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Original Article

Comparative evaluation of antimicrobial efficacy of curcumin nano suspension and curcumin suspension using agar diffusion method: An *invitro* study

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ABSTRACT

Poor water solubility and slow dissolution rate a major disadvantage of conventional formulation has been over comed by Nano drug delivery system. The aim of the present study was to develop a method for the preparation of nanoparticles of curcumin with a view to improve its aqueous-phase solubility and examine the effect on its antimicrobial properties. Nanosuspension of curcumin was prepared in two formulations with a varying ratio of by Solvent evaporation method, characterized for their anti microbial activity. Of the two formulations, F2 formulation is having greater drug content, greater *invitro* drug release thus greater anti microbial activity against gram positive and gram negative formulation.

Key words: Curcumin, nano suspension

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INTRODUCTION

Curcumin is natural polyphenol component of *Curcuma longa*. Due to its chemical structure, this molecule could be applied in several different fields, such as food, textile, and the pharmaceutical industry. It has been shown that curcumin possess anti-inflammatory and antioxidant properties[1,2]. However, its poor bioavailability, low solubility in aqueous media, instability in body fluids, and elevated degradation rate have limited the therapeutic applications of this drug. One among the various strategies, the usage of nanotechnology, has emerged to tackle these problems. Ingeneral, nanoparticle-based drug delivery systems presents important advantages, such as a long lifetime circulation, ability to improve the drug's aqueous solubility as well the bioavailability, and the capacity to overcome physiological barriers[4,5,6]. Nanoformulations is able to provide better drug delivery systems for their characteristic size. In terms of size constraints, the National Nanotechnology Initiative (NNI) defines nanotechnology in dimensions of roughly 1 to 100 nanometers (nm), but in boarder range it can be extended up to 1000 nm. Particles within that particle range reach out many targets in terms overcoming the solubility, alteration of a drug's reactivity, reaching the targeted size etc. strength, electrical properties, and ultimately, its behavior in vivo. This is the advantage over conventional therapies in the treatment of infections caused by intracellular pathogens. The very first generation of nanoparticle-based therapy included lipid systems like liposomes and micelles, which are now FDA-approved[3].

This aim of the present research had been designed to prepare the curcumin nanosuspension by Solvent evaporation method and anti microbial activity of curcumin nano suspension was compared with that of pure curcumin.

MATERIALS AND METHODS

Curcumin, purchased from Hi Media Laboratories Pvt ltd. All other chemicals are purchased from Sigma Co Pvt ltd. For the preparation of the nanoparticles solvent evaporation technique was used. Required quantity of curcumin was weighed was dissolved in methanol in the ratio of 1:2 and 1.3.The organic dispersion was subjected to vortex for 5 min at 700rpm and added to a mixture of distilled water containing Tween & Span 20 in the ratio of 1:2. This solution was subjected to ultra-sonication for 15 minutes later subjected to rotary evaporation under reduced pressure at 40-45^{o} to evaporate methanol.

The prepared Nano formulation was evaluated for drug content, loading capacity and invitro dissolution studies. The antimicrobial activity of prepared formulation was compared with pure curcumin.

Drug content: Drug content was determined by transferred 200mg of drug equivalent to a formulation into 50ml of methanol. The solution is stirred at 700rpm for 3hrs. The resultant solution was then filtered and drug content was determined by UV Spectrophotometer at 272nm.

In vitro release of the drug: 50mg of each formulation was taken in50ml of pH 6.8 phosphate buffer and placed in orbital shaker at 100rpm and for every half an hour 1 ml of sample was collected and replaced with 1 ml of buffer. These solutions were subjected to UV spectroscopy at 425 nm.

Invitro evaluation of anti-microbial activity

Preparation of microorganisms: The test organisms were inoculated individually into a tube containing 5 ml of 85% saline. Adjustments of the suspension were made in such a way that they were equivalent to 1.5×10^{8} colony-forming units.

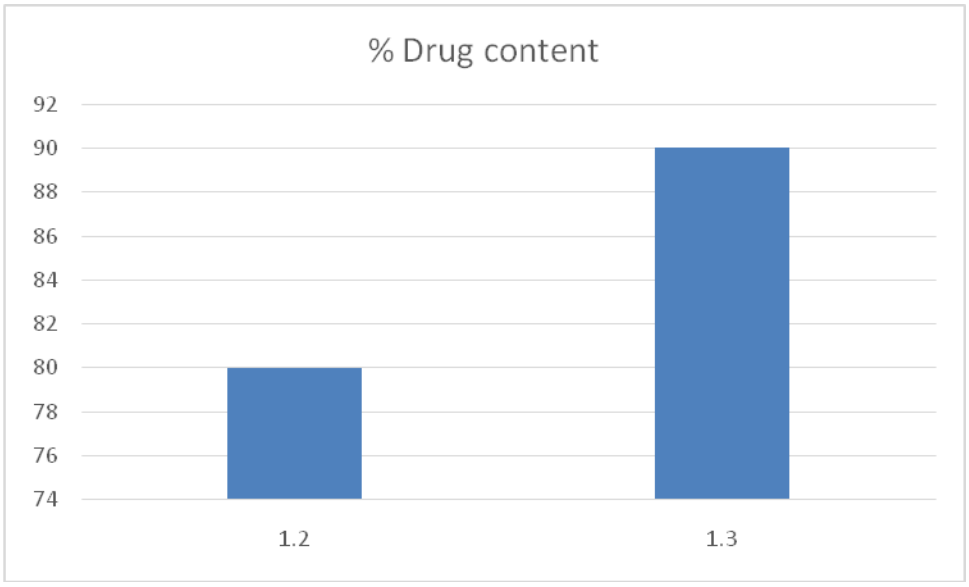
Nutrient agar medium was used for the culture of the bacteria. The three organisms – *E. coli*, *B.subtilis*, *S.aurues* and *P.aeruginosa* were used respectively.

