

Assessment of Curcumin Nanoemulsion's Cytotoxic Effect on Hepatocellular Carcinoma Cells: A Potential Therapeutic Strategy

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Polymerase Chain
Reaction.

ABSTRACT

In cancer photodynamic treatment (PDT), curcumin can be used as a photosensitizing agent in addition to its anticancer therapeutic properties. However, curcumin's limited therapeutic value in cancer treatment is due to its poor bioavailability and associated pharmacokinetics. Due to its strong anticancer effects, curcumin was prepared as a nanoemulsion with an average particle size of 17.47 nm for improved bioavailability in this in vitro investigation on the treatment of breast cancer. We evaluated this curcumin nanoemulsion's anti-tumor efficaciousness on HepG2 cell lines using the MTT assay. Surprisingly, our research showed that the IC₅₀ concentrations for cells treated with curcumin nanoemulsion alone were 600 ng/ml, while the IC₅₀ concentrations for cells treated with curcumin nanoemulsion plus laser therapy were 850 ng/ml. The produced curcumin had encouraging benefits. The created curcumin nanoemulsion has a lot of potential for use as a strong anticancer medication. In summary, PDT of curcumin nanoemulsion shows a major involvement in the death of cancer cells via many apoptotic mechanisms.

Introduction

Cancer is a fatal illness that claims lives globally. Because there aren't many treatment choices for it, doctors have a lot of challenges in treating cancer patients. Conventional chemotherapy and radiation are the main treatments available. Finding efficient cancer treatment methods is now a top focus for research. Out of all the methods investigated, photodynamic treatment (PDT) has come to light as a potentially effective technique **Das et al., 2009**. A non-toxic photosensitizer (PS) is given to a patient undergoing photodynamic therapy (PDT) if they have a lesion, which is usually but not always malignant. It is possible to apply this PS topically, locally, or systemically. Following an incubation period, red visible light (620–690 nm) is often applied to the specific lesion, which causes the production of cytotoxic species when oxygen is present. These cytotoxic species cause tissue destruction and cell death (**Kubler, 2005**).

Photodynamic therapy (PDT) is especially advantageous as a cancer treatment due to its high degree of specificity and selectivity. The PS mostly accumulates in the cancerous tissue. When light is focused on the lesion during PDT, reactive oxygen

species (ROS) are generated, leading to cellular destruction. This targeted method minimizes damage to healthy tissues, making PDT an appealing choice for cancer treatment **Mitra, (2006)**.

Curcumin, a yellow pigment extracted from the rhizomes of *Curcuma longa* (Family: Zingiberaceae), is a vital component of turmeric and is commonly used as a spice and food coloring agent. Past its culinary applications, curcumin shows striking restorative properties, including anticancer, cancer prevention agent, and calming impacts. It has likewise been displayed to incite apoptosis. In PDT, curcumin can be utilized as a photosensitizing agent, improving the viability of treatment, and diminishing secondary effects. Curcumin's tetrapyrrolic compounds with heme-like designs, conferring striking pigmentation, make it a reasonable contender for PDT. Photodynamic Treatment, with its designated approach and the flexibility of curcumin as a photosensitizer, holds guarantee as a powerful disease treatment methodology. Further exploration is justified to investigate the maximum capacity of this helpful methodology and its applications in different malignant growth types **Jayaprakasha et al., (2006)**.

Materials and methods

Preparation of curcumin nanoemulsion

Medium chain fatty substances (MCTs) got from coconut oil, Sodium dodecyl sulfate (SDS) and curcumin (Mw=368.38) Da were acquired from sigma Aldrich. Curcumin nanoemulsions were prepared according to (Silva et al., 2015b), with slight changes. At first, 0.1% (w/w) of curcumin powder was solubilized at 90°C in coconut oil for 30 min then the prepared nanoemulsion was then integrated into a fluid stage comprising of 1% (w/w) SDS disintegrated in refined water. The oil-to-watery stage volume proportion was kept up with at 1:9. The nanoemulsions were ready by pre-blending and sonication for 2 minutes utilizing sonifier equipement, trailed by high-pressure homogenization (15,000 psi, 60 minutes) utilizing a Z4 spout with a 100 µm opening.

Characterization of the prepared curcumin nanoemulsion

Morphological characterization

The morphology of nanosystems was assessed using transmission electron microscopy (TEM) with an EM 902A instrument from ZEISS, Germany, operating at 80 kV.

Physicochemical characterization of the nanocarriers

Nanosystems size and charge measurements

The droplet charge, measured as zeta potential (Zp), for the nanoemulsions, was determined utilizing a particle microelectrophoresis instrument, specifically the Zetasizer Nano ZS-90 by Malvern Instruments, based in Worcestershire, UK (Ozturk, Argin, Ozilgen, & McClements, 2014; Rao & McClements, 2013).

The particle size distribution and polydispersity index (PDI) of nanoemulsions were assessed through Dynamic Light Scattering (DLS) using the Zetasizer Nano ZS-90 by Malvern Instruments, located in Worcestershire, UK (Rao & McClements, 2013; Silva et al., 2011).

