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Development and characterization of curcumin- and 6-gingerol-based nanoemulsion for brain targeting: in vivo, ex vivo and in vitro cell line studies

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Abstract

Background Today brain tumors are the most life-threatening diseases which universally have a high death rate and reported as a second leading cause of death in not only adults but also children. Several medicinal approaches have been utilized to treat tumors, but the recommended methods had some limitations. Numerous phytoconstituents isolated from various medicinal plants have been reported to have anticancer property, but there are some major reasons for the clinical failure of these compounds such as its poor solubility, bioavailability and tissue distribution and poor absorptivity in the body. Thus, there is a strong need to design the best treatment to cancer cells with great efficiency, less side effects and with promising approach for the delivery strategies. Nanotechnology is proved to be a bridge to overcome the limitation of conventional therapies of drug administration and distribution in the cancer treatment. In the present study, curcumin- and 6-gingerol-based nanoemulsion (CGNE) has been successfully develop and characterized.

Result The FTIR spectroscopy of CGNE exhibits typical spectra of both curcumin and 6-gingerol. The pH of CGNE was found to be acidic, and the refractive index value of formulated CGNE was found to be 1.38, indicating the isotropic nature of drugs. Percentage transmittance of nanoemulsion was close to 100%. Zeta potential of optimized nanoemulsion was found to be −29 mV. During ex-vivo drug diffusion studies, 48.4% and 78% diffusion of curcumin and 6-gingerol, respectively, were observed from CGNE at the end of 6 h, and followed the anomalous release mechanism.

Conclusion The drug release mechanism of the formulated CGNE was calculated by using the Korsmeyer–Peppas equation, and the diffusion exponent '*n*' for curcumin and 6-gingerol indicated the anomalous release mechanism. In vitro cytotoxicity against the human glioblastoma cell line U373-MG, study demonstrated that the combination of curcumin- and 6-gingerol-based nanoemulsion showed the synergistic result of cell growth inhibition.

Keywords Curcumin, 6-Gingerol, Nanoemulsion, Ex vivo diffusion study, In vitro cytotoxicity, Biodistribution study

Introduction

Among the various human diseases, today worldwide cancer is one of the most leading causes of death. As per the recent report of cancer research, UK, 9.6 million patients died in 2018 out of 17 million newly detected cancer patients. If this remains up to 2040, it is estimated that there will be new 27.5 million cases of cancer patients reported per year [1]. Brain tumors are the

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most life-threatening diseases which universally have a high death rate and reported as a second leading cause of death in not only adults but also children. Several medicinal approaches have been utilized to treat tumors such as surgical elimination, chemotherapy and radiotherapy and combination treatment of any one of the recommended methods. The recommended methods had some limitations such as nonselectivity, multitrack resistance (chemotherapy), general toxicity to normal cells and nonselective drug delivery. Treatment of brain tumors is the most difficult challenge as the major obstacle in the treatment is antitumor drugs not able to cross the blood–brain barrier (BBB) in therapeutic quantities. In addition to these, some conventional drugs also possess numerous disadvantages like lack of specificity to tumor, lethal side effects in healthy tissues, etc.

Numerous phytoconstituents isolated from various medicinal plants have been reported to have anticancer property and induce apoptosis, arresting the cancer cell cycle by inhibiting the cell proliferation, by delayed metastasis and angiogenesis. Additionally, these phytoconstituents are advantageous over synthetic drugs such as low toxicity to normal cells, biocompatibility, low side effects and easy availability at reasonable price. Besides these advantages, there are some major reasons for the clinical failure of these compounds such as its poor solubility, bioavailability, and tissue distribution and poor absorptivity in the body. Thus, there is a strong need to design the best treatment to cancer cells with great efficiency, less side effects and with promising approach for the delivery strategies. Nanotechnology is proved to be a bridge to overcome the limitation of conventional therapies of drug administration and distribution in the cancer treatment. Nano-based drug delivery systems are particularly used for delivering natural phytoconstituents due to its several advantages such as enhanced bioavailability and drug stability, sustained release of the drug, and improved drug distribution, and it also helps for the delivery of two or more drugs for combinational therapy as compared with the nonencapsulated or single drugs [2–4].

Curcumin is a polyphenolic phytoconstituent obtained from the roots and rhizomes of *Curcuma longa* Linn., and 6-gingerol is another phenolic phytoconstituent obtained from the roots and rhizomes of *Zingiber officinale*. Both belong to the family Zingiberaceae. Many researchers reported both in vitro and in vivo studies for these phytoconstituents individually and showed to be potent antioxidant, anti-inflammatory and as an anticancer agent. Previously various studies reported that the combinational effects of many phytoconstituents showed the synergistic action. Curcumin is often address as wonder molecule due to its numerous potential benefits [5]. However, its therapeutic efficacy can be hindered by several challenges including poor aqueous solubility, weakness to chemical instability in alkaline environment, rapid metabolism and limited absorption from the gastrointestinal tract. Nanoparticulate systems, such as nanocarriers or liposomes, can serve as effective vehicles to deliver curcumin, optimizing its therapeutic potential and addressing challenges related to its absorption and targeting in the body. It is an innovative and well-suited solution to enhance the efficacy of curcumin in various application. The most bioactive compound present in fresh ginger is 6-gingerol [6]. The potential side effects associated with higher doses, such as heartburn, abdominal discomfort or diarrhea, highlight the importance of moderation in consumption. Additionally, the observation that ginger may have antiplatelet effects potentially increases risk of bleeding in some individual. 6-Gingerol has an optimal lipophilicity and possesses good blood–brain barrier permeation. The present study aims to develop a nanoemulsion of curcumin and 6-gingerol synergistic combination to deliver through nose as a noninvasive way for the brain tumor treatment.

Materials and methods

Materials

Curcumin and 6-gingerol were obtained as a gift from Sunpure extracts, Private Ltd., Delhi, India (Batch No. CE/801349). Tween® 80 was purchased from Loba Chem. Pvt. Ltd. (Mumbai, India), whereas acetonitrile, methanol and all other solvents and reagents used were of analytical grade.

Methods

Selection of oil, surfactant and co-surfactants

For the formulation of curcumin- and 6-gingerol-based nanoemulsion (CGNE), suitable liquid was selected by testing of a series of natural fixed and volatile oils such as castor oil, sesame oil, peanuts oil, clove oil and eucalyptus oil for both the polyphenols curcumin and 6-gingerol individually. Solubility in each oil sample was tested by visual observation by keeping the test tubes at ambient temperature 37 °C ± 0.5 °C after 24 h. Pluronic F68, Tween 80 and lecithin, glycerol, phosphatidylcholine and sodium taurocholate were tested, and Tween 80 and glycerol were selected as surfactant and co-surfactants, respectively [7, 8].

