RESEARCH ARTICLE

Curcumin Encapsulated into Biocompatible Co-Polymer PLGA Nanoparticle Enhanced Anti-Gastric Cancer and Anti-Helicobacter Pylori Effect

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Abstract

   Background: The current disadvantages (high cost, toxicity, resistance) of chemotherapy for gastric cancer opted people for alternative therapy from natural source. Curcumin (natural product) possess multiple biological activities but low bio-availability limits their uses as therapeutic. The Nano-formulation of curcumin increased the bioavailability and productivity of anti-cancer and anti-bacterial properties. The present study was initiated to determine the anti-cancer and anti-bacterial effect of Nano curcumin against gastric cancer and H. pylori. **Methods:** Curcumin loaded PLGA nanoparticles (CUR-NPs) was prepared by single emulsion solvent evaporation method. The MIC were determined using agar dilution method to find the anti-H. Pylori activity of Nano curcumin. The cytotoxicity of Nano curcumin was evaluated by MTT assay and the apoptotic effect (cell cycle arrest and morphology change) was shown by PI staining and microscopy. **Results:** The MIC of nanocurcumin and curcumin for all four H. pylori strains were 8 µg/ml and 16 µg/ml respectively. The inhibition rate of gastric cancer cells after treatment with curcumin was increased from 6% to 67% for 24h, from 8% to 75% for 48h, from 10% to 83% for 72h. In case of nanocurcumin, the inhibition rate increased from 7% to 69% for 24h, 11% to 87% for 48h and 16% to 97% for 72h. The IC_{50} of curcumin and Nano-curcumin were 24.20 µM and 18.78 µM respectively for 72 h. The population of cells in sub-G0 population increased from 4.1% in the control group to 24.5% and 57.8% when treated with curcumin and nanocurcumin respectively. After 72h of treatment with nanocurcumin, the apoptotic cells population increased as compared to native curcumin treated cells. **Conclusion:** The Nano curcumin might be used as a potential therapeutics against gastric cancer and H. Pylori. There is need of further in vivo study in order to validate CUR-NPs activity.

   Keywords: Nano curcumin- H. pylori- cytotoxicity- IC_{50}, apoptosis- gastric cancer

Introduction

   Gastric cancer is the fifth leading type of cancer and hold a third position in cancer related deaths worldwide (McGuire, 2015). The main cause of gastric cancer is infection by the Helicobacter pylori, and other risk factors are smoking, dietary factors and obesity (Chang and Personnet, 2010). The prognosis of gastric cancer is poor and most of the patients are diagnosed at an advance stage or metastatic stage. The gastric cancer is a fatal disease whose five years survival rate is less than 10% (Orditura et al., 2014). The most effective treatment of gastric cancer is surgical resection with chemotherapy or radiation therapy (Smyth et al., 2020). The risk of gastric cancer may be reduced by elimination of stomach colonializing bacteria Helicobacter pylori (Wu et al., 2019).

   Currently, systemic chemotherapy (cisplatin, capecitabine, fluoropyrimidine, topoisomerase inhibitors) have been used for the treatment of advanced stage gastric cancer (Wagner et al., 2017). Triple therapy containing two anti-microbial agents (clarithromycin and amoxicillin) with proton pump Inhibitor were suggested to eliminate *H. pylori* for early stage gastric cancer (Nahar et al., 2004). However, such therapy has not been very fruitful in clinical practice because of resistance, cost of chemotherapy and antibiotic, side effects, incomplete cure and non-compliance among patients that limits the scope of these drugs in gastric cancer.

   The drawbacks of chemotherapy and triple therapy lead to development of a new agent from natural source as these agents are greatly effective, specific cellular targets, safe and low cost (Kundu et al., 2011; De et al., 2009;

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Barati et al., 2019). Curcumin, a polyphenolic yellow compound obtained from Curcuma longa is cheap, easily available and having multiple biological activity (Walker and Mittal, 2020; Tiwari and Jain 2020; Olszewska et al., 2020; Memarzia et al., 2021). However, curcumin was less effective as it has low bio-availability and poor absorption.

Some novel methods used to overcome less bio-availability of curcumin are use of adjuvant and effective delivery system (phospholipid, liposome, micelles, and nanoparticles). Curcumin phospholipid complex showed high solubility and better usefulness in mice model (Maiti et al., 2007). Liposomal formulated curcumin prevents growth of pancreatic cancer cells and colorectal cancer cells (Li et al., 2005). Polymeric curcumin micelle increases the solubility and distribution of curcumin (Song et al., 2011). Researchers have shown the use of nanoparticles based delivery system might be suitable for hydrophobic agent like curcumin and might be possible solution of low bio-availability. Nano-formulation of curcumin with PLGA (poly (lactic-co-glycolic acid)) have shown increased bio-availability up to 22 fold than native curcumin (Tsai et al., 2011).

Nanocurcumin is now a matter of discussion among researchers as several studies have shown better efficiency of nanocurcumin over native curcumin in different cancer cells and pre-clinical trials but yet to analyse the effects of nanocurcumin on gastric cancer cell lines and bacterium *H. pylori* (Shehzad et al., 2014; Basniwal et al., 2014).

