INTRODUCTION
Cancer has afflicted people for several centuries. It is because of the early research that we hold a greater knowledge of cancer today. The word Cancer is gotten from the Greek word Karakinos, for "crab." Cancer can begin anywhere in the human body, which is comprised of trillions of cells. Typically, human cells develop and frame new cells as the body needs them. At the point when cells develop old or become harmed, they pass on, and new cells have their sport. At the point when malignant growth grows, nonetheless, this methodical cycle separates. As cells become an ever-increasing number of strange, old or harmed cells endure when they should bite the dust, and new cells structure when they are not required. These additional phones can separate ceaselessly and may frame developments called Tumor [1].

Cancer is multi-factorial group of diseases that is normally associated with wide range of deregulated effects both at the molecular and cellular levels. The old style perspective on malignant growth etiology is that hereditary modifications harm the DNA structure and initiates transformation (adjusted grouping data) bringing about non-practical proteins that lead to illness movement. Malignant growth is an infection of the qualities. A quality is a little piece of DNA, which is the expert particle of the cell. Qualities make "proteins," which are definitive workhorses of the cells. It is these proteins that permit our bodies to complete every one of the cycles that grant us to inhale, think, move, and so forth. For the duration of individuals’ lives, the cells in their bodies are developing, isolating, and supplanting themselves. Numerous qualities produce proteins that are associated with controlling the cycles of cell development and division. An adjustment (change) in the DNA atom can disturb qualities and produce defective proteins. This makes the cell become strange and lose its limitations on development. The strange cell starts to separate wildly and at last structures another development known as a "Tumor" or neoplasm [2].

Curcumin, also known as diferuloylmethane, is an active component in the golden spice turmeric (Curcuma longa) and in Curcuma xanthorrhiza oil. It is a profoundly pleiotropic atom that displays antibacterial, calming, hypoglycemic, cancer prevention agent, wound-mending, and antimicrobial exercises [3-6]. Because of these properties, curcumin has been explored for the therapy and strong consideration of clinical conditions including proteinuria, bosom malignancy, numerous myeloma, discouragement, and Non-Small Cell Lung Cancer (NSCLC). In spite of demonstrated adequacy against various test models, helpless bioavailability because of helpless retention, quick digestion, and fast foundational disposal have been displayed to restrict the restorative viability of curcumin [7].

Different names have been used to refer to CDs. These include Schardinger dextrans, cycloamyloses and cycloglucans; CDs is the most common name. CDs are composed of a number of glucopyranose units attached by α-(1-4)-linkages [8]. The glucopyranose units are designated by Greek letters to denote the number of glucose units; α representing 6 glucose units, β for 7 and γ for 8 glucose units. CDs that contain 9 to 100 glucose units are known, however, the most commonly studied CDs are those of α-, β- and γ-CD. So nowadays need of the development of cyclodextrin based Nanosponge loaded with poorly soluble anticancer drug for the enhancement the solubility and dissolution profile [9-11].
MATERIALS AND METHODS

Pre-formulation studies

Infrared spectroscopy study

The medication powder of Curcumin and cyclodextrin with KBr were readied utilizing water powered pellet press at a pressing factor of 7 to 10 tones. FTIR was checked from 400-4000 cm^-1 by utilizing perkin Elmer range GX FTIR. FTIIR study was completed exclusively for drug, record FTIR spectra of unadulterated medication and polymer [12].

Determination of analytical wave length

A standard stock arrangement of Curcumin and cyclodextrin independently in 0.1N HCl was readied having a focus 600 µg/ml. A 5.0 ml bit of stock arrangement was additionally weakened Curcumin water in a 100.0 ml volumetric flask up to check to get last concentration 30µg /ml. The standard solution of Curcumin (30 µg/ml) was checked in the scope of 600-200 nm. In 1.0 cm cell against dissolvable clear and spectra was recorded, the absorbance maxima for Curcumin was seen at 424.0 nm and for cyclodextrin was seen at 290.0 nm [13].

Melting point study

The dissolving point study was carrying out Curcumin and cyclodextrin the assistance of open slim technique or differential scanning colorimetry (DSC).

Standard calibration curve of curcumin and cyclodextrin in 6.8 pH buffer

Stock arrangement of Curcumin and cyclodextrin was set up by dissolving 50 mg of medication in 200 ml of 6.8 phosphate support. Aliquots of 1, 2, 3, 4, 5, 6 ml (5 to 30 µg/ml) were moved independently in to 50 ml volumetric cups from the stock arrangement. Volume was changed sufficient Curcumin and cyclodextrin a similar dissolvable. Absorbance of the above arrangements was taken at 424.0 nm and 290.0 nm for Curcumin and cyclodextrin individually, against the blank. Spectra of absorbance Vs concentration was plotted [15].