Formulation of Curcumin- and 6-gingerol-based nanoemulsion

The o/w type of curcumin- and 6-gingerol-based nanoemulsion was formulated with the surfactant (Tween 80) and co-surfactant (glycerol). The surfactant Tween 80 was mixed with both the drugs curcumin and 6-gingerol

(200 mg each) and stirs it for 15 min. with clove oil and prepared oil phase then sonicated for 15 min for proper solubility of drug with Tween 80. Aqueous phase was prepared by mixing glycerol into the water. The prepared oil phase was added drop wise into the aqueous phase by using syringe needle to avoid instability. The prepared nanoemulsion is further transferred for size reduction and uniformity in a high-speed homogenizer (10,000 RPM for 1 h.) (Remi Instruments Ltd., India). After the stability study of prepared nanoemulsion, polymeric solution (2%) was prepared in water, and it was added in to the prepared nanoemulsion drop wise and resultant was ultrasonicated for 15 min [9–11]. Finally CGNE-based prepared nanoemulsion sealed in suitable glass vials with rubber caps.

Physicochemical characterization of curcumin and 6-gingerol

Both the obtained curcumin and 6-gingerol individually and curcumin- and 6-gingerol-based nanoemulsion were characterized by performing FTIR study to identify the functional groups present in it [12, 13].

Fourier transform infrared spectroscopy (FTIR)

Curcumin, 6-gingerol and curcumin- and 6-gingerol-based nanoemulsion were analyzed by KBR pellet method in the wave number range of 4000–400 cm^{-1} to identify the functional groups. For the measurements, prepared pellet was loaded on holder of Fourier transform infrared (FTIR-4800, Shimadzu, Japan).

Physicochemical characterization of curcumin- and 6-gingerol-based nanoemulsion

General appearance, pH, conductivity and refractive index

The formulated CGNE where analyzed by different physicochemical parameters like general appearance, pH, conductivity and refractive index as per the procedures reported in official compendia. All the determinations were done in triplicate [14, 15].

Percentage transmittance and viscosity

Percentage transmittance of nanoemulsion was determined according to the method reported previously by using UV–visible spectrophotometer to determine the clarity of formulated nanoemulsion. The viscosity of the CGNE was measured by using Brookfield Rheometer (Brookfield RST Rheometer). Appropriate quantity of CGNE was transferred to the beaker and adjust sample to the required measurement temperature. Spindle was inserted into the beaker Start the viscometer and measured the reading [16].

Mean particle size, polydispersity index and zeta potential

Physicochemical characterization of the curcumin- and 6-gingerol-based nanoemulsion (CGNE) in terms of mean particle size and polydispersity index was determined by photon correlation spectroscopy, and zeta potential was determined by using Zetasizer Nano ZS 90 (Malvern, Malvern ltd., UK). To avoid multiscattering phenomena and to produce an appropriate scattering intensity before measurement, all samples were diluted hundred times with double-distilled water. All measurements for each sample were taken in triplicate at 25 °C [17].

Differential scanning calorimetry (DSC)

Thermal behavior of curcumin, 6-gingerol and CGNE were analyzed by using a DSC (Mettler-Toledo, Switzerland). Approximately 2 mg sample was sealed in aluminum pan and was heated at temperature range of 40 °C to 400 °C with a heating rate of 10 °C/min in DSC.

Cryo-SEM (cryo-scanning electron microscopy)

The surface microstructure of the prepared CGNE nanoemulsion was analyzed by using Cryo-SEM (JSM-7600F). For Cryo-SEM study, the CGNE nanoemulsion sample was placed on the carbon conductive adhesive tape and the mounted sample was frozen (−180 to −190 °C) in liquid nitrogen. The frozen sample was then covered with platinum particles by sputtering and allowing the surface visualization with 5 kV accelerating voltage [18, 19]

Ex vivo diffusion study

Ex vivo permeation study for the formulated CGNE was carried out on the fresh nasal tissues of goat nasal cavity obtained from the local slaughterhouse (Institutional Animal Ethical Committee Registration No. 651/PO/ReBi/S/02/CPCSEA, India). The adhered fat and mucus-free nasal tissues were mounted between the donor and receptor compartments of Franz diffusion cells having the diameter of $1cm^2$. The tissue was stabilized in phosphate-buffered saline (pH 6.4). After 20 min, the prepared CGNE nanoemulsion (50 ml) was added into the donor compartment. Simulated nasal fluid of pH 6.4 with 1% sodium lauryl sulfate was added to the receptor compartment, and the temperature of 37 °C was maintained for the whole study. At predetermined time points, 3 ml of the samples was withdrawn from the receptor compartment by replacing the sampled volume with SNF of pH 6.4 after each sampling, filtered and analyzed for drug release using a

UV–visible spectrophotometer at 282 nm and 423 nm (Shimadzu, UV-1700, Japan) [20–22].

Histopathological study

Histopathological studies were carried out by using isolated sheep nasal mucosa. Freshly isolated sheep nasal mucosa was sectioned in four pieces. The first piece was treated with PBS (6.4) solution which is termed as negative control mucosa, the second piece was treated with isopropyl alcohol which is termed as positive control mucosa, the third piece was treated with nanoemulsion, and the fourth piece was treated with water which is termed as normal control mucosa. This mucosal membrane was subjected to 5–6 h of treatment using the appropriate formulation and solution. After completing the time interval, the mucosa membrane were sectioned into thin slide and were stained with hematoxylin and eosin. The mucosa was then dissected out, and the mucocillia was examined on an optical microscope by a pathologist blinded to the study [23].

Biodistribution study

Six Wistar rats (Avg. weight 250–270 g) of either sex, bred in the animal house of RCPIPER, Shirpur, were used for the biodistribution studies. The animal ethical committees laboratory care (CPSEA) was strictly followed, and they were fed with standard laboratory diet and ordinary tap water and kept in clean cages in a twelve-hour light and dark cycle. Biodistribution study was approved and performed in accordance with the guidelines by Institutional Animal Ethical Committee (Registration No. 651/PO/ReBi/S/02/CPCSEA, India). The rats were anesthetized with the anesthetic ether and manually fixed in supine position. The prepared nanoemulsion were instilled into individual rats by nasal route of administration with LPDE tubing of 250 µl which is equivalent to 0.155 mg [24, 25].

Brain removal from Rat.

To remove the brain from rats, firstly they were anesthetized with anesthetic ether; then, they were killed humanely, and brain was carefully excised by cutting the skull. The separated brain was immediately rinsed with saline solution to remove the blood taint and blotted up with a filter paper. Finally, the brain is homogenized with one volume of saline in a tissue homogenizer to get the separated brain tissues. The obtained homogenate was analyzed by HPLC [26].