Studies on nanocurcumin against gastric cancer is poorly understood and there is a need for more studies to better understand the effect of nanocurcumin against gastric cancer and its causative agent H. pylori. Based on the earlier work on different cell lines from different researches around the world we can assume that nanocurcumin might be more effective than native curcumin and tripe therapy against *H. pylori* associated diseases. An ultimate goal of any nano-particle formulation is to display better therapeutic efficacy. With this aim, the present study was initiated with the formulation of nano-particles of curcumin that can be used for better antibacterial and anticancer activity. Keeping in mind the high rate of incident of gastroduodenal diseases and low efficiency of chemotherapy, triple therapy and native curcumin, the current study was proposed to find the antimicrobial and anti-cancer effect of nanocurcumin against gastric cancer and its causative agent *Helicobacter pylori*.

Materials and Methods

Formulation of curcumin nanoparticles

Curcumin nanoparticles (CUR-NPs) were made through single emulsion-solvent evaporation method. (Das and Sahoo, 2012) PLGA (poly (lactic-co-glycolic acid)) (100mg) was dissolved in dichloromethane (3ml), followed by addition of curcumin (10mg, dissolved in chloroform) to form emulsion. Further, emulsion was dissolved in an aqueous PVA (poly vinyl alcohol). The solution was sonicated (Vibracell Sonics) at 40 W, 4°C for 3 min and kept for overnight. The follow-on nanoparticles were ultra centrifuged at 13,500 rpm (Sorvall Ultraspeed

Centrifuge, Kendro, USA) at 4 °C for 30 min and washed with water to eliminate the extra quantity of PVA and incomplete encapsulated curcumin. The resultant nanoparticles were lyophilized (LYPHLOCK 12, MO) for 48 h.

Nanoparticle size and zeta potential measurement

The dynamic light scattering (DLS) via a Zetasizer (Nano ZS, UK) were used to determine the size and polydispersity index of the nano-formulated curcumin particles. The formulated nanoparticles (1mg/ml) were sonicated 55 W, 4°C for 30 sec (Vibracell Sonics, Newton, USA). All measurements were done in triplicates

Encapsulation efficiency of curcumin

The Agilent 1100 HPLC (Agilent technologies, Germany) was used to estimate the encapsulation efficacy of native curcumin. CUR-NPs (5 mg) was dissolved in acetonitrile (5 ml) and kept at 37°C in rocker for 48h. The mixture were centrifuged at 13, 500 rpm for 10 mins at room temperature to remove the curcumin. Thereafter, the supernatant was evaluated for curcumin with RP-HPLC having ratio of acetonitrile: water (80:20 v/v) on a flow rate of 1 ml/min, 30°C in thermostat. The percentage encapsulation efficiency of curcumin was calculated as follows

$$\% \text{ encapsulation efficiency} = \frac{\text{Amount of curcumin entrapped}}{\text{Total amount of curcumin used in formulation}} \times 100$$

Helicobacter pylori culture

The preserved *H. pylori* strain were streaked on Brain Heart infusion Agar (BHIA) plates supplemented with 7% horse serum, 0.4% Iso-VitaleX, antibiotics (trimethoprim (5mg/ml), amphotericin B (5mg/ml), vancomycin (5mg/ml) and nalidixic acid (8 µg/ml) (Sigma, USA). The streaked BHIA plates were incubated at 37°C in a double gas incubator (Heraeus Instruments, Germany) with 10%CO_2, 5% O_2, for 3 days. Colonies of *H. pylori* were recognised by colony morphology, gram staining, rapid urease test (RUT) and urease PCR. The *H. pylori* was subcultured on the similar microaerophilic condition.

Minimum Inhibitory concentration (MIC)

The agar dilution method (NCCLS, 1999) was used to determine the MIC of curcumin, nanocurcumin, metronidazole, tetracycline and clarithromycin for *H. pylori* strains used in this study. *H. pylori* strains were washed and suspended with phosphate-buffered saline (PBS) and 10µl of culture having optical density (O.D) of 0.1 at 600 nm was added to BHI agar plates containing various concentrations (0.125, 0.250, 0.5,1, 2, 4, 6, 8, 10, 12, 14, 16, 32, 64µg/ml) of curcumin, nanocurcumin, metronidazole, tetracycline and clarithromycin. The BHIA blank plates without *H. pylori* culture was used as negative control to check the contamination. All experimental and control BHIA plates were kept in double gas incubator at 37°C having microaerophilic atmosphere for 5 days. The MIC of a drug/compound was defined as the lowest concentration of drug/compound needed to prevent the visible growth of bacterial culture.

Cell culture

The gastric cancer cell line (AGS) was cultured in RPMI 1640 medium (HiMedia, India) with 10% foetal bovine serum (FBS), cocktail of antibiotics (penicillin, streptomycin and gentamicin) for 3 days at 37°C, 5% CO_2 atmosphere. The cells were given two-three passage to get sufficient amount of cells. Finally, the cells were counted by haemocytometer and 5×10^3 cells/ml (1 ml/well) were added to respective tissue culture plates for different time period at 37°C prior to treatment.