Assessing antibacterial by agar well diffusion method: The antibacterial activity of the prepared nanosuspension was determined by following the agar plate well diffusion method. Ten agar plates were sterilized and then seeded with freshly prepared samples of the pathogens. With the help of a sterile stainless steel cork borer, agar wells of 6 mm diameter each were contrived in all the agar plates used. The wells were loaded with 20 µL curcumin nano suspension and curcumin susupension. The plates were incubated at 37°C for 24 h and the zone of inhibition (ZOI; mm) that appeared around the wells was recorded.

RESULTS AND DISCUSSION

Curcumin nanosuspension of two different ratios with varying methanol concentration was prepared using single emulsion solvent evaporation method. Use of methanol for formulation of curcumin resulted in stable suspension.

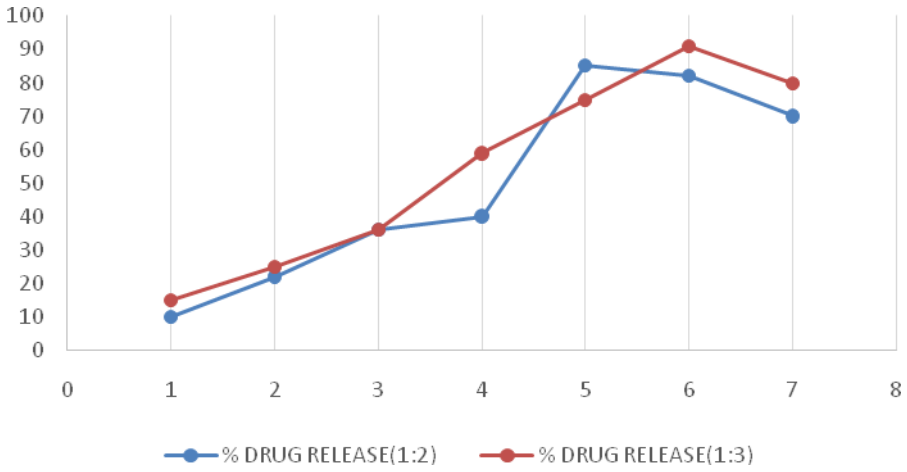
Drug content: Total drug content was determined for both the formulation (F1 and F2). From the Fig 1 it is revealed that formulation F2 was having higher drug content (i.e., 90%) indicating high solubility of curcumin in methanol[7]



Type of Formulation

Fig.1 Percentage drug content in F1 and F2 formulation

In-vitro Drug Release The most important feature of nanoparticles is the increase in the dissolution velocity, In-vitro drug release data from the nanosuspension were carried out for 60 min and graphically represented as % drug release v/s time profile (Fig. 2). The percentage drug release curve of formulation F2, showed the desired rate in phosphate buffer of pH6.8 up to 60 min.



Type of Formulation

Fig.2 Percentage drug release of F1 and F2 formulation

From that study it was found that formulation of F2 batch gave faster release behavior. This might be not only because of increase in surface area but also because of increase in saturation solubility.

Invitro anti microbial activity: Invitroanti microbial activity of the two formulations was done by agar well diffusion method and compared to the coarse curcumin suspension. It was found that F2 nano formulation was having higher antimicrobial activity than the coarse formulation. In contrast to conventional formulations, nanoformulations have characteristic dimensions <100 nm. Their uniquely small size results in novel properties, such as greater interaction with cells due to a larger surface area-to-mass ratio and versatile and controllable application[8].

TABLE 1. Comparison of antimicrobial activity of various nano formulations and pure curcumin

	Zone of Inhibition(mm)			
Formulation	***E.coli***	***B.subtilis***	***S.aureus***	***P.auruginosa***
1:2	1.2	1.4	1.5	1.9
1:3	2.5	2.2	2.6	2.7
Pure curcumin	1.5	2.0	1.5	1.9

CONCLUSION

This enhancement in activity of curcumin is due to increased solubility of curcumin resulting in its better bioavailability. Almost all the nanoformulations of curcumin have been found to be effective against Gram positive and Gram negative bacteria's. Also, most of them have shown promising antifungal activity as well, thereby establishing curcumin as a potential antimicrobial agent for further investigations. Thus the poor water soluble drugs can be formulated as nano formulations.

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Conflicts of interest; The authors declare no conflict of interest.

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