HPLC analysis

Sample preparation To 250 µl of obtained brain homogenate, 250 µl of acetonitrile as extraction solvent was added and mixed thoroughly for 10 min. The resultant was centrifuged at 3000–3500 rpm for 10 min in an ultracentrifuge. Finally, the supernatant layer was collected and 20 µl was injected in HPLC system [27, 28].

Chromatographic conditions The chromatographic separation was performed at ambient temperature with reversed-phase chromatographic method. The chromatographic conditions are mentioned in Table 1.

Data analysis The Kinetica 5.0® (Thermo Fisher Scientific Inc., US) software was used to calculate the pharmacokinetic parameters of formulated CGNE nanoemulsion. The software were used for Cmax and Tmax estimation obtained from the drug concentration vs time plot. The trapezoidal method was used to calculate the area under the concentration–time curve (AUC 0 to *t*).

After the nasal dosing, the drug targeting efficiency which is the drug that reaches the brain via olfactory pathway was calculated as per the previously reported methods of Mahajan et al. [29], by using equations (i) and (ii),

(i) Drug Targeting Efficiency (DTE)

$$\mathrm{DTE\%} = \frac{(\mathrm{AUC_{Brain}}/\mathrm{AUC_{Blood}})_{\mathrm{i.n}} \times 100}{(\mathrm{AUC_{Brain}}/\mathrm{AUC_{Blood}})_{\mathrm{i.v}}}$$

where ($\mathrm{AUC_{brain}}$ /$\mathrm{AUC_{blood}}$) i.n is the ratio of area under curve for CGNE nanoemulsion concentration in brain and blood after intranasal administration.

(ii) Direct Transport Percentage (DTP)

$$\%\mathrm{DTP} = \frac{(B_{\mathrm{i.n}} - B_X) \times 100}{B_{\mathrm{i.n}}}$$

where $B_{x¼}$ $(B_{\mathrm{i.v}}/P_{\mathrm{i.v}}.) \times P_{\mathrm{i.n}}$.

In vitro cytotoxicity (sulforhodamine B, SRB) colorimetric assay

Cytotoxicity assay of prepared nanoemulsion was carried out using human glioblastoma cell line U373-MG. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. Cells were inoculated into 96-well microtiter plates; after

Table 1 Chromatographic conditions

Particulars	Chromatographic condition
Column	15 cm column
Stationary phase	Quails BDS C18, 250 mm × 4.6 mm I.D
Mobile phase	Acetonitrile–methanol–water (50:40:10 v/v)
Data processor	EZ chrome elite chromatographic data system
Detector	Photo diode array detector
Detection wavelength	423 and 280 nm
Sample size	20 µl

cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to the addition of experimental drugs. 1 mg/ml concentration of the CGNE was prepared and stored frozen prior to use. Aliquots of 10 µl of these different phytochemical dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations, i.e., 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. In vitro cytotoxicity SRB colorimetric assay was carried according to the process reported by Van Meir, et al. [30–34].

Results

Curcumin- and 6-gingerol-based nanoemulsion was formulated by using Tween 80 as surfactant and glycerol as co-surfactant. Pre-formulation study was done on the curcumin and 6-gingerol. Independent and response variables like particle size, phase separation, polymer, surfactant and solvent concentration are applied to the formulated batches (Design Expert software version 7.0, Table 2), and a stable batch with appropriate particle size was selected for the further study. In this, the best models such as quadratic model can be selected based on the analysis of variance (ANOVA). p-value < 0.05 was considered to be statistically significant. The selected nanoemulsion batch was evaluated for organoleptic, morphological and physicochemical characteristics like particle size, infrared spectroscopy, in vivo and ex vivo study, etc.

Physicochemical characterization of curcumin and 6-gingerol

FTIR

To confirm and identify the functional groups present in the curcumin and 6-gingerol, FTIR spectroscopy was done. Figure 1a shows the FTIR spectrum of curcumin exhibited band at 3507.67 cm^{-1} (stretching vibrations) mainly attributed to phenolic hydroxyl groups.

Table 2 Formulation and characterization of nanoemulsion by design expert Software version 7.0

Batches	Factor 1 (ml) Tween 80	Factor 2 (ml) Clove oil	Factor 3 (ml) Glycerol	Factor 4 (ml) Water	Response 1 Particle size	Response 2 Centrifuge (5000 rpm)
F1	5	5	30	50	256 nm	Phase Separation
F2	10	5	30	25	65 nm	Phase Separation
F3	**5**	**3**	**15**	**27**	**202 nm**	**No Phase Separation**
F4	10	10	30	25	325 nm	Phase Separation
F5	5	10	15	50	456 nm	Phase Separation
F6	**5**	**3**	**17**	**25**	**313 nm**	**No Phase Separation**
F7	10	10	15	50	96 nm	Phase Separation
F8	5	5	30	25	147 nm	Phase Separation
F9	10	5	30	50	78 nm	Phase Separation
F10	5	5	15	25	231 nm	Phase Separation
F11	5	5	15	50	145 nm	Phase Separation
F12	5	10	30	50	189 nm	Phase Separation
F13	10	10	15	25	265 nm	Phase Separation
F14	10	5	15	25	300 nm	Phase Separation
F15	10	5	15	50	190 nm	Phase Separation
F16	10	10	30	50	298 nm	Phase Separation