Cell viability assay

The MTT assay was done to determine the cytotoxic effects of nano-curcumin and curcumin against gastric cancer cell line (AGS). The AGS cells of amount $5×10^3$ were seeded in each experimental well of 96-well plate and incubated for 24 h. Then, cells were treated with curcumin and nano-curcumin of concentration (5, 10, 15, 20, 25, 30, 40μM) for 24 h, 48h and 72h, respectively. The non-treated cell act as negative control. After treatment, old media was aspirated and 20 μL of MTT (5 mg/ml) in fresh media and was added to each well and kept in incubation for 4 h. The active cells convert the MTT into purple formazan which indicates the level of cell viability. The media was replaced with 200 μL of DMSO to liquefy the formazan crystals. ELISA Microplate Reader was used to measure the absorbance of 96 well plate at 550 nm. The assay was performed in tripliate.

Analysis of cell cycle

$2×10^5$ cells/well were seeded into 6 well plates 24 h before treatment. AGS cells were treated with curcumin and nanocurcumin of different concentration for 72 h. After treatment cells were trypsanized, washed with PBS, fixed in 70% ethanol followed by wash with PBS containing 2% fetal bovine serum (FBS) and finally stained with propidium iodide (PI) (100 μg/ml) having PBS (2%FBS) and incubated in the dark for 15 min. Afterwards, the stained cells were acquired on flow cytometer (BD LSR Fortessa) and the distribution of cells in each cell cycle were analysed in FACS diva software.

Apoptosis (morphological change) by staining with cyto9 and PI

The AGS cells death were examined using staining of cells with cyto9 and PI. After 72 h of treatment with curcumin/nanocurcumin, cells were harvested and mixed with 10μl of PI (1mg/ml) and 10μl of cyto9 (1mg/ml) (sigma. USA). The stained cells were viewed under fluorescence microscope (Leica microsystem holding GmbH, Germany). Apoptotic, necrotic and viable cells were recognised with microscopic examination on the basis of morphological change (membrane blebbing, nuclear and cytoplasmic condensation). Untreated AGS cells were kept as control.

Results

Physio-chemical characterization of curcumin nanoparticles

The physio-chemical characteristic of the formulated CUR-NPs was evaluated by Dynamic Laser Scattering analysis. The size of prepared CUR-NPs was uni-modal with diameter of ~ 175 nm, polydispersity index of ~ 0.1 and zeta potential of ~ −16.4 mV (Figure 1, Table 1). The CUR-NPs are negatively charged due to the presence of

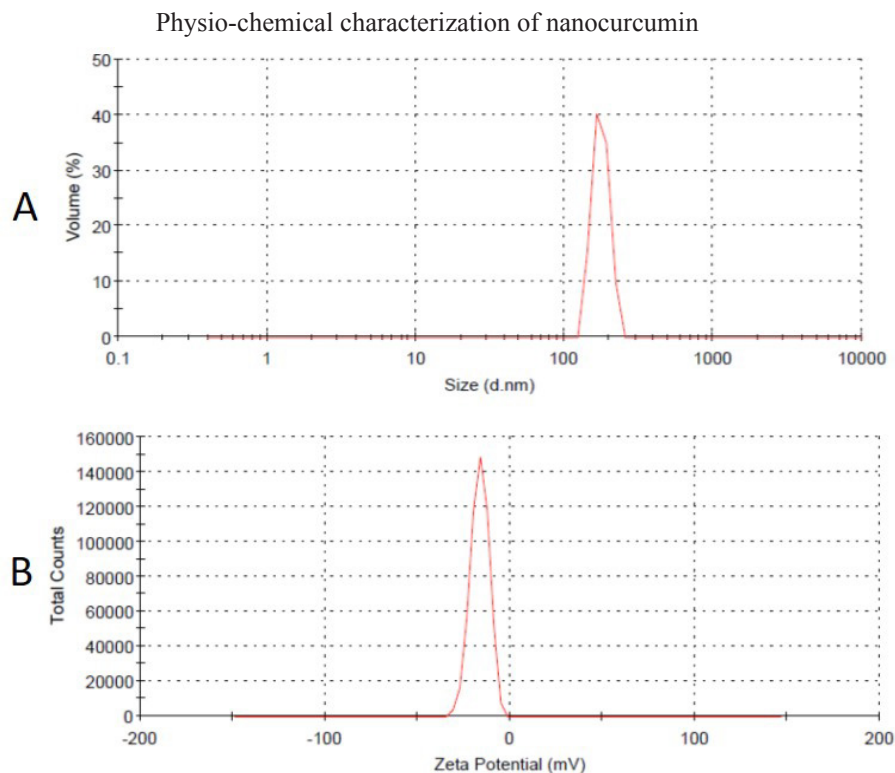Physio-chemical characterization of nanocurcumin

Figure 1. (A), nanocurcumin size measurement by Zetasizer; (B), Zeta potential of nanocurcumin measured by Zetasizer

Table 1. Physico-Chemical Characterization of Nanocurcumin

Particle Size (nm)[a]	Poly dispersity index[b]	Zeta potential (mV)[c]	Entrapment Efficiency (%)[d]
~175 ± 2.1	~0.1 ± 0.004	~ −16.4 ± 0.38	~80 ± 2.1