Particle size measurement

Particle size analysis was carried out by malvonizer apparatus and sieve analysis method.

Preparation of curcumin nanosponges

Cyclodextrinnanopreparation (CDNS) was prepared using pyromelliticdianhydride as a crosslinker for beta cyclodextrin monomer as per the previously reported procedure. Beta-cyclodextrin (0.0054 M) was disintegrated in dimethylsulfoxide containing triethylamine. The arrangement was added with pyromelliticdianhydride (0.001073 M), blended and let to respond at room temperature for 3 h. The item was Soxhlet removed utilizing CH3)2CO for 24 h and dried at 60 °C±0.5 for 24 h. The pre-arranged nanosponges contain 1:2M proportion of cyclodextrin and crosslinker. The system was continued utilizing a higher measure of pyromelliticdianhydride (0.0215 M) to get ready nanosponges having a molar proportion of 1:4 (cyclodextrin: crosslinker). In view of our previous investigation, pyromelliticdianhydride cross-connected beta cyclodextrin having cyclodextrin to crosslinker molar proportion 1:2 was utilized for stacking curcumin. Cyclodextrinnanopreparation and curcumin were taken in 1:1 w/w proportion. Curcumin arrangement was arranged utilizing dichloromethane. To this arrangement, nanosponge was added and dissipated under mixing to eliminate the dissolvable. The resultant curcumin loaded nanosponges were dried at 50±0.5 °C for 24 h. The dried items got were contracted as CUR-CDNS for curcumin loaded nanosponges [16].

Characterization of CD based curcumin nanosponges

Surface morphology

Scanning Electron Microscope (SEM) (Model JSM 5200, Japan) was used to characterize surface morphology of prepared nanosponge. The examples were ready by engrossing the nano wipe on twofold size sticky tape which adhered to aluminum wound and gold covered under vacuum utilizing a falter coater. Tests were presented to vacuum for 5-10 min. at 40 mA and explored at speeding up voltage of 15 kV and 10 kV [17].

Transmission electron microscope (TEM)

The surface morphology of CUR-CDNS was studied by TEM (transmission electron microscope, Technai 20 G2, FEI, The Netherlands). The fluid scattering of the example was set on the target X-ray tube and a wide-angle goniometer. The X-ray source was-beam tube was worked at a capability of 45 kV and a current of 40 mA. The scope of sweeps was performed from 10 °C to 80 °C±0.5C at a speed of 4 °C each moment at additions of 0.05 °C [21].

X-ray crystallography

X-ray diffraction patterns of curcumin, CD nanosponge and C-CD-NS were determined using a diffractometer equipped with a rotating target X-ray tube and a wide-angle goniometer. The X-beam source was-beam tube was worked at a capability of 45 kV and a current of 40 mA. The scope of sweeps was performed from 10 °C to 80 °C±0.5C at a speed of 4 °C each moment at additions of 0.05 °C [21].
Drug content

Curcumin content in nanospoanges accurately weighed amount of nanosponge product was dissolved in methanol, sonicated, filtered, diluted suitably and analyzed using UV spectrophotometry at 424 nm for curcumin [22].

Solubility study

Solubility of CU, physical mixture, and CU-NS (obtained from different ratios of drug and NS) was determined by shake flask apparatus. Momentarily, overabundance of CU, actual blend, and CU-NS were added to HPLC grade water in fixed glass compartments at 25 °C. The examples were disturbed at 100 rpm for 24 h in the shaker water shower (EQUITRON® 147, Medica Instrument Mfg, 148 Co., India), then, at that point centrifuged at 10,000 rpm for 10 min. The supernatants were separated (0.45 µm 149 PVDF needle channel, Millipore MIllex-HV). The filtrate was appropriately weakened with methanol and broke down by switch stage HPLC comprising of a Waters. A C18 Luna® RP-HPLC (250 152 mm × 4.6 mm, 5 µm) (Phomomenexnc., CA, USA) insightful section was utilized for the assessment. The improved portable stage was made out of ammonium acetate derivation support (pH 4.0) and acetonitrile (42:58 v/v), with 1 ml/min stream rate. Tests of 20 µl were broke down for evaluation of CU at 424 nm utilizing PDA locator [23].

