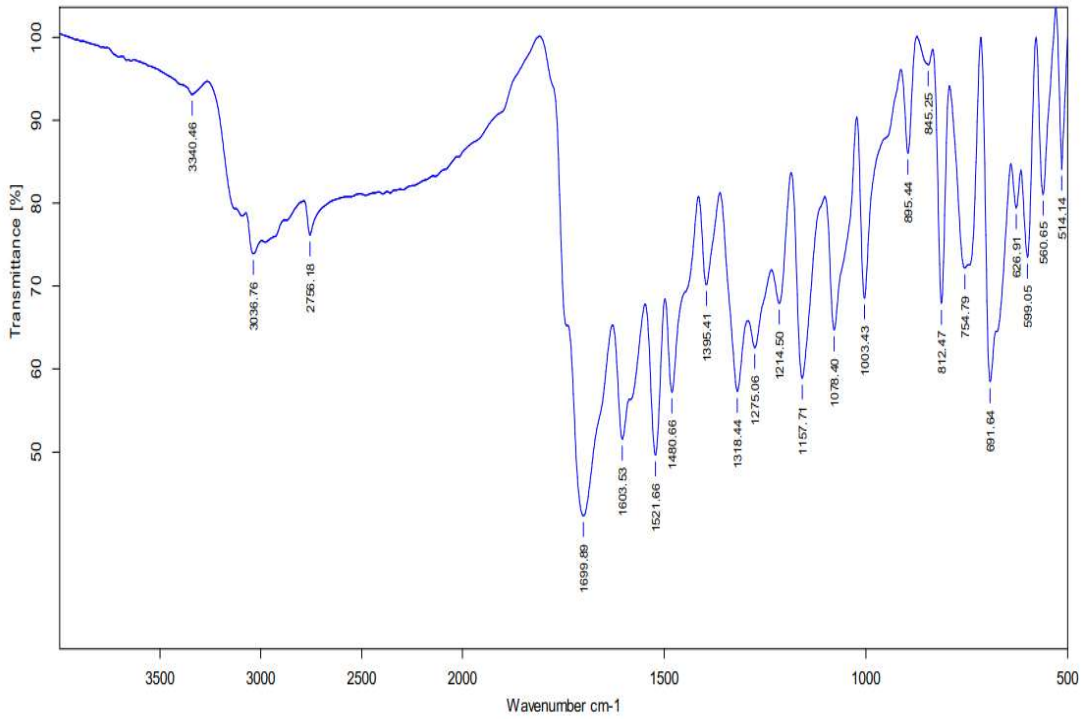

Figure 2: FT-IR spectrum of naringin

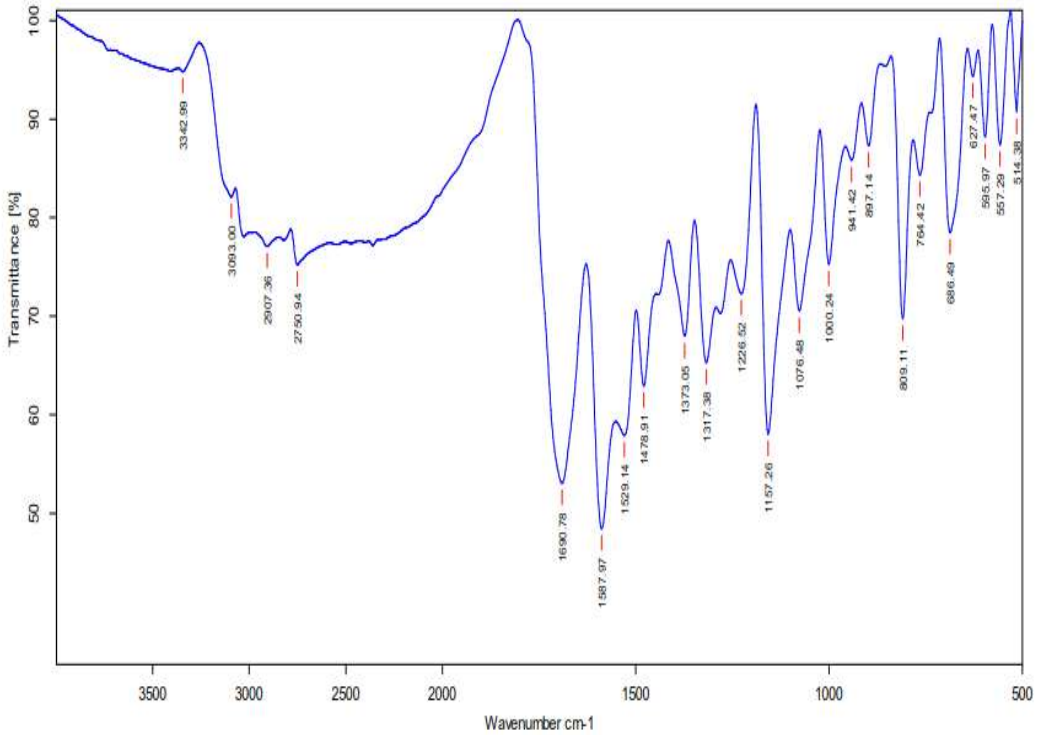

Figure 3: FT-IR spectrum of physical mixture of zein, gum acacia and naringin

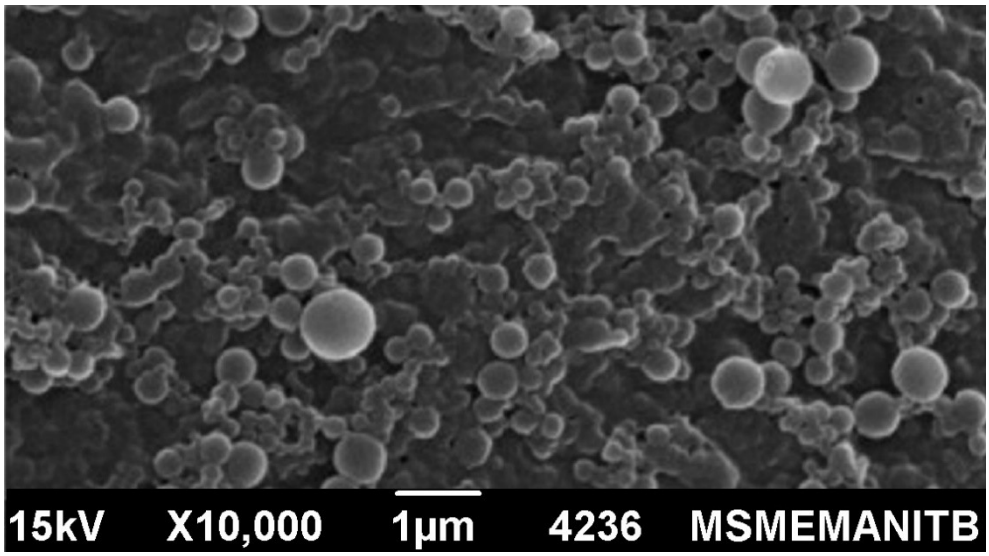

Figure 4: SEM photomicrograph of zein nanoparticles

Optimization of zein nanoparticles

Five ratio of zein to gum acacia were used for determining the optimum ratio resulting in stable nanoparticles. It was found that when the ratio of zein/gum acacia was 10:1 and 5:1, the solution turned highly turbid on achieving pH 4.0 suggesting formation of very large particles. On the other hand reducing the zein/gum acacia ratio to 1:2 and 1:3 resulted in formation of large particles as indicated by

a whitish solution formed on pH maintenance. Nevertheless, it was found that a 1:1 ratio of zein/gum acacia resulted in small and stable particles as indicated by a bluish solution.

Since the 1:1 ratio of zein/gum acacia resulted in small stable particles, this ratio was used keeping stirring speed and pH constant, varying the time of stirring. It was found that when the contents were stirred for 15 min, milky solution was formed suggesting large particles. On the other hand, an almost translucent, slighty whitish solution was obtained on stirring for both 30 and 45 min. Since increased stirring time did not result in significant change in particle size of the nanoprecipitate, a stirring time of 30 min was considered optimum.

Three stirring speeds were studied to optimize the speed required to produce stable nanoparticles. It was observed that at speed of 200 rpm, the precipitation of particles was not in the nano size range whereas at 400 and 1000 rpm, the particles produced were of almost similar size. Also it was found that the size distribution of the particles obtained at 1000 rpm stirring was very high (0.896) as compared to that at 400 rpm (0.453). Hence a stirring speed of 400 rpm was considered as optimum to produce the most stable zein nanoparticles.