[a], Particle size was measured by Zetasizer; [b], Zeta potential was measured by Zetasizer; [c], Polydispesity index was measured by Zetasizer; [d], Entrapment efficiency was measured by RP-HPLC
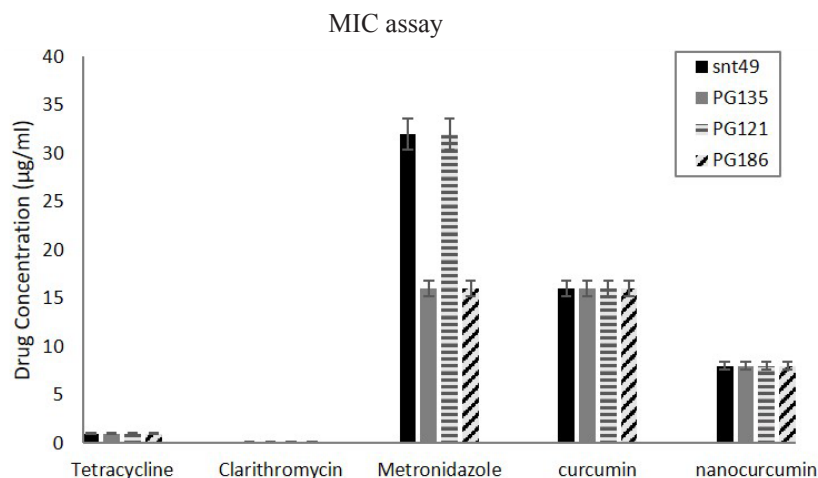

Figure 2. The anti-*H. Pylori* Effect of Tetracycline, Clarithromycin, Metronidazole, Curcumin and Nanocurcumin against *H. pylori* Strain snt49. The vertical axis represent drug concentration and horizontal axis represent drug tested. The results were repeated in triplicate with standard error

the carboxyl groups on their surface. The encapsulation efficiency of curcumin was ~ 80 % as estimated through HPLC (Table 1). Using PLGA polymer, similar kind of size and zeta potential were observed (Das and Sahoo, 2012). Therefore, use of emulsifier TPGS1000 in the PLGA based formulation has resulted in small sized curcumin loaded particles that is highly useful for better cellular uptake and enhanced therapeutic activity. Many groups have worked towards enhancing the solubility and pharmacokinetics of native curcumin by utilizing PLGA polymeric nanocarrier system (Anand et al., 2010) or in combination drugs (Das and Sahoo, 2012). Further, curcumin encapsulated in glycerol mono-oleate based nanoparticles as well as micellar formulations has demonstrated better bio-therapeutic efficacy, compared to native curcumin (Mohanty et al., 2010).

Antimicrobial activity of nanocurcumin

The anti-*H. pylori* activity of Nano formulated curcumin, native curcumin and three antibiotics (metronidazole, tetracycline and clarithromycin) were determined by evaluating MIC against clinical isolates of *H. pylori.* Bacterial cells were considered to be resistant when the MIC was >8 µg/mL for Mtz, > 2 µg/

mL for Tet and Cla. The MIC were obtained after treating *H. pylori* strains with different concentration (0.125, 0.250, 0.5,1, 2, 4, 6, 8, 10, 12, 14, 16, 32, 64µg/ml) of curcumin, nanocurcumin, metronidazole, tetracycline and clarithromycin for 72h in three independent trials as listed in Table 2. The MIC of tetracycline and clarithromycin for all four *H. pylori* isolates were less than 1 µg/ml and 0.125µg/ml respectively which showed that these four strains were sensitive to these two drugs (tetracycline and Clarithromycin). All these four *H. pylori* strains were found to be resistant to metronidazole as their MIC was more than 8 µg/ml. The MIC of metronidazole for two *H. pylori* strain were 32 µg/ml and for other two were 16 µg/ml, respectively. The MIC of curcumin for all four strains were 16 µg/ml. The MIC of nanocurcumin for all four strains were 8 µg/ml which is lower than native curcumin (Figure 2). The MIC data of tested drugs suggested that nanocurcumin has more ability to inhibit *H. pylori* strain compared with curcumin and metronidazole but still it is less efficient than tetracycline and clarithromycin for these four tested *H. pylori* strains. We therefore suggest that nanocurcumin may have a potential role as anti-*H. pylori* activities in comparison with curcumin and metronidazole.

Table 2. Minimum Inhibitory Concentration (MIC) of Tested Drug (Curcumin, Nanocurcumin, Amoxicillin, Clarithromycin and Metronidazole) against *H. pylori*

