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Curcumin Nanoformulation: Antioxidant,Antibacterial, and Toxicity Assessment

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Abstract

Nanotechnology-based techniques becomea privilege to the phytochemicals which have poor bioavailability but potential biological activities. Curcumin is categorized among them that having vast medicinal properties but its hydrophobicity limits its clinical acceptance. The study aimed to evaluate the antioxidant and antibacterial potentials of synthesized and characterized curcumin nanoformulation (CN) and to evaluate its toxicity on human red blood cells and invertebrate brine shrimps. The curcumin nanoformulation was synthesized and confirmed by dynamic light scattering and chromatographic methods. The antioxidant potential of the synthesized CN was analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while antibacterial activity was assessed by following the agar well diffusion protocol using Gram-positive and Gram-negative bacteria. The hemolysis assay and brine shrimp lethality assay were performed to evaluate the *ex vivo* toxicity of the synthesized CN.The data revealed that the synthesized CN had a very small particle size (6.06 ± 1.7 nm) with polydispersity index and zeta potential value of 0.219 and -11.8 ± 3.89 mV respectively. The antioxidant and antibacterial potential of the synthesized CN were improved as compared to the pure curcumin. The hemolytic and brine shrimp lethality activity of the synthesized CN wassignificantly minimal as compares to its respective positive controls. These findings suggested that the synthesized CN with potential antioxidant and antibacterial activities and almost no toxicity could be a promising therapeutic option in various antimicrobial and free radical toxicity treatments. Moreover, after a detailed safety analysis of synthesized CN, it can be applied in various formulations and treatments.

Keywords: Antibacterial, Antioxidant, Brine shrimp, Curcumin, DPPH, Hemolysis.

Introduction

Curcumin is plant based bioactive polyphenolic agent found in the *Curcuma longa* plant(1,2). It was most studied and valuable spice in the ancient history of Indiafor its excellent medicinal properties such as antioxidant(3), antimicrobial(4), antimalarial(5), anticancer(6), anti-inflammatory(7)and wound healing(8).Although its wide range of medicinal properties,

it has poor aqueous solubility andlimited bioavailability, as evidenced by poor levels in serum, limited tissue distribution, along with rapid metabolism that further greatly restricts its pharmacological approaches(9).Researchers have investigated the practice of curcumin nanoformulation as an efficient drug delivery system to circumvent these limitations. Since curcumin is a potent therapeutic drug, it is desirable to synthesize novel formulation methods to boost itspharmaceutical potential.

Nanoformulations have particle size varies from 1-100 nm and have key qualities due to their size and surface area(10).Curcumin nanoformulation can improve curcumin's solubility, stability, and bioavailability, perhaps resulting in greater therapeutic effects. Curcumin nanoformulations have been receiving considerable interest in the field of cancer research in the last few years. Numerousfindings have shown that curcumin nanoformulations have the potential to be an effective anticancer agent(11–15). Curcumin nanoformulation can cause cancer cells to undergo apoptosis(16),inhibit tumor development(17)we propose previously developed, self-assembling dextran-curcumin nanoparticles for the treatment of prostate cancer in combination therapy with Doxorubicin (DOXO, and prevent metastasis(18). Curcumin nanoformulations have been proven to improve the efficacy of traditional chemotherapy and radiation therapy(19–21)developing nanobiomaterials for combination of radiotherapy and chemotherapy is required for more powerful and successful cures. Because of the amazing X-ray sensitization proficiency of Bi based nanoparticles, in this work, we synthesized and used Bi2S3 as an enhancer of X-ray radiation therapy, and furthermore, Bi2S3 served as carrier of curcumin (CUR.