The effect of pH of solution on nanoprecipitation was studied by carrying the process at four different pH. In highly acidic pH, neutral pH and alkaline pH the particles appeared unstable within a few minutes of storage and resulted in aggregation. At moderate pH of 4.0, the particles appeared to be stable for longer duration of time and hence a pH of 4.0 was selected for optimized zein nanoparticles. Zein has an isoelectric pH of 6.2 and keeping pH below it helps in it retaining the positive charge on surface.[17] Gum acacia has negative charge and the formation of zein/gum acacia complex results in an overall negative charge on the surface resulting in stable particles **[18]**.

The most optimum conditions for nanoprecipition using zein and gum acacia was scribed as zein/gum acacia ratio of 1:1, stirring time 30 min at 400 rpm and pH of 4.0. These conditions were used to preparation of naringin loaded nanoparticles.

Preparation and characterization of naringin loaded nanoparticles

Naringin was added to the zein solution in three ratio 1:20, 1:10 and 1:5 and the nanoprecipitation was achieved using the optimized conditions. The particles obtained were evaluated for various parameters as per reported methods. The yield of the nanoparticles was found to be 78-84%

whereas the encapsulation ranged from 39.1 to 78.4%. The particle size was found to be 191.5 nm to 321.7 nm with polydispersity index ranging from 0.516 to 0.891.

The results obtained for each naringin loaded zein nanoparticle formulation are presented in **Table 1**.

Table 1: Evaluation of formed nanoparticles

S. No.	Naringin-zein ratio	Yield (%)	Particle size (nm)	PDI	Zeta Potential (mV)	%EE
1	1:20	84	191.5	0.516	-23.1	78.4
2	1:10	83	237.9	0.613	-20.9	56.7
3	1:5	78	321.7	0.891	-17.1	39.1

The highest entrapment and smallest particle size was obtained when 1:20 ratio of naringin-zein was used. Hence it was considered the best formulation and this formulation was used for studying the drug release and antioxidant activity.

***In vitro* release of naringin from zein nanoparticles**

The release of naringin was studied by dialysis method for a duration of 24 h in phosphate buffer. It was observed that the release of naringin from solution increased steadily for 6 hours and then it started to decline from the 5th hour till the end of 12th hour. A maximum of 46.49% naringin was released in 4 hours. The decline in cumulative drug percentage may be due to the degradation of naringin in solution after 4 hours (since it has a half-life of approximately 3 hours). On the other hand, when encapsulated in zein nanoparticles 82.41% naringin was released steadily over 24 hours **(Figure 5)**.

