Nano-curcumin: A potential herbal alternative with enhanced anti-*E. gingivalis* efficacy

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Abstract

Curcumin is a bioactive component derived from Curcuma longa and well known for its medicinal properties. The major disadvantage associated with the use of curcumin as a medicine is its low systemic bioavailability due to its poor aqueous solubility. These limitations can be overcome by the converting the curcumin to nanocurcumin. The present study was to prepare the nano-particles of curcumin with improved aqueous solubitity and to investigate their efficacy in vitro against protozoa Entamoeba gingivalis, presence of which in the oral cavity is associated with poor oral hygiene leading to advanced periodontal disease.

Curcumin nanoparticles of particle size in the range 70–80 nm with the aqueous solubility of up to a maximum of 3 mg/mL were prepared. The anti-amoebic activities of nanocurcumin were tested against Entamoeba gingivalis strain (ATCC 30927) in vitro. The cultures were observed under an inverted microscope and numbers of the trophozoites were counted. The inhibition caused by the curcumin, nanocurcumin and metronidazole at various concentrations was calculated by counting the viable trophozoits using the hemocytometer under the microscope. The inhibition results showed more than 80% killing of E. gingivalis , which is comparable with that of standard drug Metronidazole demonstrating that nanocurcumin can act as a potential anti-parastics herbal drug.

Keywords: Nano-Curcumin, Bioavailability, Anti-amoebic, *Entamoeba gingivalis*, Efficacy.

Introduction

Curcumin or diferuloylmethane is a polyphenol extracted from the rhizomes of turmeric (*Curcuma longa*) along with demethoxy curcumin and bisdemethoxy curcumin and contains a diarylheptanoid structure. It has a long history of therapeutic use in Indian and Chinese medicines for the treatment of flatulence, dyspepsia, liver disorder, jaundice, urinary tract diseases, wound, inflammation, cancer and many other diseases[1-4]. It has also been used in the treatment of intestinal parasites and of parasitic skin infection[4]. Curcumin has been suggested to be useful as anti-HIV[5], antibacterial[6] andantifungal treatment[7]. Curcumin also acts against parasites[8-11], both drug-sensitive and drug resistant parasite strains through its unique biomolecular mechanisms. Antiparasitic activities of curcumin are achieved through effects on transcription of genes and modulation of various protein factors.

Despite the efficacy of curcumin in number of pathogenic organism including bacteria, virus, fungus and parasities, its application is limited due to its poor aqueous solubility, low bioavailability[12] and rapid metabolism in the gastrointestinal tract[13,14].

To overcome these drawbacks, curcumin was used either with adjuvants or as in binding form in liposomes or nanoformulations[3,15]. Although, these methods increase its bioavailbility significantly , use of all these methods involves addition of foregin particles likes liposomes, nanoformulation which raise an issue of their toxicity along with inclusion of complicated process of their synthesis leading to overall increased cost. Therfore an alternative process to increase the aquaous solubility and efficacy of curcumin without any extensive expertise and cost is always in demand.

Entamoeba gingivalis (E. gingivalis) is a mouth colonizing protozoa with no identified cyst form and has been associated with poor oral hygeine and causing mild to severe periodontal diseases like gum bleeding, degree of decayed and loose teeth[16]. The prevalence of this parasite in the people suffering from periodontal inflammation has been reported 11.3% to 56.5%[16-22]. The treatment of this protozoa with herbal extract can be a good solution instead of other chemical medicine.

In our previous stuides, we prepared nanocurcmin and demostrated its effects as an anticancerous drug successfully[23]. The study proved that nano form of curcumin is even more effectivitive for the treatment of cancer as compared to the normal curcumin. The present work demonstrated the anti protozonal effects of nanocurcumin on a human oral parasite, *E. gingivalis*.

Material and Methods

Materials: Curcumin [(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] was purchased from Sigma Chemicals. Dimethyl sulphoxide (DMSO) used for curcumin nanoparticle preparation was purchased from Merck Chemicals, India. 0.22µM filters (GVMP 01230, Millipore) were used for filteration of aqueous solution of nanocurcmin. Other chemicals and reagents were purchased from Hi-Media Ltd., Mumbai, India.