F3 & F6 -Optimized stable batches with no phase separation

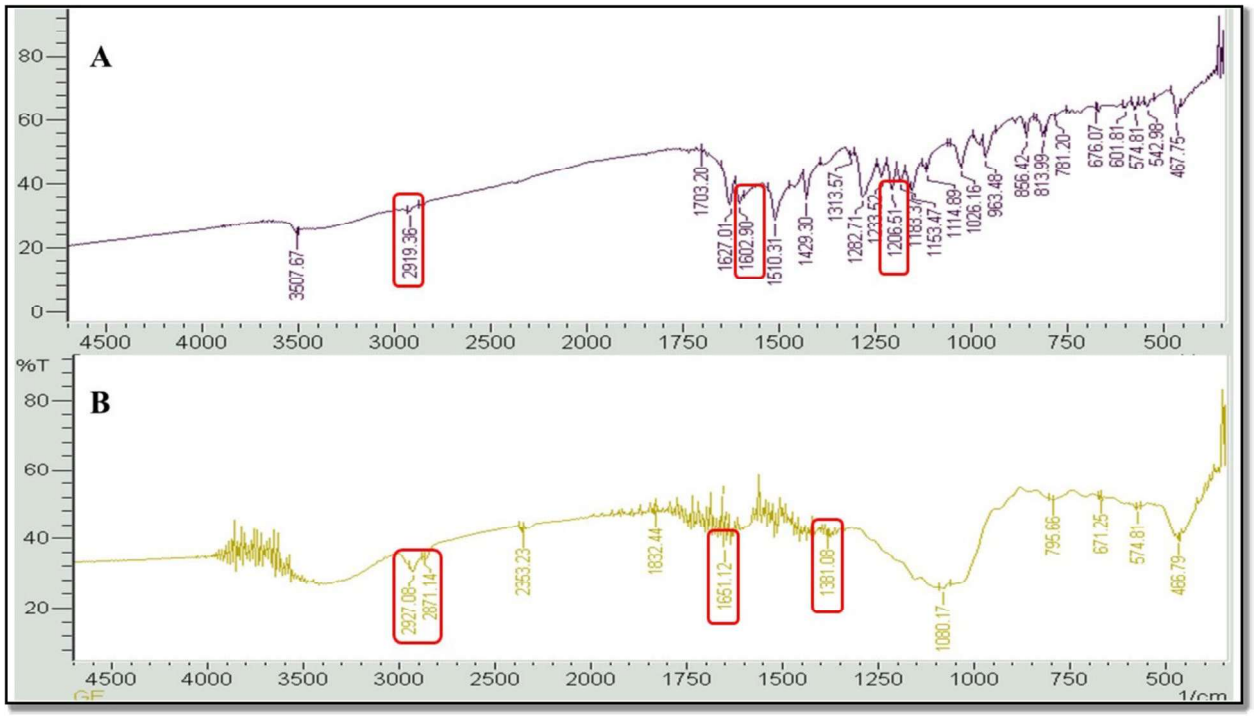

Fig. 1 **a** FTIR spectrum of curcumin and **b** the FTIR spectrum of 6-gingerol

The peak appeared at 2919.36 cm^{-1} is of the asymmetric valence vibrations of the methyl group, and the peak at 1602.90 cm^{-1} is due to ketone and carbonyl C=O stretching of curcumin. Bands at 1510 and 1233 were mainly associated with the C=C bond and the phenolic C–O group, respectively. Figure 1b shows the FTIR spectrum of 6-gingerol exhibited band at 2927 and 2871 cm^{-1} attributed to CH_2 stretching and band at 1651.12 and 1381.08 cm^{-1} mainly attributed to C=O (stretch) and C–H, respectively. A characteristic band at 1080.17 corresponds to the stretching of –CHOH of 6-gingerol [35, 36].

Physicochemical characterization of nanoemulsion From all formulated nanoemulsion batches, batch no. F3 was found to be very stable as neither any phase separation, creaming and film forming nor any cracking effect was noticed/ recorded in this batch. So, batch no. F3 was subjected to the further analysis.

General appearance, pH, conductivity and refractive index The formulated CGNE nanoemulsion appeared as bright yellow-colored emulsion with sweet odor and aromatic to spicy taste. The pH of nanoemulsion was acidic in nature and appeared as 5.73 ± 0.89. To assess the type of formulated nanoemulsion either as w/o or o/w, its conductivity was measured. The conductivity of optimized nanoemulsion was found to be 61.41 ± 1.8 µs/cm, indicating the oil-in-water-type nanoemulsion formulation. The refractive index values of nanoemulsion and placebo nanoemulsion were found to be 1.38 ± 0.04 and 1.37 ± 0.98, respectively.

Percentage transmittance and viscosity Percentage transmittance of the optimized nanoemulsion was found to be 97.08 ± 0.6% which is close to 100%. Viscosity of the optimized nanoemulsion was found to be 48 ± 1.92 cP. The nanoemulsion viscosity is an important criterion, and low viscosity value ensures easy of packing and handling [16].

Mean particle size, polydispersity index and zeta potential To determine the mean particle size and polydispersity index, it is important to forecast physical stability and in vivo drug release (Fig. 2a). The mean particle size and polydispersity index of optimized nanoemulsion were found to be 202.1 nm and 0.327, respectively. Zeta potential value is the indicator of stability of formulated nanoemulsions. Zeta potential of optimized nanoemulsion was found to be −29 mV (Fig. 2b), indicating that dispersion will have greater long-term stability.

Differential scanning calorimetry (DSC) The thermal properties of curcumin, 6-gingerol and CGNE were studied by DSC (Fig. 3). The enthalpy changes in

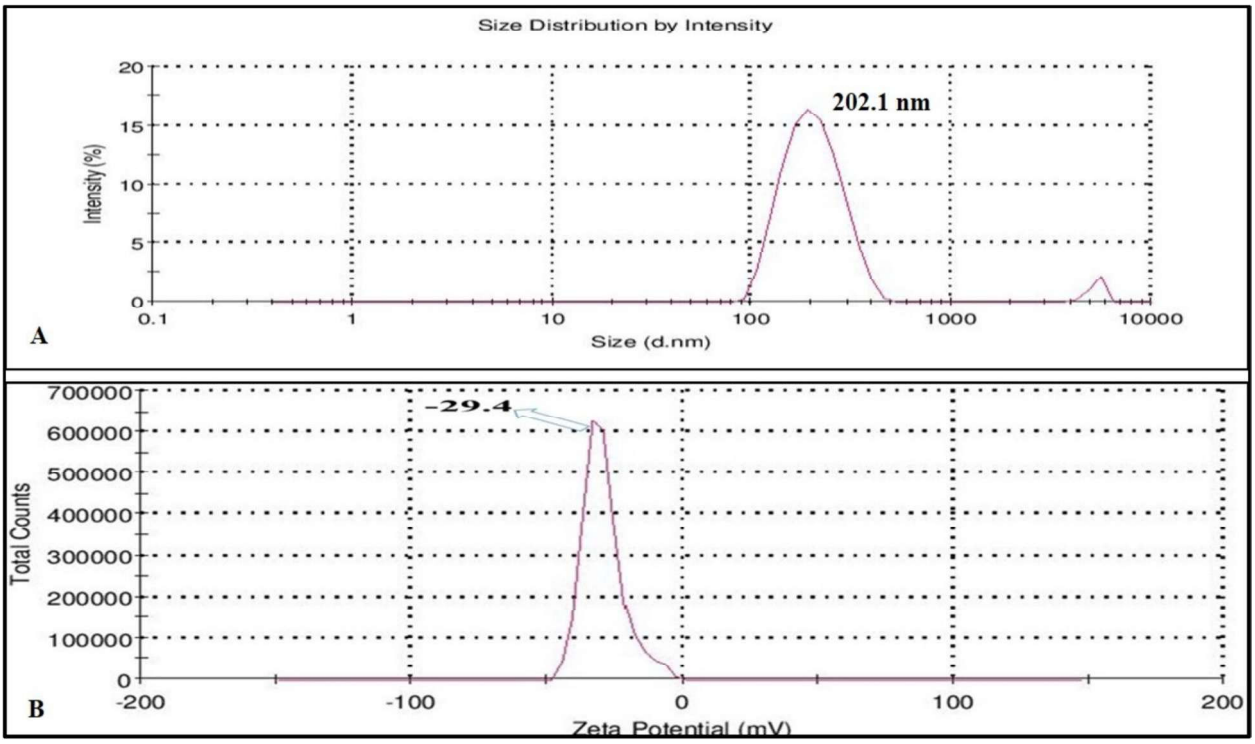

Fig. 2 **a** Mean particle size and **b** zeta potential of optimized nanoemulsion