Curcumin nanoformulations have broad spectrum antibacterial potential against various bacterial species due to the disruption of the bacterial cell membrane, leading to bacterial death(22–25).Furthermore, curcumin nanoformulations outperform free curcumin in terms of antioxidant activity. This has prompted significant interest in the practice of curcumin nanoformulations as a possible therapeutic management for various oxidative stress-related diseases, involving cancer, diabetes, and neurological disorders(26).This area has drawn considerable interest in current history, and many researchers have found that curcumin nanoformulations have potential antioxidant properties(27–30)but it is practically water-insoluble and has low bioavailability; a possible solution to this obstacle would be formulations of curcumin nanoparticles. Surfactants such as tween 80 can be used to stabilize low-solubility molecules preventing particle aggregation. The objectives of this study were the preparation of a suspension with curcumin nanoparticles in tween 80, the testing of pure curcumin solubility and of a simple mixture of curcumin with tween 80 and nanosuspension in water and ethanol as solvents, and finally the assessment of the antioxidant activity. We prepared the nanosuspension by injecting a curcumin solution in dichloromethane at low flow in water with tween 80 under heating and ultrasound. The analysis of particles size was conducted through dynamic light scattering; the non-degradation of curcumin was verified through thin-layer chromatography. The analyses of antioxidant activity were carried out according to the DPPH method. The method applied to reduce the particles size was efficient. Both the curcumin suspension and nanosuspension in tween 80 increased its solubility. Curcumin and the formulations presented antioxidant activity.",”container-title”:”Food Science and Technology”,”DOI”:”10.1590/1678-457X.6515”,”ISSN”:”0101-2061, 1678-457X”,”-journalAbbreviation”:”Food Sci. Technol”,”language”:”en”,”note”:”publisher: Sociedade Brasileira de Ciência e Tecnologia de Alimentos”,”page”:”115-119”,”source”:”SciELO”,”title”:”Production, solubility and antioxidant activity of curcumin nanosuspension”,”URL”:”http://www.scielo.br/j/cta/a/MngxC7yjbmBGnY4PGwQkKkh/?lang=en”,”volume”:”35”,”author”:[{“-family”:”Carvalho”,”given”:”Deivis de Moraes”},{“family”:”Takeuchi”,”given”:”Katiuchia

Pereira”},{“family”:”Geraldine”,”given”:”Robson Maia”},{“family”:”Moura”,”given”:”Celso José”,”-dropping-particle”:”de”},{“family”:”Torres”,”-given”:”Maria Célia Lopes”}],”accessed”:{“-date-parts”:[[“2023”,4,22]]},”issued”:{“date-parts”:[[“2015”,3]]}},”label”:”page”},{“id”:1760,”uris”:[“http://zotero.org/users/8294352/items/72ZQW-67F”],”itemData”:{“id”:1760,”type”:”article-journal”,”abstract”:”Abstract Encapsulation of bioactive compounds has been carried out to improve bioavailability and to protect them against harm conditions. However, encapsulation processes are often aggressive and it is important that encapsulated substances keep their biological activity. In this work curcumin was nanoencapsulated using dichloromethane as solvent and ultrasound as dispersion device. Nanoparticles were obtained using different curcumin concentrations and encapsulants (PLLA and Eudragit S100.

Despite the potential therapeutic benefits of curcumin nanoformulations, their toxicity needs to be evaluated to ensure their safe use during drug development.Various curcumin nanoformulations were evaluated for their biocompatibility as well as *in vitro* and *in vivo* toxicity before clinical application(31–33).The ability of a substance to cohabit with the biological cells without generating any detrimental consequences is referred to as biocompatibility. Toxicity evaluation, on the contrary side, examines the possible harm that a material may produce to a biological system. Moreover, due to the associated harmful effects of greater doses and longer exposure, toxicity studies of curcumin nanoformulations are continues to be an emerging topic of research.

The study aimed to assess the potential biological activities of synthesized curcumin nanoformulation (CN) such as antioxidant and antibacterial activities for its therapeutic applications. Further, this study is also designed to assess the preliminary toxicity of the synthesized CN if any.

Materials and methods

Materials

Curcumin, TPGS, methanol, chloroform, 0.2µm filters, andTLC silica gel plate were procured from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Formic acid, toluene, ethyl acetate, ascorbic acid, 2,2-diphenyl-1-picrilhhydrazyl (DPPH), dimethyl sulfoxide (DMSO), phosphate buffer saline (pH 7.4), andnutrient agar from Himedia (India), bacterial cultures of*Staphylococcus aureus*(NCIM 2079; equivalent ATCC 6538P)*, Bacillus subtilis*(MTCC 736)*, Pseudomonas aeruginosa* (NCIM 2200; equivalent ATCC 9027),and *Escherichia coli*(NCIM 2065; equivalent ATCC 8739) were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India.

Preparation of curcumin nanoformulation (CN)

To obtain curcumin nanoformulation, 0.01% stock solution of curcumin (0.01 g/ml) in methanol) was added into 0.1% solution of TPGS (0.1g/mlin chloroform) in successive titration with magnetic stirring. After evaporation of all the solvent the dried film was rehydrated with autoclaved MiliQwater. The sample was then centrifuged at 14000RPM for 20-25minutes at 4˚C in cooling centrifuge. Supernatant was collected and filtered through 0.2µm filter to eliminate any unencapsulated curcumin particles. Filtrate was considered as a curcumin nanoformulation, and it was stored in glass vials at 4˚C under dark condition.