Equipments: Scanning Electron Microscope (EVO-18, Carl Zeiss, Germany) was used to study the surface morphology of curcumin nanoparticles; Dynamic Light Scattering (DLS, Malvern Zetasizer S90 series) to analyse the the particle size of the prepared nanocurcumin. Ultrasound device- ultrasonic cleaner TPC-25 (from Roop Telesonic) was used for the preparation of curcumin nanoparticles. Rotavapour (R-210) equipped with heating bath (B-491) and vacuum pump (V-700) was used to concentrate the nanocurcumin sample and to further breakdown the particle size. UV-visible spectrophotometer (Spectro UV-vis Dual beam and Auto Cell UVS-2700, Labomed, INC,Germany) was also used.

Preparation of nanocurcumin: Nano particles of curcumin were prepared by the methods of Gera et al[24] with modification: curcumin (100 mg, 0.27 mmol) was dissolved in dimethyl sulfoxide (20 mL) to prepare the curcumin solution. 1mL of this solution was sprayed into boiling water (50 mL) dropwise with a flow rate of 0.2 mL/min in 5 minutes under ultrasonic conditions with an ultrasonic power of 100W and a frequency of 30 KHz. After addition was complete, the contents were sonicated for 10 minutes and then stirred at room temperature for about 20 minutes when a clear orange colored solution was obtained.

The solution was concentrated under reduced pressure at 50 ºC and finally freeze dried to obtain an orange powder. Maintaining the drop flow was significant for obtaining uniform sized nanoparticles. It was observed that addition of curcumin solution in dimethyl sulfoxide to water at a rate faster than 0.2 mL/min. led to particle aggregation.

Characterization of Curcumin Nanoparticles: The synthesized nanoparticles were characterized by UV-visible spectroscopy, Fourier-transform infrared (FTIR), Dynamic light scattering (DLS) and Scanning electron microscopy (SEM) analysis. For UV-vis spectroscopic studies, nano particles of curcumin [1mg/ml] were used to measure the absorption spectra. The FTIR spectra of curcumin and nanocurcumin were recorded on a FTIR analyzer [BRUKER] to find out the chemical grup present in both the samples.

The mean particle diameter of curcumin nanoparticles was measured by dynamic light scattering (DLS). A scanning electron micrograph (SEM) of the aqueous dispersion of curcumin nanoparticles was recorded to characterize the surface morphology and size of nano-particles.

Prepartion of working solution of Nanocurcumin, curcumin and standard antiparasitic drug: To obtain working concentrations of nanocurcumin 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml, powder of above prepared nanocurcumin was dissolved in water. Similar concentrations of normal curcumin were also prepared. Metronidazole (Procured from local Pharmacy shop) (500mg) was dissolved in water to prepare solutions of concentrations 12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml.

Culture Medium for *E. gingivalis*: *E. gingivalis* is the most frequent human oral protozoa[16]. The parasite can be detected by examining under light microscope directly using wet mount with normal saline[17]. In the current study TYSGM-9 (Trypticase-Yeast Extract-Serum-Gastric Mucin) medium was used. The samples, after inoculation in the medium for culture for *E. gingivalis* were incubated at 37˚C for 48-72 hours.

This media was prepared from potassium phosphate dibasic 2.8 gm, potassium phosphate monobasic 0.4 gm, sodium chloride 7.5 gm, casein digest peptone 2.0gm, yeast extract (BBL) 1.0 gm and distilled water 970 ml[17].

Anti parastic Efficacy of nanocurcumin: Antiparastic efficacy of nanocurumin was evaluated against standard strain of *E. gingivalis* (Brumpt (ATCC 30927). The results were compared with that of normal curcumin and standard drug of choice for the treatment of parasitic infections.

For efficacy study, 2×10^5 cells/ml trophozoits of *E. gingivalis*, in triplicate, were incubated in TYSGM-9 media added with normal curcumin, nanocarcumin and metronidazole drug of concentration (12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml) individually for each. DMSO was used as a positive control in a 96-well tissue culture plates (200 µl/well) at 37°C under aerobic conditions for 24 hours. After incubation is completed, plates were chilled for 15 min and trophozoites from each well were examined and counted with an inverted microscope to observe the inhibition of the protozoal growth.

For this, the plates were chilled for 15 min to detach the trophozoites from the plate surface. The numbers of viable cells from every well were counted, using trypan blue and a haemocytometer. The results were calculated as the percentage of growth inhibition when compared with the controls grown .

Results and Discussion

Characterization results of the prepared Nano-curcumin: The preparation based on a wet-milling technique involved spraying the curcumin solution in a volatile organic solvent into hot water under ultrasonication followed by concentrating the aqueous solution under reduced pressure and then freeze-drying it to obtain a powder. When resuspended in water, the lyophilized powder formed a very fine dispersion and appeared to be soluble, unlike curcumin, which is completely insoluble in water, with undissolved flakes clearly visible in the suspension. Dry, lyophilized powder of nanocurcumin was found to have good physical and chemical stability, was readily dispersible in water andcould be stored at room temperature for over 6 months without any decomposition or aggregation.