Loading efficiency

The loading efficiency (%) of curcumin loaded in C-CD-NS was determined as follows: A known measure of C-CD-NS was taken and broken up into total ethanol (Analytical Reagent 99.9 % v/v) and the medication content was investigated utilizing UV spectrophotometer (Shimadzu UV 2101 PC) at 424 nm after shaking. The loading efficiency was calculated from the following Equation [23].

\[
\text{Loading efficiency} = \left( \frac{\text{Amount of curcumin incorporated in CD nanoparticles}}{\text{Amount of curcumin initially added}} \right) \times 100
\]

Differential scanning calorimetry (DSC)

Thermal characteristics of CU, NS, physical mixture, and the nanospoange complex of CU were analyzed using DSC (DSC 821e, Mettler-Toledo International Inc., and Switzerland). Each example (3-5 mg) was warmed in a creased aluminum skillet somewhere in the range of 25±0.5 °C and 300±0.5 °C at a filtering pace of 20±0.5 °C/min and under a nitrogen stream of 40 ml/min. Instrument was recently adjusted with void search for gold and indium for heat rate [24].

Drug release study

In vitro release study Franz diffusion cell with a diameter of 2.4 cm was used for the in vitro release study. The receptor compartment was added with 30 ml arrangement containing phosphate cradled saline and methanol (1:1) and permitted to equilibrate at 32 °C utilizing a hot plate with an attractive stirrer. Receptor stage was isolated from the benefactor stage by a layer channel type 0.2 µm, Sartorius AG W-3400 Goettingen Germany, recently absorbed receptor liquid for 24 h. The fluid scattering of CUR, CUR-CDNS were ready and utilized for in vitro discharge test. The benefactor compartment was then added with 5 ml watery scattering of test. The contributor receptor interface and every one of the openings are fixed utilizing parafilm to forestall vanishing. The receptor medium was kept up with at 32 °C and blended at 200 rpm utilizing attractive stirrer. Tests (2 ml) were removed from the receptor stage at foreordained time periods min, supplanted with the new medium. Removed examples were reasonably weakened and examined by HPLC strategy utilizing C18 section (4.6 mm I.D × 100 mm × 5 µm; Shimadzu) with the portable stage containing methanol and 0.5% acidic corrosive (75:25) at a stream pace of 0.5 ml/min (r²=0.999, 1 ng to 10 µg). The experiment was carried out in triplicate for each test sample [25].

In vitro cytotoxicity test

Cytotoxicity of curcuminnanosponge and their combination were studied in MCF-7 (human breast adenocarcinoma) cell lines. CUR-CDNS samples were mixed in ratios 1:3, 1:1 and 3:1 by weight and labeled as V1, V2 and V3. CUR-CDNS were labeled as C. Distinctive fixation going from 1µg/ml to 125µg/ml were ready for each test tests C, V1, V2, V3 and utilized for cytotoxicity test. Cell responsibility was evaluated utilizing MTT ((3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide) examine strategy. Cell culture having 1.0 ± 105 cells/ml was arranged utilizing Dulbecco’s changed fundamental medium containing 10% Fetal cow-like serum (FBS). 100 µl of sub refined cell suspension was added to each well of a 96 well microtiter plate, added with test tests of various fixations and hatched for 72 h at 37 °C in 5% CO2 air. After brooding, each all around was added with 20µl of MTT (2 mg/ml) in MEM without phenol red and hatched for 3 h. The supernatant medium was taken out and added with 50 µl of isopropanol. The plates were perused at 540 nm utilizing microplate reader. Cell feasibility is determined as the proportion of test cell absorbance to control cell absorbance and communicated in rate. Fixation needed to repress cell development by half was produced from the portion reaction bend for test tests C, V1, V2 and V3. Clear nanosponge test was utilized as a control test. The data was analyzed by Compusyn software to compute the combination index (CI) value to evaluate the synergetic effect [26].

Stability studies

The stability study was carried out for Nano sponge formulation as per ICH guidelines. The nanospoange were placed in screw capped glass container and stored at ICH storage (40 °C±2 °C/75%RH±5%RH) condition for a period of 60 d. The samples were analyzed for physical appearance and for the drug content.

| Table 1: ICH guidelines for stability study |
|--------------------------------------------|
| Study | Storage condition | Time period |
|-------|-------------------|-------------|
| Long term | 25 °C±2 °C/60%RH±5RH | 12 mo |
| Intermediate | 30 °C±2 °C/65%RH±5%RH | 6 mo |
| Accelerated | 40 °C±2 °C/75%RH±5%RH | 6 mo |