Particle size, polydispersity index (PDI) and zeta potential

The particle size of CN was measured by dynamic light scattering measurement in the Zetasizer Nano-ZS (Malvern Panalytical, Malvern, United Kingdom). 1 mg/ml of CN was dissolved in nanopure water and 10 µl of this suspension was diluted to 1 ml for analysis. Zeta potential and PDI were measured by Zetasizer Nano ZS90 (Malvern Panalytical, Malvern, United Kingdom) at ambient temperature. For this assessment, 1 ml of the CN was taken in clear

disposable zeta cells to know the surface charge of the synthesized CN. All measurements were performed in triplicates.

Thin layer chromatography (TLC) analysis

Thin layer chromatography was performed by the method of Rohman et al(34)safety, and efficacy. In this study, thin layer chromatography (TLC. It was carried out to determine if the curcumin molecules were degraded throughout the CN synthesis. The curcumin and TPGS were taken as standards while synthesized CN as a sample. We used the methanol: formic acid: toluene: ethyl acetate (0.7:0.8:3:4 v/v/v/v) as mobile phase and TLC Silica gel plate as a stationary phase. The Rf value was calculated according to the given equation:

High performance thin layer chromatography (HPTLC) analysis

High performance thin layer chromatography (HPTLC) was implemented by following the method of Kushwaha et al(35). It isa powerful analytical technique used to obtain a chromatographic imprint ofthe important molecule. The samples were applied on to the HPTLC plate precisely using a Hamilton microliter syringe and the plate was left to dry. The plates were then placed in a glass chamber (20 cm × 20 cm) containing 10 ml of mobile phase of methanol : formic acid : toluene : ethyl acetate (0.7:0.8:3:4 v/v/v/v) for 20 minutes at ambient temperature. The dried plate was scanned at 254 nm using Cammag TLC scanner IV with visionCATS system (version 2.5.18262.1).

2,2-diphenyl-1-picrilhydrazyl (DPPH) assay

The antioxidant activity of the synthesized CN was performed by the protocol of Naksuriya and Okonogi(36).100 µM of methanolic solution of DPPH was prepared and kept in dark at 4ºCbefore analysis. 1 mg/ml stock solution of curcumin, CN, TPGS, and ascorbic acid (positive control) were diluted to varying concentrations (10-100 µg/ml) in methanol or distilled water according to their solubility and added in 12-well plate in triplicate concentration with blank and positive control. 200 µl of 1.5 M Tris-HCL was added to each well and subsequently 1 ml DPPH was added then kept it covered with alluminium foil for 30 minutes then the absorbance was taken at 540 nm using microplate readers(AgilentBioTek, California, USA).The DPPH inhibition activity was calculated using the given equation:

Antibacterial activity

The antibacterial efficacy of the synthesized CN was analyzed by an agar well diffusion protocol given by Valgas et al(37).The antibacterial activity of the synthesized CN was tested against Gram-positive cocci *Staphylococcus aureus,* Gram-positive rods *Bacillus subtilis,* Gram-negative rods *Pseudomonas aeruginosa* and, Gram-negative rods *Escherichia coli*. The bacterial cultures were prepared from the stock cultures and final concentration was adjusted to 10^8 CFU/ml. The bacterial suspensions were inoculated onto the surface of the agar medium plates. Wells of around 6-8 mm in diameter were created in the agar using a sterile cork borer. The samples of DMSO (1%), curcumin, synthesized CN, and TPGS were loaded at 10mg/ml concentrations into the wells. The plates were incubated for 24 hours at 37˚Cthat allowed the growth of bacteria and diffusion of the samples. After the incubation period, the plates were observed for the presence of a zone of inhibition around the wells and measured in millimeters using ruler.