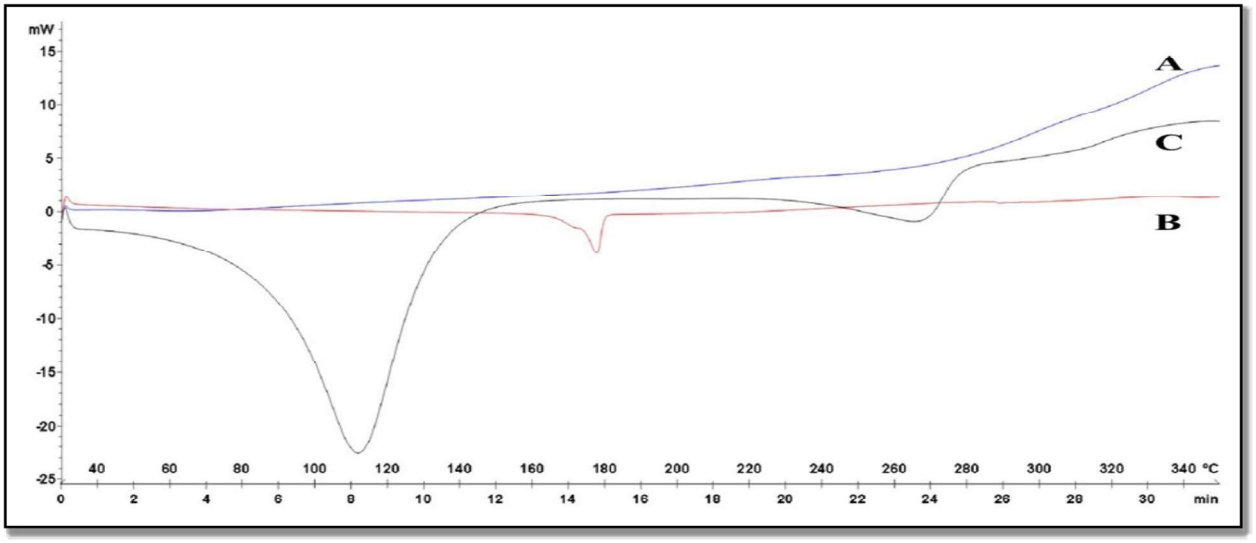

Fig. 3 DSC thermogram with broad endothermic peak: **a** curcumin, **b** 6-gingerol, **c** curcumin- and 6-gingerol-based nanoemulsion

the nanoemulsion as a function of temperature were measured by DSC. The thermogram of curcumin and 6-gingerol (Fig. 3a, b) shows broad endothermic peak at 173.07 and 189.53 °C, respectively, with 160 and 179 °C onset and 184 and 190 °C endset, respectively [37]. Figure 3c shows DSC thermogram of nanoemulsion showing broad endothermic peak at 111.10 °C with onset at 87.11 °C and endset at 131.91 °C. This indicates the changes appeared in the thermal behavior of curcumin and 6-gingerol suggest that both the drugs curcumin and 6-gingerol were entirely encapsulated and molecularly dispersed.

Fourier Transform Infrared spectroscopy (FTIR) FTIR spectrum of CGNE nanoemulsion was recorded and compared with individual curcumin and 6-gingerol spectrum, as shown in Fig. 4. The prepared nanoemulsion spectrum exhibited some similar peak and some shifting of peak pattern which confirms the presence of both curcumin and 6-gingerol in the prepared nanoemulsion. The peak at 3600–3000 cm^{-1} attributed to the hydroxyl groups of both curcumin and 6-gingerol. The peak appeared at 2932 cm^{-1} is of the asymmetric valence vibrations of the methyl group, and the peaks at 1624.12 and 1247 cm^{-1} are due to ketone and carbonyl C=O stretching and the phenolic C–O group of curcumin. The peaks appeared at 1624.12, 1419.66 and 1247 cm^{-1} mainly attributed to C=O (stretch), C=C (stretch) and C–H, respectively. A characteristic band at 1108.14 cm^{-1} corresponds to the stretching of -CHOH of 6-gingerol [35].

Cryo-SEM (cryo-scanning electron microscopy) Cryo-SEM image of CG nanoemulsion was found to be in spherical shaped with 200 nm size (Fig. 5d). Similar type of results of size analysis was obtained by Photon correlation spectroscopy [18, 19].

Ex vivo drug diffusion study The percent drugs release of curcumin- and 6-gingerol-based nanoemulsion was studied by ex vivo diffusion method through the dialysis membrane in simulated nasal fluid at pH 6.4 (Fig. 5a, b). At predetermined time interval, samples were withdrawn from the receptor compartment, filtered and analyzed for drug release by using a UV–visible spectrophotometer at 282 nm and 423 nm.

At the end of the 6 h, percent drug release of curcumin and 6-gingerol was observed to be 48.4% and 78%, respectively (Fig. 5c). The prepared CGNE nanoemulsion showed the biphasic release pattern as in the case of 6-gingerol initially burst release and followed by sustained release for the next few hours. This type of release may be observed due to low melting point of the 6-gingerol.

Histopathological study

Histological studies showed isopropyl alcohol (ISA)-treated positive control mucosa (Fig. 6a), phosphate-buffered saline (pH 6.4)-treated negative control mucosa (Fig. 6b), water-treated normal control mucosa (Fig. 6c) and CGNE-treated mucosa (Fig. 6d). The histopathology examination demonstrated no difference in the mucosal structure or occurrence of any mark/lesion with CGNE-treated mucosa compared to the positive control group.