The enhanced aqueous solubility of nano-sized curcumin particles could be attributed to its larger surface area, which promotes dissolution. Similar results have been

demonstrated in previous studies[23,24] where reduction in the particle size of active ingredients to nanoparticle size has shown improvement in its efficacy, solubility andbioavailability.

UV-visible Spectroscopy: The prepared Nano-curcumin sample was subjected to UV-visible spectroscopy analysis and the graph was obtained. The results indicated the formation of curcumin nanoparticles and characteristic peak at 435nm (Fig. 1).

Dynamic Light Scattering (DLS): DLS used to determine the size distribution profile of nano particles in a suspension or solution via a laser or a monochromatic light source. DLS of an aqueous dispersion of nanocurcumin revealed the formation of nanoparticles with an average hydrodynamic diameter of 77.9 nm.

FTIR Spectra: The FTIR spectra of curcumin and nanocurcumin particles were studied. The curcumin nanoparticles have shown absorption peaks at 1664 cm^{-1} and 1325 cm^{-1} relating to amide I and III of C=O stretching, N-H/C-N stretching and CH_2 wagging coupled with OH groups of curcumin respectively. All the above observations found in the FTIR spectra confirm the presence of silimar peaks in curcumin and curcumin nanoparticles (Fig. 2).

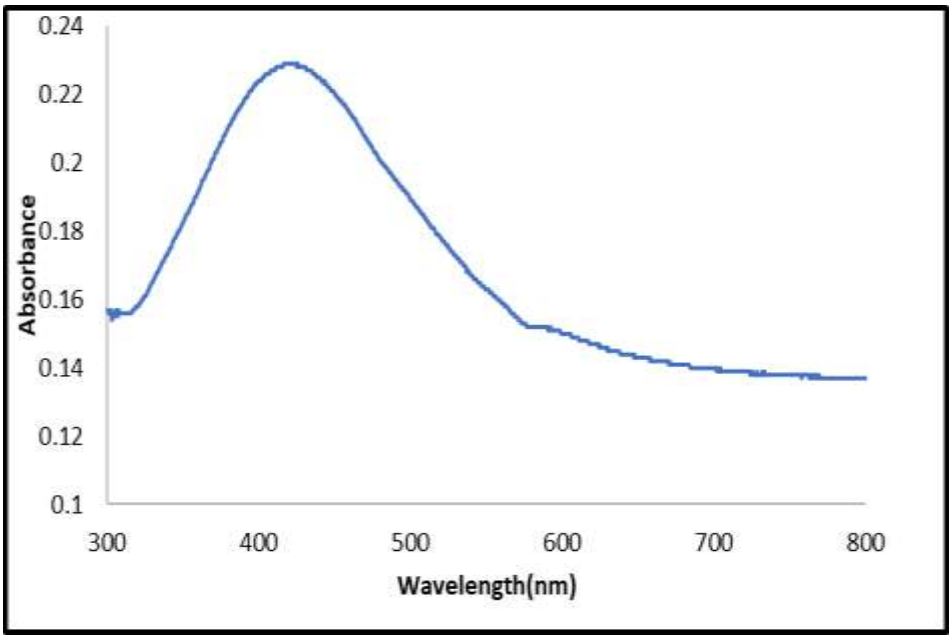

Figure 1: UV-VIS Spectroscopy of Nanocurcumin

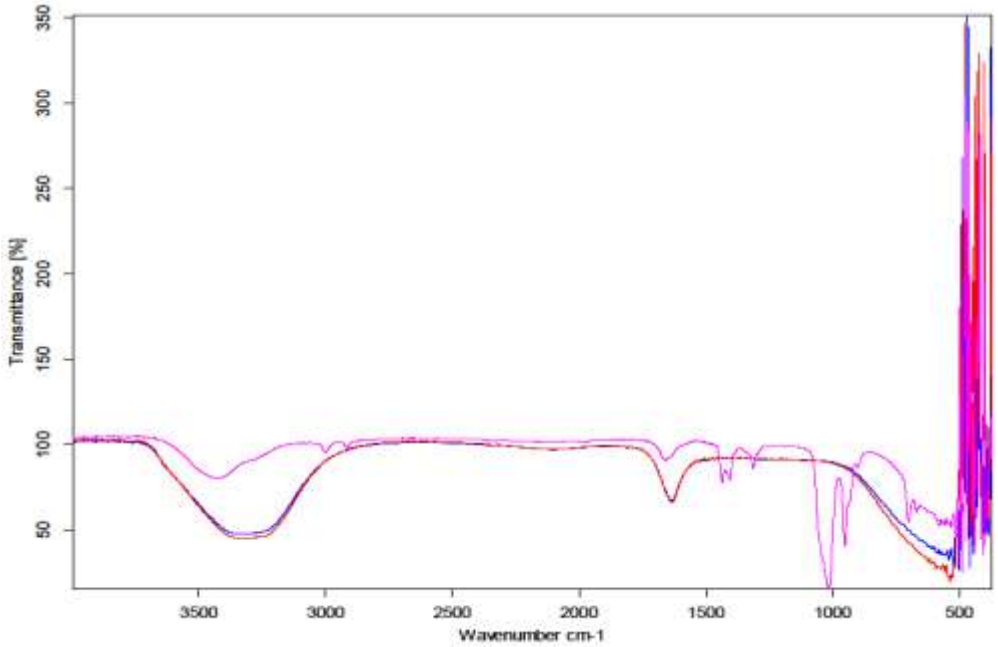

Figure 2: FTIR spectra of Nano curcumin