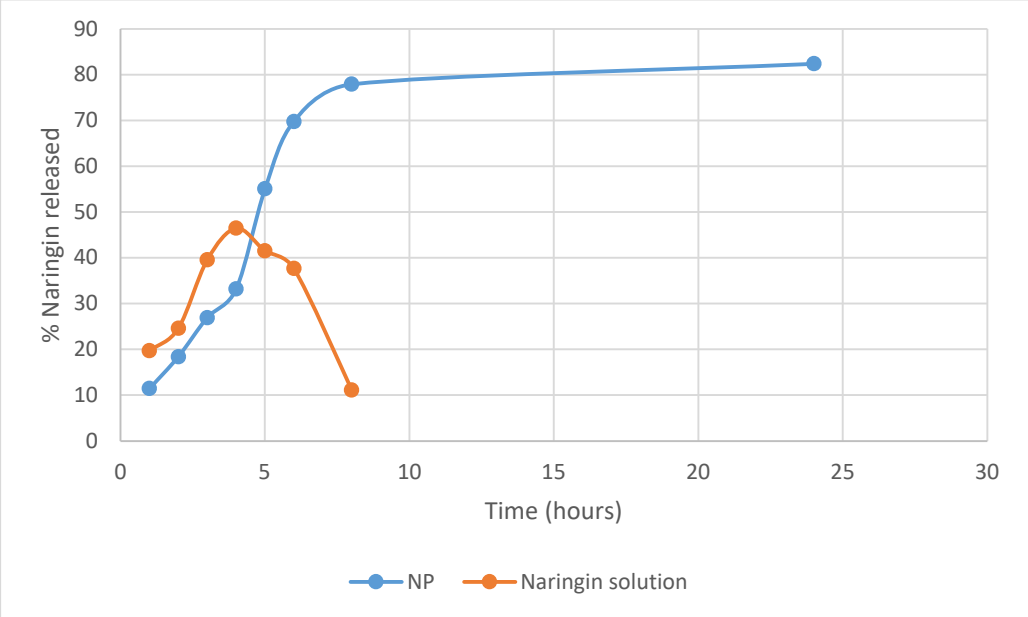

Figure 5: *In vitro* release of naringin from solution and zein NP

After 4 h, 46.49% naringin was released from solution while only 33.24% naringin was released from zein nanoparticles indicating the ability of nanoparticles to protect the encapsulated naringin. Nevertheless, with time the nanoparticle shell loses its integrity due to formation of aqueous channels leading to swelling of the nanoparticles aiding gradual drug release through such pores **[19]**.

***In vitro* antioxidant activity of naringin NP**

The *in vitro* antioxidant activity of naringin loaded zein nanoparticles was compared with that of naringin solution. The antioxidant action was assessed by DPPH radical scavenging assay method **(Figure 6)**.

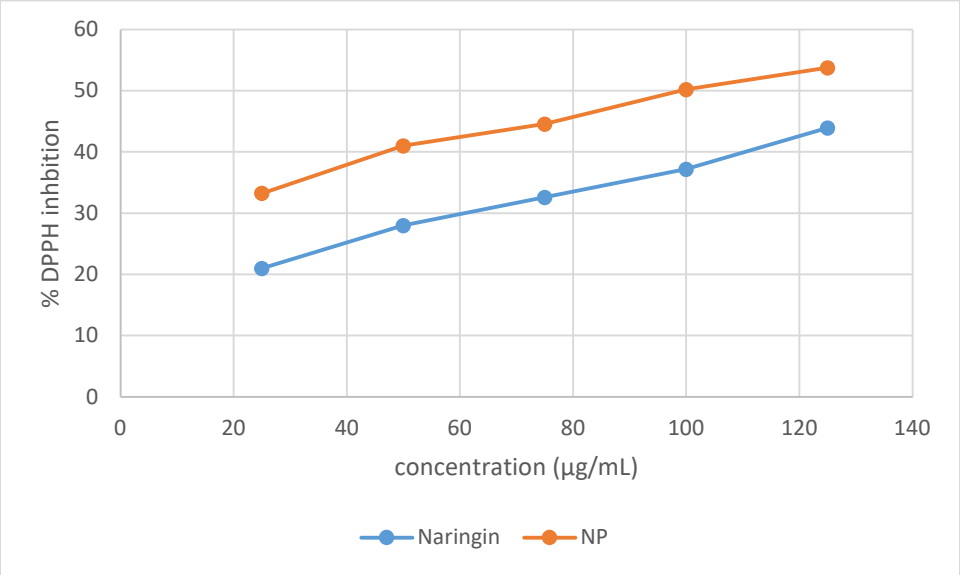

Figure 6: Antioxidant activity of naringin and naringin loaded nanoparticle

As it can be visualized from **Figure 6**, the anti-oxidant activity of the nanoparticle was higher than the naringin solution at all concentrations. This could be due to the stabilization of naringin within the nanoparticle shell **[20]**. The IC_{50} value was calculated from the plot and was found to be 142.24 µg/mL and 99.58 µg/mL for naringin and the naringin loaded zein nanoparticle respectively.

CONCLUSION

The primary hypothesis for the current work was that incorporation of drugs into shell core nanoparticles prepared by natural substances like zein and gum acacia could be helpful in stabilizing the drug and improving its bioavailability and efficacy. Naringin is widely regarded as potent antioxidant and is reported to be active against several ailments like cancer and diabetes among others.

Naringin was successfully incorporated into zein nanoparticles stabilized by gum acacia and an improved release and antioxidant profile naringin was found from the nanoparticle formulation. The results led to the conclusion that zein nanoparticles are highly effective in improving the stability, bioavailability and antioxidant action of naringin.

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