Brine shrimp toxicity assay

The brine shrimp toxicity assay was analyzed by using method of Meyer et al(38). Briefly, the brine shrimp cysts were added into a petri plate filled with artificial sea water (3.3% w/v). The cysts were hatched after the overnight incubation at an ambient temperature, and the hatched larvae, known as nauplii, were used for assay. Further, 10 nauplii were added into each well of the 12-well plate with artificial sea water. The synthesized CN with concentrations of 1 to 10 mg/ml was added into 12-well plate and in-

cubated for overnight in the presence of visible light. After incubation, the live and dead nauplii were calculated for each dose and control (artificial sea water). The percentage of lethality was calculated using given equation:

Hemolysis activity

The hemolytic efficiency of the synthesized CN was assessed by following the protocol of Ayubi et al(32).Fresh blood sample (5ml) was collected in heparinized tubes and centrifuged at 2000 RPM for 5minutes. After discarding plasma, the red blood cells were washed three times with phosphate buffer saline. The 2% solution of red blood cells was prepared in phosphate buffer saline for the analysis. To study the hemolytic activity, 500 µL of the red blood cells solution was added to each microcentrifuge tube containing10mg/mlof curcumin, TPGS, and synthesized CN. Phosphate buffer saline was taken as a negative control and Triton X-100 (10% v/v) as a positive control. The samples were incubated at 37ºC for 3-4 hours on shaker, following the centrifugation at 2000 RPM for 5minutes and the release hemoglobin content was scanned at 540 nm. Hemolysis (%) was calculated by the given equation:

Statistical analysis

All the experiments were performed and analysed three times. The analyzed data were calculated as mean ± standard deviation (SD) and analyzed by GraphPad Prism 8 software (version 8.0.1 for Windows 2010;GraphPad Software, Inc., San Diego, CA).One-way analysis of variance (ANOVA) was performed by standard procedures. Significant differences between means were determined by Dunnett's multiple comparison tests,and $P< 0.05$ was regarded as significant value.

Results and Discussion

Particle size,polydispersity index (PDI) and zeta potential

Particle size, polydispersity index (PDI), and zeta potential are important parameters that can affect the stability, cellular uptake, and biological activity of curcumin nanoformulation(39–41).Smaller particle size tends to have improved bioavailability and cellular uptake due to their increased surface area and ability to cross membranes(42).The analysis was carried out using dynamic light scattering determined the average particle size of synthesized CN as 6.06 ± 1.77 nm with 91.9% intensity (Fig. 1A). It indicated that the synthesized CN has significantly smaller particle size than other curcumin nanoparticles with particle size of 9 nm(43)and 10 nm(44).Fig. 1 showed the low polydispersity (PDI) value of the synthesized CN 0.219,it was between the 0 (monodisperse sample) and 1 (polydisperse sample) compared to the other curcumin nanoformulations with PDI values of 0.596(45).Such low PDI value of nanoparticles indicated more homogeneous and stable nanoparticle population(46)or lipidic carriers, are being extensively employed to enhance the bioavailability of poorly-soluble drugs. They have the ability to incorporate both lipophilic and hydrophilic molecules and protecting them against degradation in vitro and in vivo. There is a number of physical attributes of lipid-based nanocarriers that determine their safety, stability, efficacy, as well as their in vitro and in vivo behaviour. These include average particle size/diameter and the polydispersity index (PDI.The synthesized CN has zeta potential value of -11.8 ± 3.89 mV (Fig. 1B) which was comparable with the -12 mV zeta potential value of nanocurcumin(47) indicated moderate stability of curcumin nanoparticles.

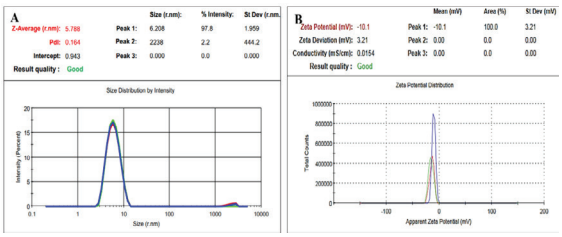

Fig. 1 Particle size and Polydispersity index (PDI) A) Zeta potential B) analysis of synthesized curcumin nanoformulation (CN).

Thin layer chromatography (TLC)

The thin layer chromatography analysis of the synthesized CN was carried out using pure curcumin as a standard to verify whether there had been any alterations in curcumin during its nanoformulation synthesis procedure. Fig. 2 shows the TLC chromatogram of curcumin, D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and synthesized curcumin nanoformulation (CN).The synthesized CN showed two spots, one spot matched with the same Rf value of curcumin (0.91) and second matched with the same Rf value of TPGS (0.98) (Fig. 2). The Rf value of curcumin was also comparable with results found by Fatima et al(48).The sharp spot of curcumin and light spot of TPGS in the lane of synthesized CN showed that curcumin was dominant(49)as compared toTPGS in the synthesized CN. It also suggested that the synthesis process of curcumin nanoformulation did not degrade curcumin and TPGS. Additionally, curcumin rendered its biological activities due to maintained chemical structure without any degradation during the synthesis procedure.