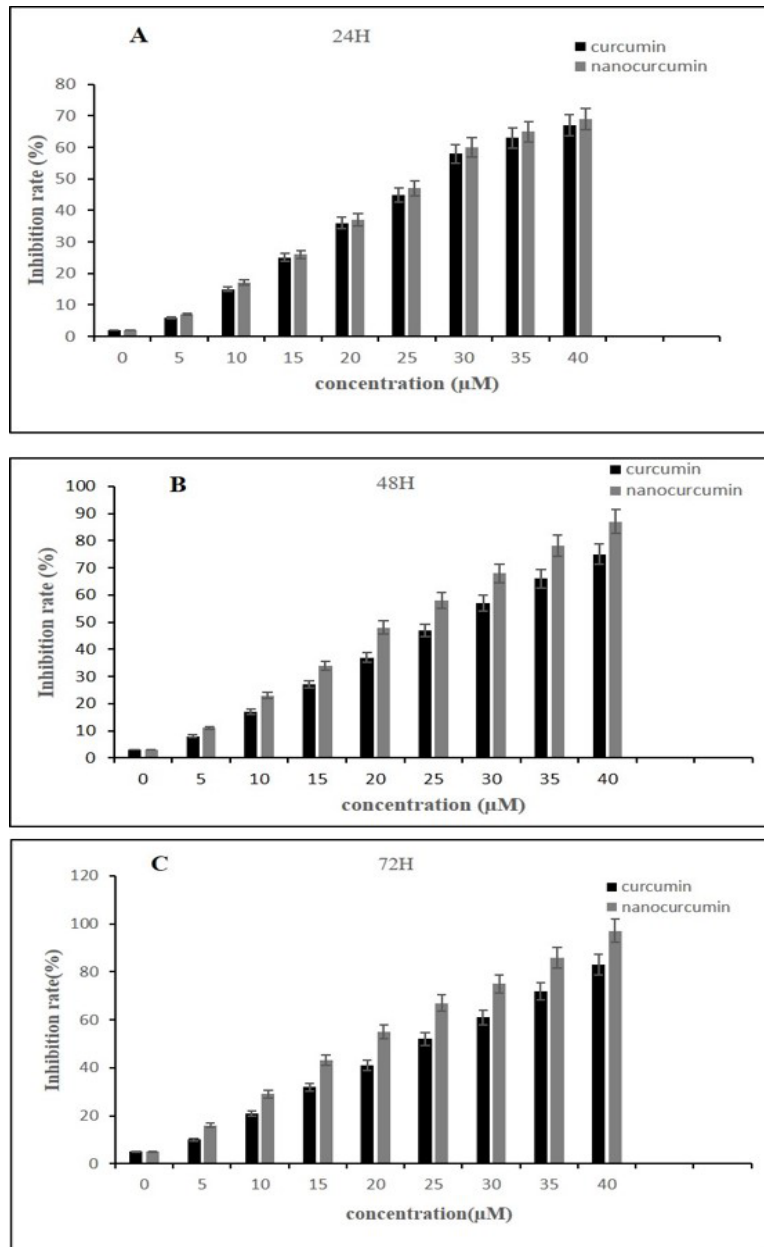| Bacteria (*H. pylori* strain)	Curcumin (µg/ml)	Nanocurcumin (µg/ml)	metronidazole (µg/ml)	Tetracyclin (µg/ml)	Clarithromycin (µg/ml)
Snt49	16	8	32	1	0.125
PG135	16	8	16	1	0.125
PG186	16	8	16	1	0.125
I-121	16	8	32	1	0.125

MTT assay

Figure 3. The AGS Cell Inhibition after Treatment with Curcumin and Nanocurcumin. The Y axis indicates the cell inhibition and X axis indicates the concentration of curcumin and nanocurcumin. A, The cells were treated for 24h; B, The cells were treated for 48h; C, the cells were treated for 72h

Inhibition of gastric cancerous cells proliferation

The anti-proliferative activity of nanocurcumin and curcumin were determined using cell proliferation assay for 24h, 48h and 72h, respectively. The change in colour of MTT from yellow to purple indicates the viable cells. The viability of AGS cell decreased after treatment with wide range of drug concentration (5-40μM) as compared

Table 3. IC_{50} Value of Curcumin and Nanocurcumin against Gastric Cancer Cells (AGS) at at 24, 48 and 72H

Incubation Time	Curcumin	nanocurcumin
24h	28.26μM	27.31 μM
48h	26.75μM	22.10μM
72H	24.20μM	18.78μM