Biodistribution study by HPLC analysis

The profiles of the levels of CGNE in the brain showed that the maximum concentration was reached around

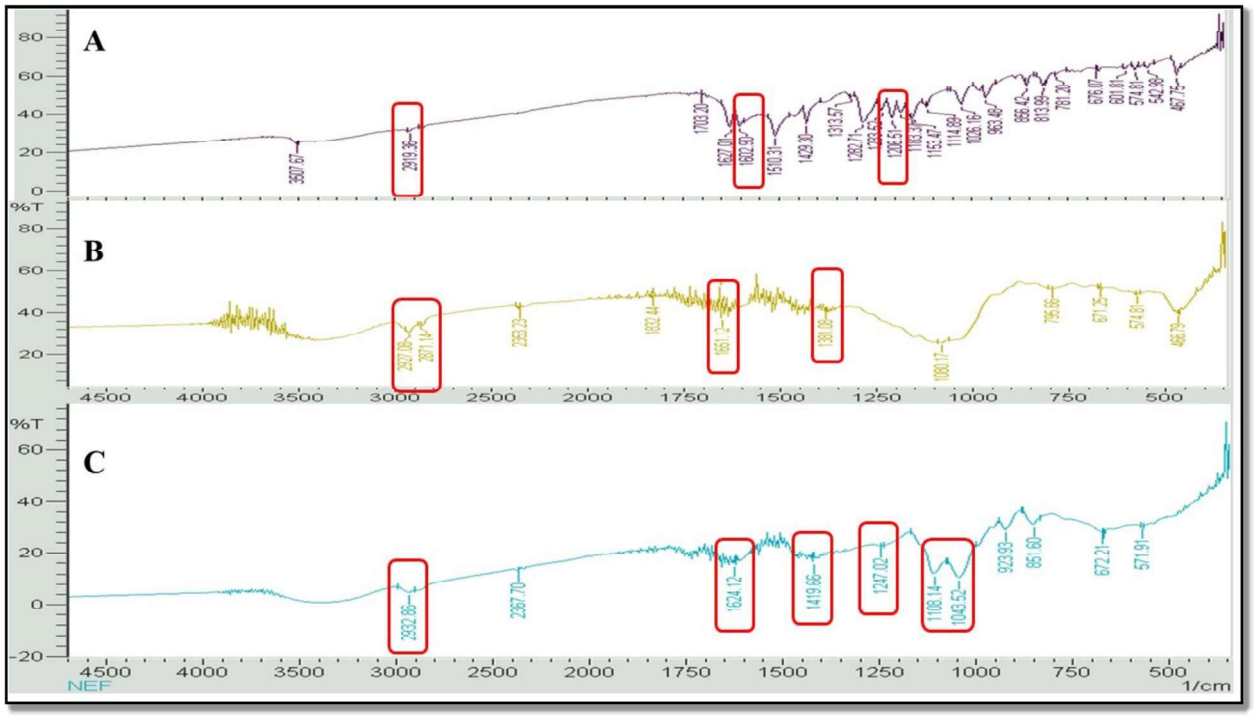

Fig. 4 a FTIR spectrum of curcumin, **b** FTIR spectrum of 6-gingerol and **c** FTIR spectrum of curcumin- and 6-gingerol-based nanoemulsion

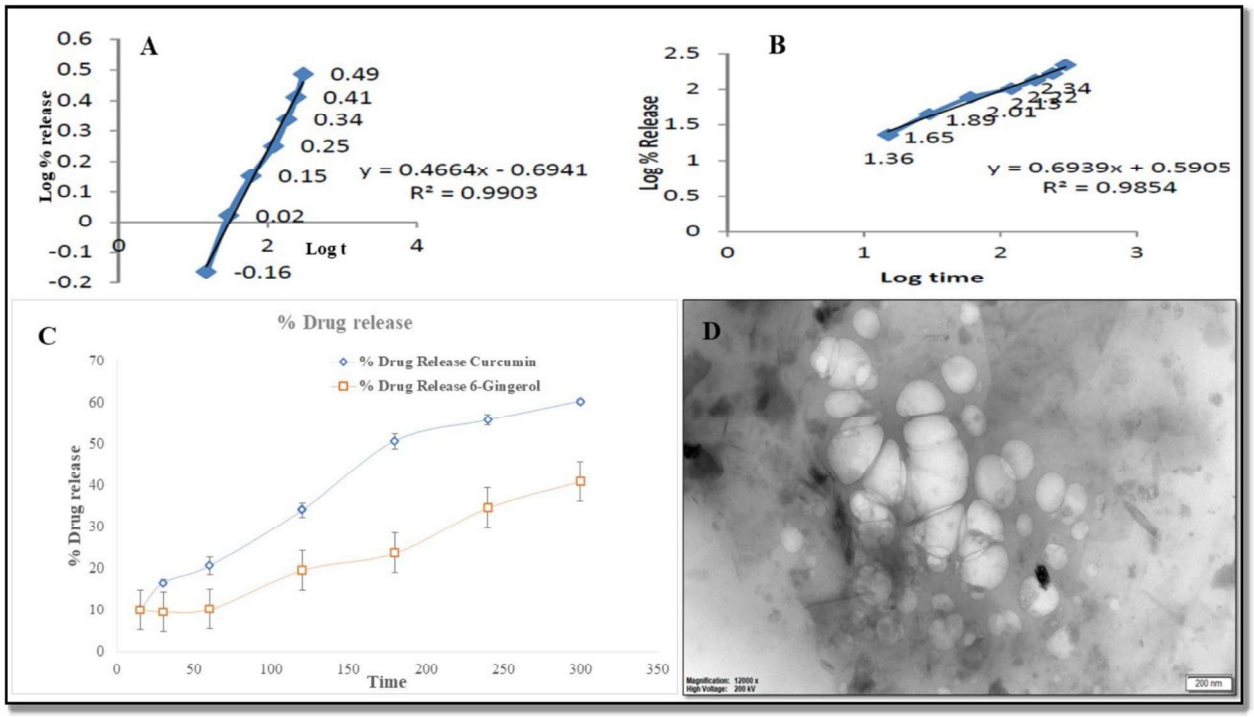

Fig. 5 **a** Korsmeyer–Peppas plot demonstrating mechanism of drug release—curcumin, **b** Korsmeyer–Peppas plot demonstrating mechanism of drug release—6-gingerol, **c** percent drug release of curcumin- and 6-gingerol-based nanoemulsion and **d** Cryo-SEM of CGNE