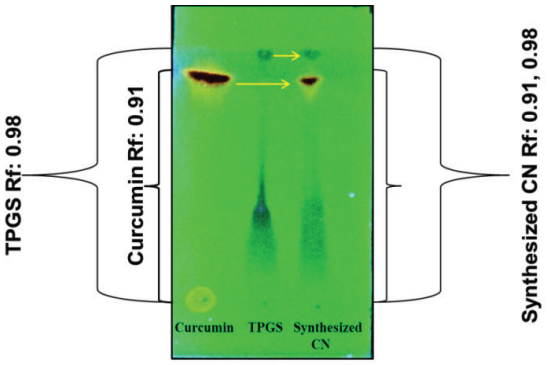

Fig. 2 TLC chromatogram of the curcumin, TPGS, and synthesized curcumin nanoformulation (CN) with Retention factor (Rf) values.

High performance thin layer chromatography (HPTLC)

The high performance thin layer chromatography analysis of the synthesized CN was carried out to confirm the presence of curcumin and TPGS in the synthesized CN which supports the data obtained from TLC analysis. Fig. 3 showed the HPTLC chromatogram of curcumin, TPGS, and synthesized CN indicates that the two peaks obtained in the chromatogram of the synthesized CN (blue) were matched with the peak of curcumin (red) and peak of TPGS (pink). The result confirmed that the presence of curcumin and TPGS does not hindered by the synthesis procedure of curcumin nanoformulation. It also described the synthesis procedure might also preserved its biological properties.

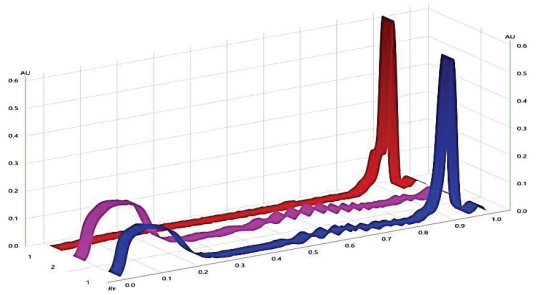

Fig. 3 The peaks of curcumin (red), TPGS (pink), and synthesized curcumin nanoformulation (CN) (blue) showed HPTLC chromatogram.

Antioxidant activity of the synthesized CNby 2,2-diphenyl-1-picrilhydrazyl (DPPH) assay

The DPPH assay is a widely used antioxidant parameter to evaluate the free radicle scavenging efficiency of the antioxidants, and the degree of DPPH inhibition is directly proportional to the antioxidant potential of the tested compounds. In this study, the DPPH inhibition action of the synthesized CN was tested and compared with ascorbic acid, curcumin, and TPGS. Fig. 4showed that the synthesized CN exhibited highest DPPH inhibition of 98% than ascorbic acid (83%) (positive control), curcumin (56%), and TPGS (68%) at 50 µg/ml concentration (Fig. 4). This result could be described by the unique properties of the synthesized CN, such as nanosize, increased solubility that could facilitate their interaction with reactive oxygen species (ROS) and other free radicals by neutralized their damaging effects(50–52).

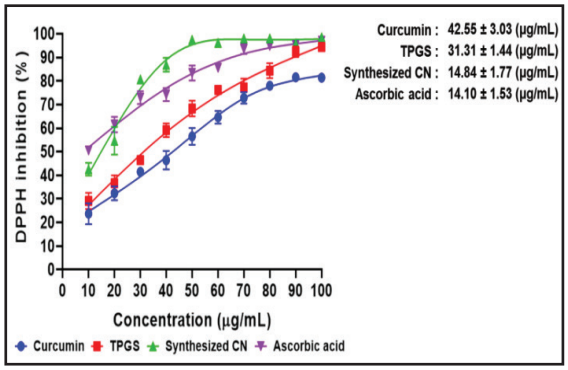

Fig. 4 Evaluation of DPPH inhibition (%) activity of curcumin, TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate), synthesized curcumin nanoformulation (CN), and ascorbic acid with their IC_{50} (µg/ml) ± standard deviation (SD) values.