to untreated cells. The inhibition rate of curcumin increased from 6% to 67% for 24h, from 8% to 75% for 48h, from 10% to 83% for 72h. In case of nanocurcumin, the inhibition rate increased from 7% to 69% for 24h, 11% to 87% for 48h and 16% to 97% for 72h as indicated in Figure 3. The maximum inhibition rate of curcumin was found to be 83% at 40μM concentration for 72h and for nanocurcumin it was 97% at 40μM for 72h. Our result indicated that both curcumin and nanocurcumin have time and dose dependent anti-proliferative effect on AGS cells in the range of 5-40μM for 24h, 48h and 72h of treatment. Our results showed that the cytotoxic effect of nanocurcumin was almost equal at 24h, slightly higher at 48h and significantly higher at 72h compared to curcumin. Half of maximum inhibitory concentration (IC_{50}) of both curcumin and nanocurcmin were determined and listed
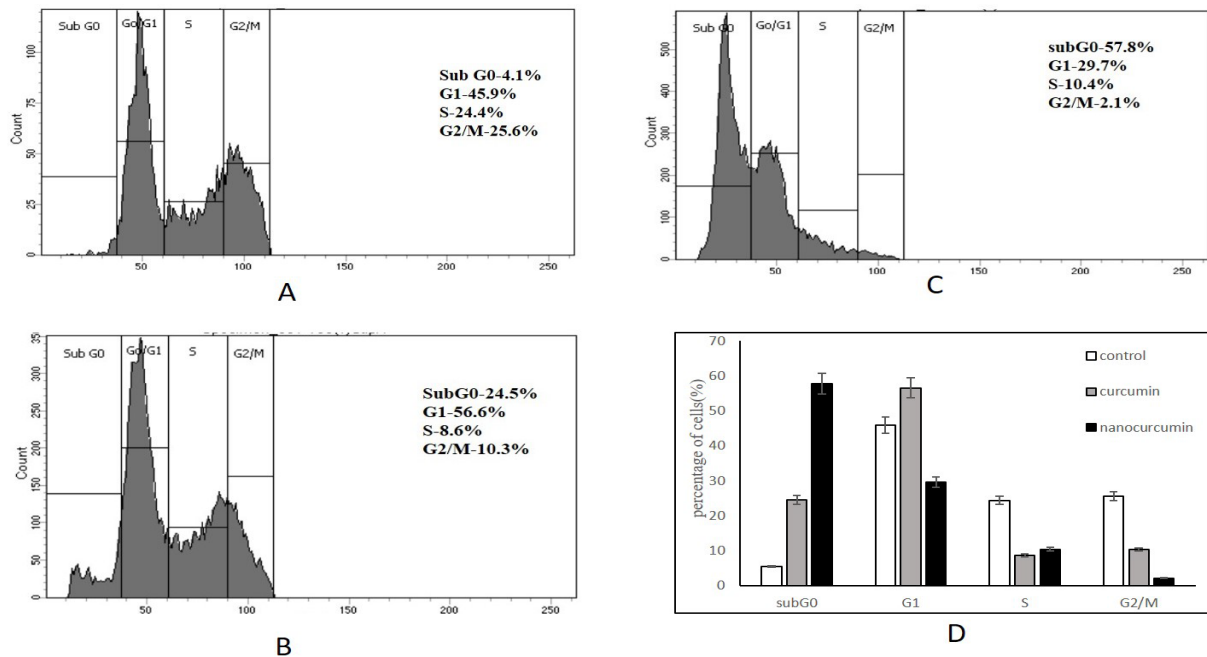
Cell cycle analysis

Figure 4. Cell Cycle Analysis with PI Focussed on Sub G0 which Represents Death Cell Population. The distribution of percentage of cells in the sub G0, G1, S and G2/M phase is shown. A, control (untreated); B, treated with curcumin at IC_{50} concentration; C, treated with nanocurcumin at IC_{50} concentration for 72H

in Table3. The IC_{50} value of curcumin and nanocurcumin against AGS cells were (28.26 μM, 27.31μm) at 24 h, (26.75 μM, and 22.10 μM) at 48h and (24.20 μM, 18.78 μM) at 72h. The lower IC_{50} value of nanocurcumin (18.78 μM) at 72h compared to native curcumin (24.20 μM) showed that nanocurcuin has better toxicity than curcumin on gastric cancer cell lines (AGS), which can be elucidated by high solubility but slow rate of diffusion of curcumin from nanoparticles.

Cell cycle arrest

Cells were treated with curcumin and CUR-NC for 72h to analyse the distribution of cells in each cell cycle. Our results show that flow cytometry showed the distribution of cells in three phase of cell cycle (G1 vs S vs G2M) and may spot apoptotic cells with fractionated DNA as shown in Figure 4(A-D). The result indicated that curcumin/nanocurcumin arrested the cell cycle of AGS cells in the sub-G0 phase that represent apoptotic

cells and induces apoptosis after treatment for 72 h. Cells without treatment were taken as controls. Cells treated with curcumin at IC_{50} concentration shows distribution of cells in sub-G0 phase (24.5%) while nanocurcumin treated cells at IC_{50} concentration shows cells distribution in sub-G0 phase (57.8%). Our result shows the increase in sub-G0 population of treated AGS cells, from 4.1% in the control group to 24.5% and 57.8% when treated with curcumin and nanocurcumin respectively. We found that nanocurcumin treated cells induced more apoptosis (24.5%) compared to curcumin treated induced apoptosis (57.8%). Our data showed that nanocurcumin is more efficient and specific than native curcumin in delaying the cell cycle at sub-G0 phase.

Morphology change by PI/Cyto9 staining

Curcumin and nanocurcumin treated AGS cells were stained with PI/cyto9 for 72 h to determine the apoptosis by morphology change. The curcumin and nanocurcumin

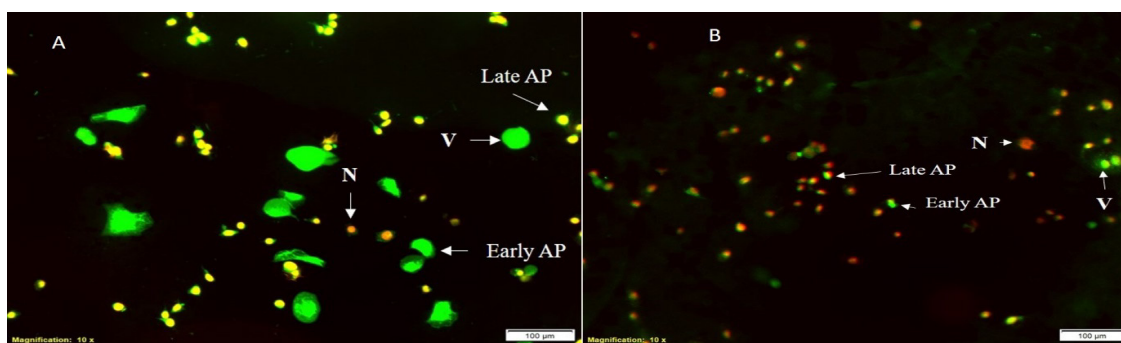Morphology change by PI/Cyto9 staining

Figure 5. PI and Cyto9 Staining of Curcumin/Nanocurcumin Treated AGS Cells. V, viable Cells; AP, Apoptotic cells; N, Necrosis cells; A, Curcumin at IC_{50} concentration treated cells for 72H; B, nanocurcumin treated at IC50 treated cells for 72H; Curcumin at IC_{50} concentration treated cells for 72H