RESULTS AND DISCUSSION

Table 2: Physical parameter

| S. No. | Parameter | Result |
|--------|-----------|--------|
| 1      | Color     | A yellowish amorphous powder |
| 2      | Odour     | Characteristic |
| 3      | Taste     | Slight pungent |

A standard stock arrangement of Curcumin and cycloextrin independently in 0.1N HCl was readied having a focus 600µg/ml. A 50 ml bit of stock arrangement was additionally weakened Curcumin water in a 100.0 ml volumetric flask up to check to get last concentration 30µg/ml. The standard arrangement of Curcumin (30 µg/ml) was checked in the scope of 600-200 nm. In 1.0 cm cell against dissolvable clear and spectra was recorded, the absorbance maxima for Curcumin was seen at 424.0 nm and for cycloextrin was seen at 290.0 nm.
X-ray diffraction patterns of curcumin powder were determined using a diffractometer outfitted with a pivoting objective X-beam tube and a wide-point goniometer. The X-beam source was a beam tube worked at a capability of 45 kV and a current of 40±1.40 mA.

**DSC study**

The DSC test was done on curcumin was presented below

**Table 3: DSC Study of curcumin**

| S. No. | Stage   | Temperature (°C) |
|--------|---------|------------------|
| 1      | Onset   | 180.26±0.5 °C    |
| 2      | Peak    | 181.20±0.5 °C    |
| 3      | End set | 182.42±0.5 °C    |

Where n=3, mean±SEM

**Table 4: Melting point of pure drug**

| S. No. | Melting point (°C) | Average melting point (°C) |
|--------|-------------------|-----------------------------|
| 1      | 182±0.5           |                             |
| 2      | 180±0.5           | 181.00                      |
| 3      | 181±0.5           |                             |
The average melting point of pure drug is 181 °C which is complied with Stander melting point of drug. From all the FTIR Study and actual perception it was presumed that there was no huge Drug-excipients collaboration was noticed. The consequences of FTIR study shown that there is no adjustment of medication’s liquefying top. So we can reason that medication and other excipients are viable which one another.

Calibration curve of curcumin in 6.8 phosphate buffer
Phosphate buffer pH 6.8 was used for the preparation of curcumin concentration and absorption was measure by Shimadzu UV-1601 UV/Vis double beam spectrophotometer. The λ max of curcumin was found to be 424 nm.

Solubility study
Solubility studies were carried out by each adding excess of drug in screw-capped vials each containing 50 ml different solubility medium such as water, 1.2 pH buffer, 3.0 pH buffer, 6.8 pH buffer, 7.4 pH buffer. The suspensions were continuously stirred on electromagnetic stirrer (Remi, India) at 37±0.5 °C and 300 rpm for three hours. The suspensions were filtered through 0.22 μm membrane filter. The filtrates were suitably diluted and analyzed, spectrophotometrically (Shimazdu-1700, UV/Visible spectrophotometer) for the dissolved drug at 424 nm.

Table 5: Solubility data of curcumin

| Medium         | Solubility (mg/50 ml) |
|----------------|-----------------------|
| Distilled Water| 235±0.5               |
| 1.2 pH buffer  | 22±0.5                |
| 3.0 pH buffer  | 180±0.5               |
| 6.8 pH buffer  | 23±0.5                |
| 7.4 pH buffer  | 241±0.5               |

Where n=3, mean±SEM

Preparation of nanosponge

Shape analysis
The shape analysis of the nanosponge, drug loaded nanosponge and CD conjugates nanosponge obtained is shown in figure. It was evident from SEM photo micrographs that nanosponge, drug loaded nanosponge and CD conjugates nanosponge were discrete, spherical or oval curcumin slightly rough surface. No significant change of shape was found in drug loaded nanosponge and CD conjugates nanosponge as compare to nanosponge.

Aspect ratio
Aspect ratio was done for the nanosponge’s spherocity, for the stream property. Hot stage magnifying instrument was utilized for the estimation of the stature and width of the nanosponge Aspect proportion was determined from following equation:

\[
\text{Aspect ratio} = \frac{\text{Length of nanosponge}}{\text{Width of nanosponge}}
\]

Table 6: Aspect ratio of different batches

| S. No. | Length (nm)     | Width (nm)     | Aspect Ratio     |
|--------|-----------------|----------------|------------------|
| 1      | 1420.0±0.05     | 1368±0.05      | 1.037±0.05       |

From the above result aspect ratio of the nanosponge is 1.037.