Antibacterial potential of the synthesized CN

The antimicrobial efficiency of the synthesized CN was examined against *Pseudomonas aeruginosa*, *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* using the agar well diffusion assay. The bacterial growth was inhibited by the antibacterial actions of curcumin, synthesized CN, and TPGS in terms of zone of inhibition (Fig. 5). The zone of inhibition for tested bacterial species was measured and evaluated the antibacterial potential of the synthesized CN (Fig.6). The results showed that the synthesized CN exhibited greater zone of inhibition (21 mm) against Gram-positive cocci *S.aureus*. It could be explained by the action of synthesized CN on bacterial cell membrane disruption by leakage of intracellular contents leading to the cell death(53,54).*P.aeruginosa* and *B.subtilis* had moderate sensitivity towards synthesized CN based on the zone of inhibition of 18 mm and 17 mm respectively. This could be explained by the inhibition of certain enzyme that involved in the bacterial cell survival(54,55). Moreover, the synthesized CN can cause inhibition of bacterial cell proliferation by blocking the assembly dynamics of FtsZ in the Z ring(56,57). The present results also showed that synthesized CN was least effective on Gram-negative rods *E.coli* (16 mm). One possible mechanism for the least effect of synthesized CN against *E.coli* could be related to the structure of cell wall, which contains lipopolysaccharides (LPS) that act as a protective barrier against external agents, including antimicrobial compounds. Additionally, *E.coli* is known to have efficient efflux pumps, which can actively pump out foreign compounds, including antimicrobial agents, from the bacterial cell(58).This may also contribute to the reduced efficacy of the synthesized CN against *E.coli*. The effectiveness of the synthesized CN against the tested bacterial species showed as:*S. aureus* >*P.aeruginosa* > *B. subtilis* >*E. coli*(Fig. 5 and 6).

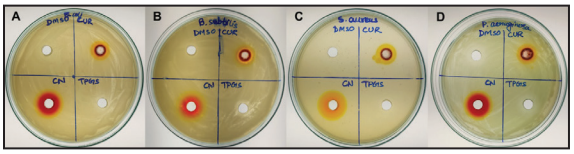

Fig. 5 Antibacterial activity of the DMSO (vehicle control), curcumin, synthesized curcumin nanoformulation (CN), and TPGS against *E. coli* (A) *B. subtilis* (B) *S.aureus* (C) *P. aeruginosa* (D).

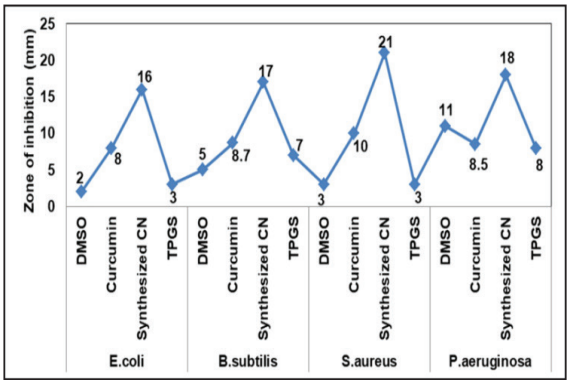

Fig. 6 Zone of inhibition of the DMSO (vehicle control), curcumin, synthesized curcumin nanoformulation (CN), and TPGS.

Brine shrimp toxicity analysis of the synthesized CN

The brine shrimp toxicity assay is affordable and accessible assay for assessing toxicity of nanomaterials that might be applied in various treatments(59–61).The brine shrimp toxicity

assay is widely used simple and inexpensive to check the toxicity profile of the chemicals. The assay could also be used in the preliminary phases of the drug design or for environmental monitoring to assess the effective toxicity of the chemical. The LC_{50} values of potassium dichromate or $K_2Cr_2O_7$ (positive control), curcumin, TPGS, and synthesized CN were shown in the Fig. 7. The LC_{50} values of the tested compounds wereevaluated according to the Meyer and Clarkson toxicity index(38,62). According to this toxicity index, the compounds with less than 1 mg/mlLC_{50} values are considered as toxic, and LC_{50} values higher than 1 mg/ml are considered as non-toxic(38,62,63)utilizing brine shrimp (Artemia salina Leach.Results of the present study showed that the LC_{50}values of $K_2Cr_2O_7$ was 0.363 ± 0.025, curcumin was 4.462 ± 0.660 mg/ml, synthesized CN was 8.076 ± 0.616 mg/ml and TPGS was 8.702 ± 0.272 mg/ml (Fig. 7). Hence, tested compounds pure curcumin, synthesized CN, and TPGS were non-toxic.