Scanning Electron Microscopy (SEM): The results for both the samples, normal curcumin as well as nanocurcumin samples were obtained, compared, analysed and recorded. In case of curcumin powder, no uniform particle structure was observed and the particle size was quite big ranging from 1-2µm in diameter. In contrast to curcumin powder, nano-particles of curcumin were found to be somewhat sperical in shape with particles size ranging from 70-90 nm, indicating the successful preparation of curcumin naoparticles (Fig. 3).

Anti-parasitic efficacy of nanocurcumin: In present study, *in vitro* effect of nanocurcumin and curcumin on the growth of *E. gingivalis* was investigated and compared with the standard drug metronidazole. The results of growth inhibition are shown in fig. 4, as the inhibition of the parasite by nano curcumin was comparable to that of standard drug metronidazole. The nanocurcumin showed the significant inhibition by killing the parasitic upto 85% as compared to 94% by metronidazole at the concentration of 100ug/ml while normal curcumin inhibited only 24% at same concentration ($p<0.05$).

Even at very low concentration of 12.5ug/ml, nanocurcumin killed 41% of parasite which is in line with the standard drug (killed 55%) while normal curcumin inhibition at this dose was almost negligible (killed only 7% parasites). This might be due to poor aqueous solubility and bioavailability of the curcumin compared to nanocurcumin; the reuslts were compared to that reported earlier[3,23].