both are effective in stimulating apoptosis in AGS cells in a time dependent manner. We found that curcumin treated AGS cells showed higher viable cells population than apoptotic cells but in case of nanocurcumin, the apoptotic cell population was much higher than viable cells as shown in Figure 5(A, B). Our finding shows that nanocurcumin was more cytotoxic towards AGS cell. Cells treated with IC_{50} value of curcumin showed green and bright green colour nuclei indicating the live and early apoptosis in AGS cells. Cells treated with IC_{50} value of nanocurcumin exhibited bright green and bright orange colour nuclei indicating the early apoptosis and late apoptosis in AGS cells. Necrotic cells were red in colour where PI penetrated the nuclear matter. After 72h of treatment with nanocurcumin, the apoptotic cells population increased as compared to native curcumin treated cell population.

Discussion

Curcumin has potential to prevent gastric cancer cells by inhibiting the oncogenic pathway and it may act as therapeutic candidate to eliminate the *H. pylori* from gastric disorder patients (Vetvicka et al., 2016; Sarkar et al., 2016; Bahrami and Ferns, 2020). Several pre-clinical and clinical studies have been done on use of curcumin against various diseases but still it is not in practice commercially for the therapy of diseases like other drugs because curcumin have some drawbacks like low bioavailability and poor absorption (Anand et al., 2007; Prasad et al., 2014).

An ultimate goal in any nanoparticle formulation is to display better therapeutic efficacy. In the present study, we formulated curcumin loaded bio-compatible PLGA nanoparticles to increase the bio-availability and efficiency of native curcumin that can be used for better anti-bacterial and anti-cancer activity. PLGA co-polymer is FDA approved for drug delivery and functional foods as it is bio-degradable and bio-compatible (Mundargi et al. 2008). PLGA based nano-delivery system protects the entrapped curcumin from gastric hydrolysis and degradation. Further, polymeric nanoparticle enables the encapsulated drugs to be released either by diffusion or swelling or combination of these processes (Misra et al; 2009). Formulation of nanocurcumin reduces the particles size of native curcumin to nano-scale that leads to improvement in the cellular uptake and bio-availability (Feng, 2004). The small size of CUR-NP ~175nm is a vital factor, and has a demonstrative consequence on the stability, cellular uptake, and drug release parameters (Gratton et al; 2008; Champion et al., 2008). The Poly dispersity index (PDI) of nanocurcumin is stable after loading of curcumin in to PLGA nanoparticles. The entrapment efficiency (EE) is an important factor which prompted the drug therapeutic effect. The great EE of CUR-NP showed that the single emulsion-solvent evaporation method is appropriate for the loading of curcumin into polymer PLGA nanoparticles. Polymeric nanoparticles are effectively used for useful delivery of 5 flurouracil, preodontal tetracyclin and insulin.

The anti-*H. pylori* properties of curcumin was first shown by Mahady et al., (2002) followed by other studies to project curcumin as potential candidate against *H. pylori* (Sarkar et al., 2016) but with limitations. To overcome the drawbacks of curcumin, nanoparticles based delivery system was used and found a better efficacy in several cancerous cell lines (Möller et al., 2021). The nanoparticles based delivery of curcumin is poorly understood against *H. pylori* infection. We determined the anti-*H. pylori* properties of curcumin using curcumin loaded PLGA nanoparticles. The particles size reduction of curcumin lead to a major improvement in the anti-*H. pylori* activity. The MICs of CUR-NP was lower than native curcumin for all four *H. pylori* strains as shown in (Figure 2, Table 2). Nanocurcumin showed effective antimicrobial actions against *H. pylori* strains compared with native curcumin. By taking into consideration of MICs data, we found that the toxicity of nanocurcumin is better than native curcumin on *H. pylori* strains and this can be related with size reduction. Our anti-microbial results are in agreement with earlier findings where author prepared nanoparticles using different methods (Jain et al. 2009). The anti-bacterial properties of curcumin was enhanced when it was used as curcumin loaded PLGA nanoparticles against several bacteria especially gram negative bacteria and suggested that CUR-PLGA-NPs may be considered as novel antibacterial agents (Gao et al., 2020). Nanoparticles encapsulated antibiotics reported improved efficacy, reduced toxicity and prolong half-life of these antibiotics (Yacoby and Benhar, 2009). Efficiency of the nanoparticles in eliminating Helicobacter pylori was studied using mucoadhesive gliadin nanoparticles (Umamaheshwari et al 2004). All these four strains were pathogenic as they are positive for *cagA, vacA and dupA* gene. We hypothesized that nanocurcumin might have more anti-*H. pylori* potential than native curcumin by inhibiting the growth of *H. pylori* strains.