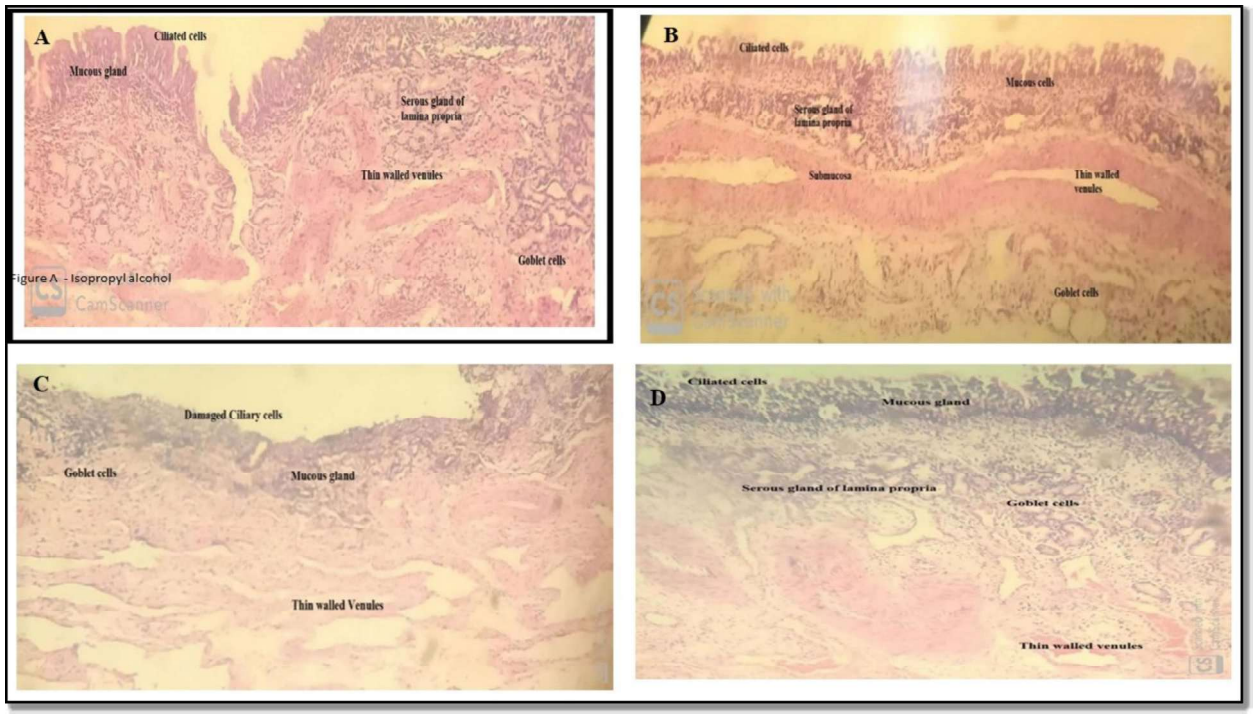

Fig. 6 Histopathological study. **a** Nasal mucosa with ISA acting as positive control, **b** nasal mucosa with figure as ISA acting as positive control, **c** normal control/blank control and **d** CGNE-treated mucosa

180 min after the administration of the CGNE and an initial absorption phase biodistribution study was performed in Wistar rat to investigate the crossing of CGNE across BBB. As brain is covered outside by protective BBB, this complex arrangement made treatment complicated as it restricts the entry of many compounds together. Our study focuses mainly on BBB crossing. Numerous research projects aim to explore the bioavailability of anticancer medications throughout the central nervous system. Our main goal is to determine CGNE bioavailability across the blood–brain barrier. The chronological profile of curcumin- and 6-gingerol-based nanoemulsion concentration in brain and plasma was shown to be higher following intranasal (IN) administration of drug-loaded NE as compared to intravenous (IV) injection of pure drug suspension (PDS), according to the results of biodistribution experiments (Fig. 7b). Our study's initial discovery was that intranasal delivery of a curcumin- and 6-gingerol-based nanoemulsion permitted CNS absorption. The profiles of the levels of CGNE in the brain showed that the maximum concentration was reached around 180 min after the infusion of the CGNE and an initial absorption phase. The drug concentration in the brain was shown to be higher for IN-delivered of after the first 30 min CGNE [11256.33 ± 5.68 ng/g] than PDS [3063 ± 321 ng/g]. As time progressed, the concentration increased, and thus, after 90 min, IN-delivered CGNE showed higher accumulation [660123 ± 7.21 ng/g] of drug in the brain compared with PDS [5694 ± 1224 ng/g]. The highest concentration was observed in the brain after IN administration of CGNE. The C_{max} was found [1013900 ± 8.71 ng/g] at t_{max} of 120 min, whereas for PDS, the C_{max} was [6686.33 ± 136 ng/g] at t_{max} of 180 min, after IN administration. The bioavailability of CGNE administered by nasal route was found to be 46.62% for the doses studied, based on the AUC data measured over a 0–180 min period. This would have been made possible by the CGNE quick absorption and extended stay in the rat nasal cavity, which allowed for intranasal transport to the brain [29]. The nasal route involves the medication entering the brain through the upper nasal cavity's trigeminal and olfactory nerves. Alongside this finding are also showing drug targeting efficiency 1.069 ± 0.012%, direct transport percentage 99.76 ± 0.03%, total body clearance 9.60 ± 0.01 mL/min/kg, t_{max} 0.02284 ± 0.01 h, terminal elimination half-life ($t_{1/2}$) 1.03 ± 0.06 h and apparent volume of distribution [2.20 ± 0.05 mL/kg] [19, 29].

In vitro cytotoxicity [Sulforhodamine B, (SRB)] colorimetric assay Various concentration (10–80 µg/ml) of curcumin and 6-gingerol combination-based nanoemulsion was analyzed by using human glioblastoma cell line U373-MG. The GI 50% (growth inhibitory 50) value of the curcumin and 6-gingerol combination-based nanoemulsion (< 10 µg/mL) indicates higher cytotoxicity and less cellular viability (Fig. 7a).