Scanning electron microscopy
These studies were done for the nanosponge’s shape and surface study. Following study gives perfect idea about shape and surface of the nanosponge prepared.
Fig. 8: Scanning electron micrographs of (A) Nanosponge (B) Drug loaded nanosponge and (C) CD-conjugates nanosponge

Fig. 9: Aspect ratio of different batches of nanosponge

Fig. 10: Scanning electron micrographs of shape and surface size of nanosponge
Zeta potential

Particle size analysis exhibits that CUR-CDNS is nanosized with size ranging between 450 and 550 nm having unimodal distribution exhibiting good homogeneity. Zeta potential noticed for CUR-CD-NS were more negative contrasted with separate plain CUR (−20.1±1.57) demonstrating solidity of the nanodispersion. The nanochannels shaped in CDNS due to crosslinking of CD outcomes in the permeable design which gives various cooperation destinations notwithstanding the hydrophobic cyclodextrin cavities. These nanocavities help in more noteworthy entanglement of curcumin in CDNS.

In vitro release profile of curcumin

In vitro profile exhibits the release pattern of curcumin from CUR-CDNS in comparison with the plain CUR. Plain curcumin showed a lag phase of 3–4 h while the previous delivery was seen from CUR-CDNS. The arrival of curcumin from CUR-CDNS at every hour was fundamentally more noteworthy than their separate plain CUR (P<0.001). Curcumin discharge was upgraded to very nearly 10 folds toward the finish of eighth hour. Hydrophilic property of the CDNS and the expanding capacity of pyromellitic dianhydride cross-linked cyclodextrin and nanoscale molecule size helps in wetting, solubilization bringing about quicker and improved delivery from CUR-CDNS. For the comparison purpose nanospores are betacyclodextrins crosslinked with carbonate bonds. The polymer formed is nanoparticulate in nature, that the solubility of itraconazole was enhanced more than 50-folds with a ternary solid dispersion system [27] but present work increased solubility as well as also increased the drug release profile of poorly water soluble drugs. They offer unique advantage of controlled release and are biologically safe.

In vitro cytotoxicity activity

Cytotoxicity and synergism CUR-CDNS showed a dose-dependent effect on MCF-7 cell proliferation analyzed by MTT method. In vitro cell culture toxicity assays were carried out on different cell lines (i.e., HELEA; MCF7, COS) by using the MTT test. Cells were incubated for between 24 and 72 h and their viability determined. After incubation with nanospores no cytotoxicity was observed [27]. Mix of CURCDNS at 3:1 proportion displayed a huge expansion in cytotoxicity impact in a fixation subordinate way (*P<0.05) when contrasted and the single treatment of CUR-CDNS. Development hindrance of the cells at 1:3 mix proportions was not essentially unique in relation to CUR-CDNS single treatment at all fixations and like 1:1 mix proportion at a lower focus. Notwithstanding the two blends (1:3 and 1:1 proportion) showed a critical contrast (*P<0.01) against. Clear nanospores didn’t show any cytotoxic impact on cell societies. The IC_{50} worth of CUR-CDNS was seen to be 10.11μg/ml (n=3). Treatment with a combination of CUR-CDNS at 1:1 and 1:3 ratios resulted in an IC_{50} value of 14.98 μg/ml. Other in vitro methods for evaluating the toxicity of new materials include the determination of its haemolytic properties. When nanospores were incubated with human erythrocytes for 90 min, no haemolytic activity was observed up to a concentration of 15 mg/mL showing that the nanospores possess good blood compatibility [27]. So the Cyclodextrin-based nanospores were found better compatibility and stability for in vitro cytotoxicity method.
Characterization based stability study of CUR-CD-NS

Fig. 13: Characterization graphs of formulation–control (*25 °C±2 °C) and stability (8 w at *40 °C±2 °C) a-Differential scanning calorimetric thermograms (DSC); b-Fourier transform Infra-red spectra (FTIR); C-HPLC peaks

CONCLUSION
Cyclodextrin nanosponges (CDNS) were fabricated using pyromelliticdianhydride as a crosslinker and loaded with curcumin. These nanosized curcumin nanosponges (CUR-CDNS) showed greater in vitro release than respective plain curcumin. In vitro cytotoxicity study and combination index analysis showed the synergistic effect of CUR-CDNS against MCF-7 cells. The present study reveals that the combination of curcumin results in higher cytotoxicity against breast cancer cells and suggests cyclodextrin nanosponges as an effective nanocarrier for the delivery of curcumin.

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AUTHORS CONTRIBUTIONS
All the authors have equally contributed to this manuscript.

CONFLICT OF INTERESTS
The authors declare no conflict of interest, financial or otherwise.

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