In vitro studies against a several human cancer cell lines showed that nanocurcumin exhibits more therapeutic efficacy than native curcumin (Shehzad et al., 2014; Basniwal et al., 2014). The cell lines like DU145, PC3, and LNCaP showed better uptake of nanocurcumin as comparable to native curcumin (Mukerjee and Vishwanatha, 2009). Our MTT assay on gastric cancer cell reflects that CUR-NP demonstrated a lower IC_{50} value in comparison to native curcumin. The toxicity of compound (curcumin/nanocurcumin) differs on its IC_{50} value. The IC_{50} of nanocurcumin 18.78μM which was lower than IC_{50} of curcumin 24.20 μM for gastric cancer cell line (AGS) in 72 h (Table 3). The lower values of IC_{50} designate that nanocurcumin revealed more toxicity against AGS cells than native curcumin. Based on our data, we can assume that the Inhibition of AGS cells for nanocurcumin was higher than native curcumin at every concentrations used (Figure 3). The formulation of nanocurcumin in aqueous dispersible will remove the toxic compounds like DMSO from the native curcumin, which led to toxic side effects. The finding of our study regarding anti-cancer effect of curcumin loaded PLGA nanoparticles was supported by other researchers using the polymeric nanoparticles but in different cancer cell lines (Mohanty et al., 2010; Das and Sahoo, 2012; Anand et al., 2010). Various studies have shown that curcumin

loaded PLGA nanoparticles promoted neuronal uptake, neuroprotective against SK-N-SH cells and induce reverse cognitive deficit, neurogenesis in Alzheimer's diseases model (Doggui et al., 2012; Tiwari et al., 2013). The Nano formulated PLGA-curcumin showed better result than native curcumin in inhibiting degenerative changes and delaying the death of mice in cerebral malaria (Dende et al., 2017). Recently, Sufi et al., (2020) showed that curcumin loaded PLGA nanoparticles may be effective against colon cancer as Nano-formulation preserved the curcumin from degradation in varied ranges of pH. Previously reports on curcumin-loaded (PLGA-TPGS) nanoparticles showed improved efficiency of curcumin in reducing the growth of hepatocellular cancer cells and demonstrated high anti-tumour efficiency and low toxicity which promised a novel candidate for the therapy of liver cancer (Chen et al., 2019). Recent study conducted on the outcome of CUR-NP on SK-OV-3 ovarian cancer cells demonstrated that the physicochemical properties like stability in the presence of light, was enhanced compared to native curcumin and showed robust apoptotic effects on tumour cells (Duse et al., 2019). Therefore, based on our findings, we proposed that CUR-NPs might be a superior therapeutic method than native curcumin for healing gastric cancerous cells.

Different anti-cancer compounds arrested the cell cycle at different stage (G0/G1, S, and G2/M phase) and induce apoptosis (Torres and Horwitz, 1998; Murray, 2004). Hence, the impact of nanocurcumin and curcumin on the cell cycle of AGS cell was resolved to recognize the mode of action of nanocurcumin/curcumin in reducing AGS cell growth. This study showed that both curcumin and nanocurcumin can arrest the cell cycle at G0/G1 phase in a dose dependent way. Cell cycle and apoptosis are two critical factors in the regulatory of cell growth. The integrity of genome of any cells depends upon the cell cycle checkpoints which protect dividing cells from the potentially lethal outcomes of DNA damage. Study on cell cycle checkpoint found that the G0/G1 checkpoint is a potential target for anticancer treatment. It checks cells with damaged DNA from replicating and proliferation and permits for the repair of DNA damage. However, faults in the G0/G1 phase may force a cell to go for apoptosis, and factors to enhance the apoptotic effect may rise the cytotoxicity of chemotherapy (Wang et al., 2009). Nanocurcumin is a potent inhibitor of cell proliferation by delaying the cell cycle at subG0 phase. The current strategy for the progress of novel cancer treatments is blockage of the cell cycle (McDonald and EI-Deiry, 2000; Buolamwini 2000). We concluded that the apoptotic cell populations increased with incubation of curcumin loaded PLGA NPs as compared to native curcumin.

In summary, we reported the formulation and characterization of curcumin loaded PLGA nanoparticles and determined their anti-cancer and anti-bacterial properties against gastric cancer and *H. Pylori*. The obtained results showed enhanced potent anti-cancer and anti-bacterial activities of Nano-curcumin in comparison to native curcumin which may open new approach in cancer therapy by overcoming the limitation of conventional therapy. There is need of further in vivo study in order to validate CUR-NPs activity

Author Contribution Statement

Jawed Alam: Conceptualization, execution, analysis of data and writing of the article. Fahima Dilnawaz: Execution and writing of parts of methodology section. Tahziba Hussain: Review, editing and final approval of manuscript. Asish Kumar Mukhopadhyay: review and editing. Durg Vijay Singh, Sanjeeb Kumar Sahoo, Sanghamitra Pati: Guidence. All authors have read and agreed to the published version of the manuscript

Acknowledgements

Ethical Issues
There is no ethical issues as the present research work doesn't include any human or animal tissue samples. The entire work has been carried out using cell lines as in vitro model system.

Conflicts of interest
The authors declare that they have no conflicts of interests.

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