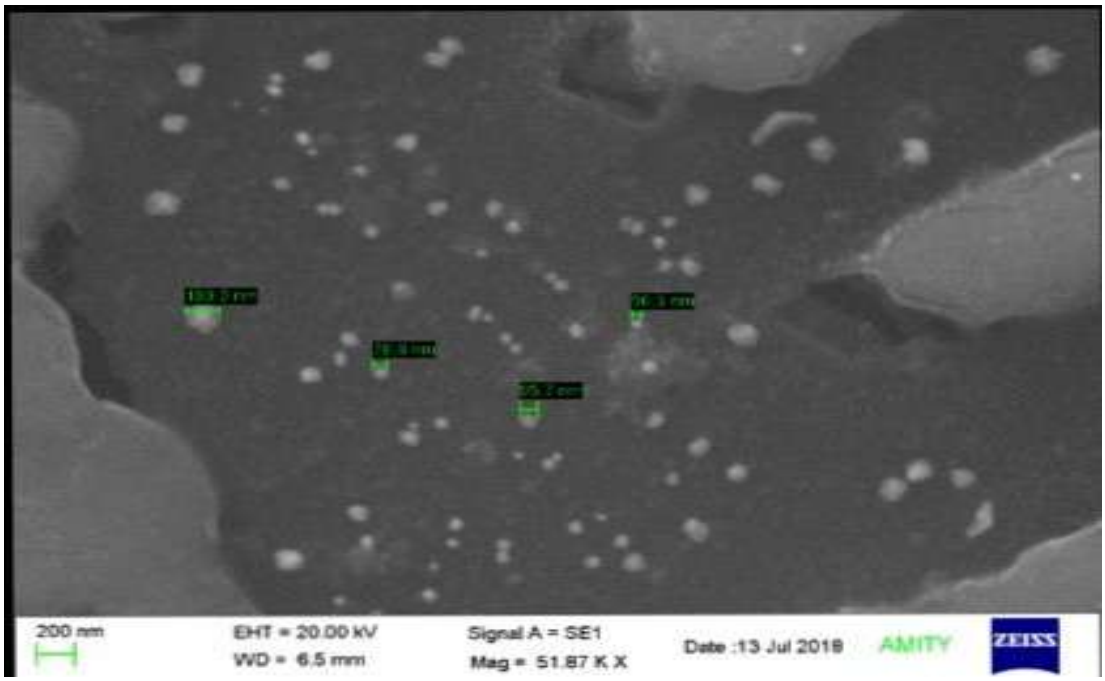

Figure 3: SEM of Nano curcumin

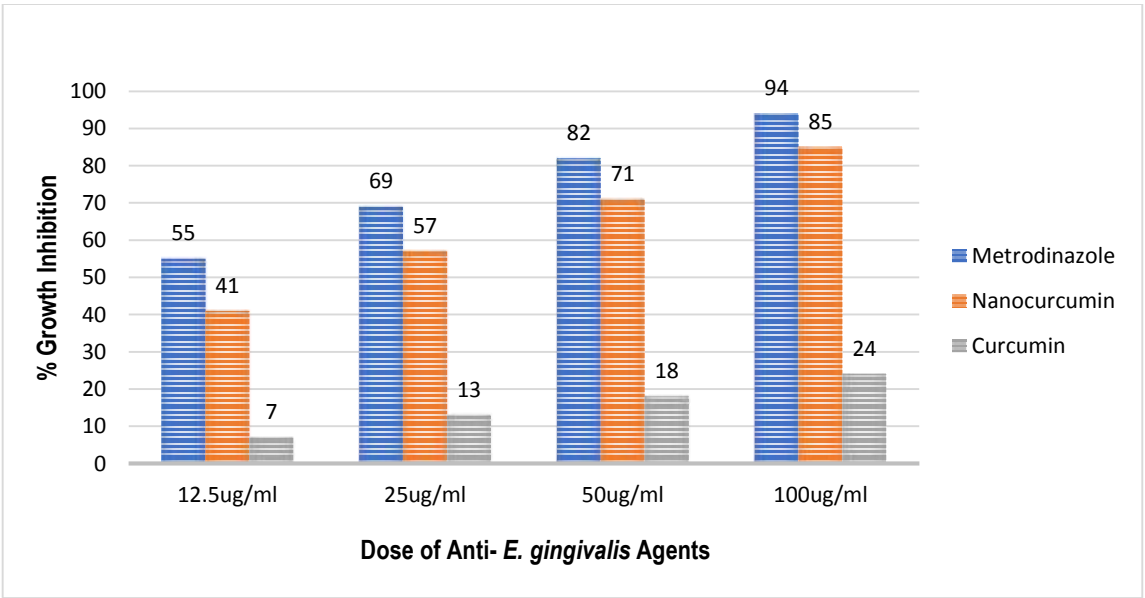

Figure 4: Anti- *E. gingivilis* effect of various Drugs/compounds

E. gingivalis is a mouth colonizing protozoa with no identified cyst form and has been associated with poor oral hygeine causing mild to severe periodontal diseases like gum bleeding, degree of decayed and loose teeth. The prevalence of this parasite in the people suffering from periodontal inflammation has been reported 11.3% to 56.5%[16-20].

In our study also, nearly 94% killing of *E. gingivalis* was observed using standard drug as reported earlier by other researchers which ensured metronidazole killed the parasite[16,22] while our nanocurcumin showed almost 85% inhibition of *E. gingivalis* , on other hand normal curcumin was unable to show any significant anti-*E. gingivalis* efficacy (nearly 20 % at higher dose).

Additional advantage of our study over other studies is that we have used aquous preparation of nanocurcumin which is more bioavailable and effective compared to organic and nanoencapsulated formulation of herbs/ antimicrobial agents[23,24].

Conclusion

In conclusion, nanocurcumin has the potential anti-E. gingivalis activity and results showed more than 80 % inhibition of growth using nano-curcumin was comparable to the inhibtion caused by standard drug metronidazole, being used for the treatment of parasitic infections. The study provide a herbal and cost effective natural herbal drug that can be used against this parasite to reduce periodontal diseases and maitain the oral hygeine by natural way.

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