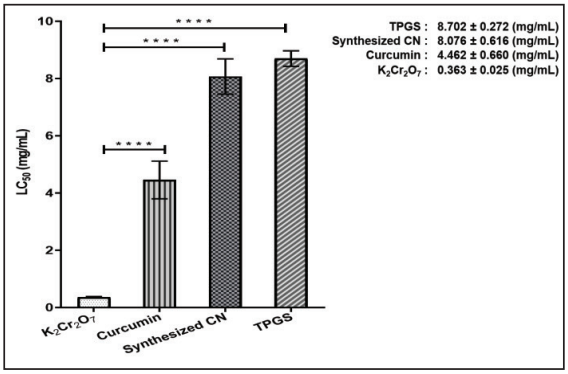

Fig. 7 LC_{50} graph of brine shrimp toxicity analysis. Comparison of $K_2Cr_2O_7$ with curcumin, synthesized CN and, TPGS gives statistical significant *P* values. Where, ****P<0.0001 and n=3. Statistical Analysis was performed using multiple comparisons (one way ANOVA) with Tukey's test.

Hemolytic activity of the synthesized CN

Hemolysis assay is a commonly used test to evaluate the potential toxicity of compounds on red blood cells. The assay examined the released hemoglobin from the lysis of red blood cells. The hemolytic activity of the synthesized CN was shown in the Fig. 8. The results revealed that control (untreated) red blood cells showed 0% hemolysis, phosphate buffer saline (negative control) showed 0.13%, dimethyl sulfoxide (vehicle control) showed 1.79%, curcumin showed 1.20%, TPGS showed 0.56%, and synthesized CN showed only 0.23% of hemolysis. While, triton X-100 (positive control) showed 97.97%of hemolysis.The % hemolysis of alone curcumin and alone TPGS were slightly more than the synthesized CN which was synthesized from curcumin and TPGS. Moreover, the % hemolysis of tested compounds was negligible when compared to triton X-100. According to the regulatory guidelines provided by ISO-10993-4(64), if the compound causes less than 2% hemolysis, it is considered as non-hemolytic compound and safe for the *in vivo* application. It indicatedthat the synthesized CN could be considered as non-hemolytic and safe for the further *in vivo* applications.

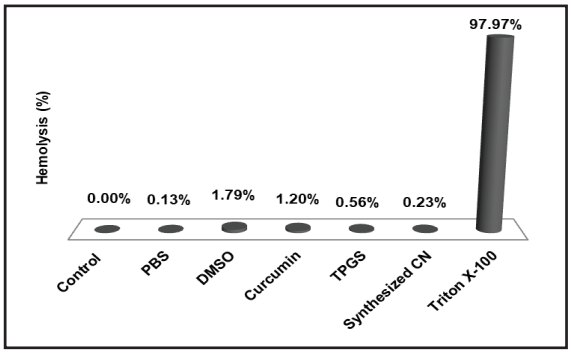

Fig. 8 Hemolytic activity of the various test compounds.

Conclusion

The study results showed that the curcumin nanoformulation (CN) was successfully synthesized and characterized using parameters like particle size, PDI value and zeta potential. The TLC and HPTLC analysis of synthesized CN confirmed that curcumin molecules did not degrade during the synthesis process and hence retained the biological properties of

curcumin as well. The synthesized CN showed significant antioxidant activity, greater than the pure curcumin, TPGS, and ascorbic acid which indicated that reduction in the particle size might improve the antioxidant potential. Moreover, the synthesized CN exerted high antibacterial efficacy against Gram-positive cocci *S. aureus.* Thus, enhanced biological activitiesof the synthesized CN were explained by the conversion of curcumin into its nanoformulation with unique qualitiessuch as size and increased biological potential. Furthermore, the study highlights the safety profile based on the results obtained from the brine shrimp toxicity analysis and hemolytic analysis ensured that the synthesized CN could be potential candidate for the therapeutic treatment and prevention of the bacterial infections and oxidative stress-related diseases. The great antibacterial and antioxidant potencies of the synthesized CN with no *in vitro* toxicity made them an attractive alternative to conventional curcumin formulations. In addition, subsequent to a comprehensive biological potential and safety analysis of the synthesized CN, it is referred as a suitable for the therapeutic interventions.

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