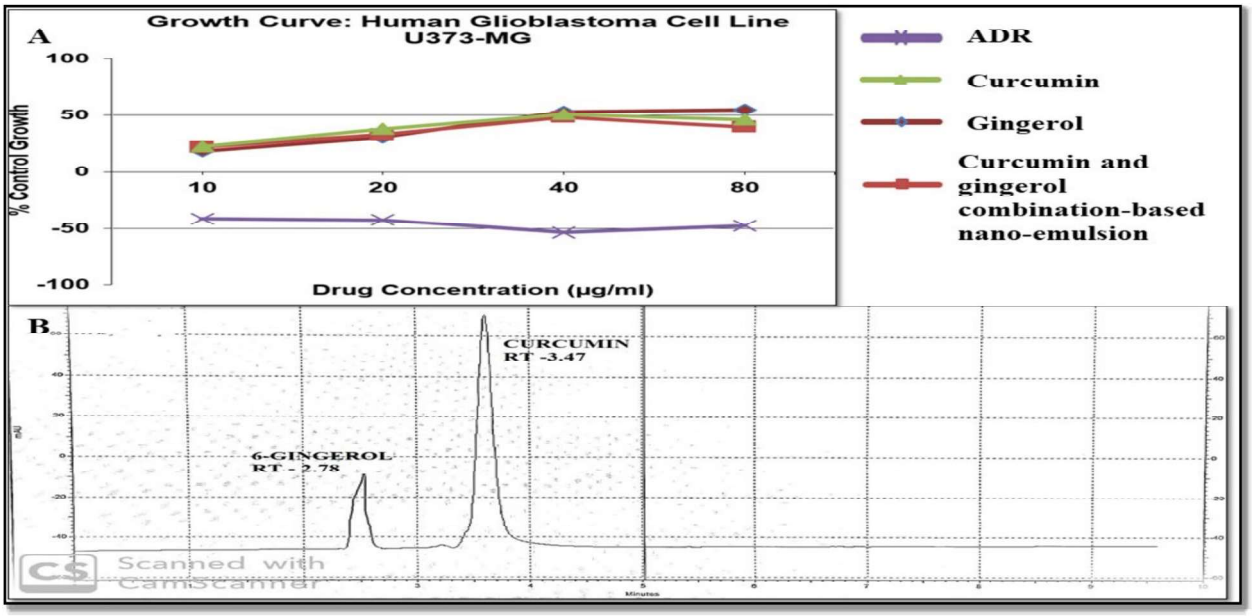

Fig. 7 **a** In vitro cytotoxicity (sulforhodamine B, SRB) colorimetric assay, ADR: adrenomycin, and **b** chromatogram of biodistribution study by HPLC

Discussion

FTIR spectroscopy of curcumin and 6-gingerol individually performed to confirm its purity and identity. The FTIR peaks (Fig. 1a) related to the phenolic and aromatic groups confirm the identification of curcumin, and in Fig. 1b, appeared peaks confirm the presence of alcohol, carbonyl and methylene groups in the long alkyl chain of 6-gingerol [35, 36].

Based on independent and response variables, batch no. F3 was selected for the further study. The selected CGNE batch appeared as bright yellow-colored emulsion, and the pH of formulated nanoemulsion was found slightly acidic in nature. The refractive index value of formulated nanoemulsion indicated the isotropic nature of drugs. The refractive index value assists to know probable interactions between the incorporated drugs and the used excipients. The comparable refractive index values of placebo and formulation indicate clarity and transparency of formulated nanoemulsion [16].

Higher percentage transmittance of the optimized nanoemulsion indicated the clear, transparent appearance and uniform distribution of oil droplets in water of the optimized nanoemulsion. The repulsive forces between negatively charged particles prevent their aggregation or flocculation. Stability of the nanoemulsions is the crucial factor where the dispersion of particles is essential for the efficient drug delivery. The negative value of zeta potential is the indicator of stability of formulated nanoemulsions. Larger negative value of zeta potential indicated the good physical stability of nanoemulsion due to the electrostatic repulsion of individual particles [19].

By comparing FTIR spectrum of curcumin- and 6-gingerol-based nanoemulsion and individual spectra of curcumin and 6-gingerol, no significant changes were observed. This indicates that both the curcumin and 6-gingerol were compatible in the formulated nanoemulsion. Further presence of the phenolic and aromatic groups of curcumin and the alcohol, carbonyl and methylene groups of 6-gingerol in the CGNE confirms the successful development of curcumin- and 6-gingerol-based nanoemulsion. The drug release mechanism of the formulated CGNE was calculated by using the Korsmeyer–Peppas equation from the plot of log (Mt/M). The diffusion exponent '*n*' for curcumin and 6-gingerol was found to be 0.46 and 0.69, respectively, indicating the $0.45 < n < 1$ to the anomalous release mechanism (Fig. 4a, b). The histopathology examination confirmed the biocompatibility and safety of the CGNE as there was no significant difference in the mucosal structure or occurrence of any mark/lesion with CGNE-treated mucosa compared to the positive control group [19].

Biodistribution study result exhibited that the concentration in the brain and plasma of formulated CGNE after intranasal administration was more as compared to pure drug suspension. This may be due to the fast absorption of formulated CGNE due to its extended residence time in the nasal cavity of rats which allowed for intranasal transport to the brain. To prove nose-to-brain direct transport efficiency, % DTP and % DTE were calculated and the obtained results confirm the better drug concentration in the brain through the upper nasal cavity's trigeminal and olfactory nerves. These results are in high agreement with related reports which shows that curcumin- and 6-gingerol-based nanoemulsion rises nose-to-brain uptake of drugs, and similar finding was reported by Mahajan and Patil 2021, for the curcumin- and quercetin-based nanoemulsion via nose-to-brain delivery [19].

In vitro cytotoxicity of curcumin and 6-gingerol combination-based nanoemulsion was analyzed by using human glioblastoma cell line U373-MG, and the study demonstrated that the combination of curcumin- and 6-gingerol-based nanoemulsion showed the synergistic result of cell growth inhibition as compared to the individual curcumin and 6-gingerol. These findings are in relevance with the reports by Mahajan and Patil 2021 that curcumin- and quercetin-based nanoemulsion gave promising use for brain tumor treatment [19, 29].

Conclusion

In the present study, curcumin- and 6-gingerol-based nanoemulsion has been successfully develop and characterized. The *FTIR* spectroscopy of CGNE exhibits typical spectra of both curcumin and 6-gingerol. The pH of CGNE was found to be acidic and the refractive index value of formulated CGNE was found to be 1.38, indicating the isotropic nature of drugs. Percentage transmittance of nanoemulsion was close to 100%, indicating the clear, transparent appearance and uniform distribution of oil droplets in water. Zeta potential of optimized nanoemulsion was found to be −29 mV, indicating good long-term physical stability of nanoemulsion due to the electrostatic repulsion of individual particles. *Ex vivo* drug diffusion study of curcumin and 6-gingerol at the end of the 6 h was observed to be 48.4% and 78%, respectively. The diffusion exponent '*n*' for curcumin and 6-gingerol was found to be 0.46 and 0.69, respectively, indicating the $0.45 < n < 1$ to the anomalous release mechanism. In the in vitro cytotoxicity against the human glioblastoma U373-MG cell line, the combination of curcumin- and 6-gingerol-based nanoemulsion showed the synergistic result of cell growth inhibition as compared to the individual curcumin and 6-gingerol. However, further detailed studies are required to explore the exact mechanism of action of the combination of the synergistic

action shown by the curcumin and 6-gingerol in treatment of carcinomas.

Abbreviations

CGNE	Curcumin- and 6-gingerol-based nanoemulsion
FTIR	*Fourier* transform Infrared spectroscopy
HPLC	High-performance liquid chromatography
BBB	Blood–brain barrier
IN	Intranasal
IV	Intravenous
SRB	Sulforhodamine B
PDS	Plain drug suspension
DSC	Differential scanning calorimetry
Cryo-SEM	Cryo-scanning electron microscopy

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Ujwaldip V. Deore was invovled in conceptualization, supervision and writing—original draft. Ms. Pooja S. Murkute was responsible for formal analysis, methodology and investigation. Both the authors read and approved the final manuscript.

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The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

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Not applicable.

Consent for publication
The authors declare no conflict of interest.

Competing interest
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