Results

Transmission electron microscope (TEM)

The TEM images (Fig. 1a) revealed that the morphology of the prepared Nano-CUR, showcasing spherical shapes with a consistent and uniform size distribution. The average particle size was determined to be 17.47nm.

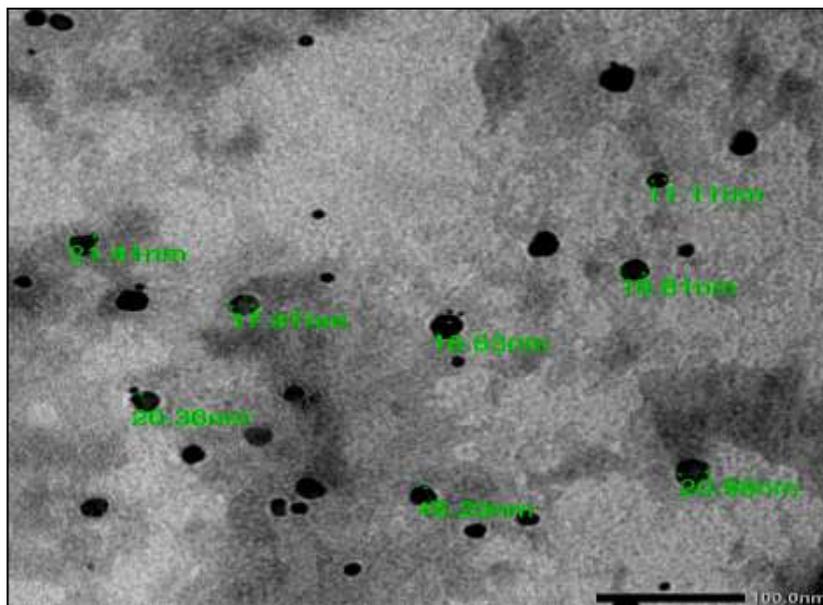


Fig. (1): TEM of curcumin nanoemulsion

Zeta potential and Zeta seizer analysis

The zeta potential of the prepared nanoemulsion was determined using Malvern, UK. recorded zeta potential was -76.8 mV, and an average hydrodynamic potential of 173.7 nm was observed (Fig. 2 A-B).

FTIR spectroscopy of curcumin nanoemulsion

The FTIR spectrum and vibrational characteristics of functional groups for both pure curcumin and the nanoemulsion are presented in Fig. (3).

In Figure (3), the absorption bands in the FTIR spectrum of pure curcumin are observed at 3511 , 1628 , 1510 , and 1282 cm^{-1} , corresponding to the vibrations of ν (OH), ν (C=C), [ν (C=O), δ CC), and δ (CC=O)], and δ (C-O-C), respectively. In particular, the fact that there are no peaks in the important carbonyl region (1800 –

1650 cm^{-1}) suggests that curcumin mostly exists in the keto-enol tautomeric form.

Upon formation of the nanoemulsion, spectral changes are evident, as depicted in Fig. (3B). The FTIR bands for the nanoemulsion appear at 3452 , 1637 , and 1213 cm^{-1} , corresponding to the vibrations of ν (OH), ν (C=C), and [ν (C=O), δ CC), and δ (CC=O)], and δ (C-O-C), respectively. These variations in the spectrum indicate the impact of nanoemulsion formation on the molecular structure of curcumin.

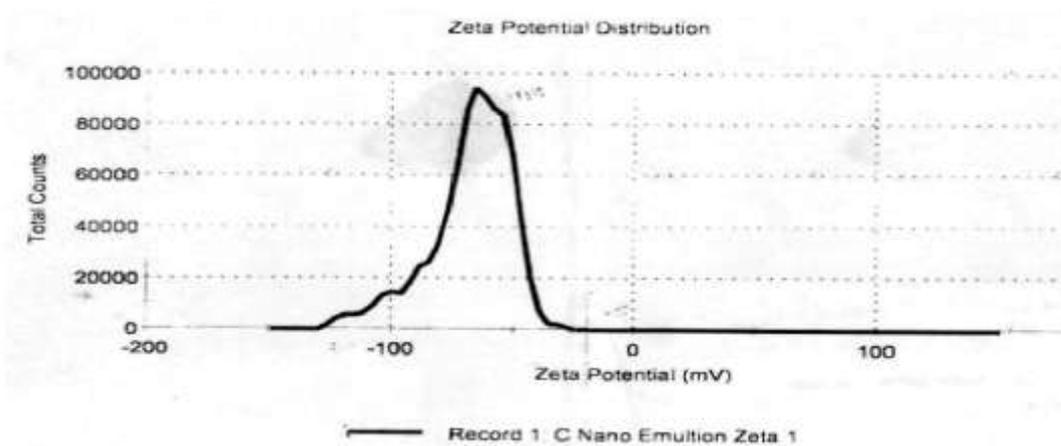


Fig. (2): Zeta potential of curcumin nanoemulsion

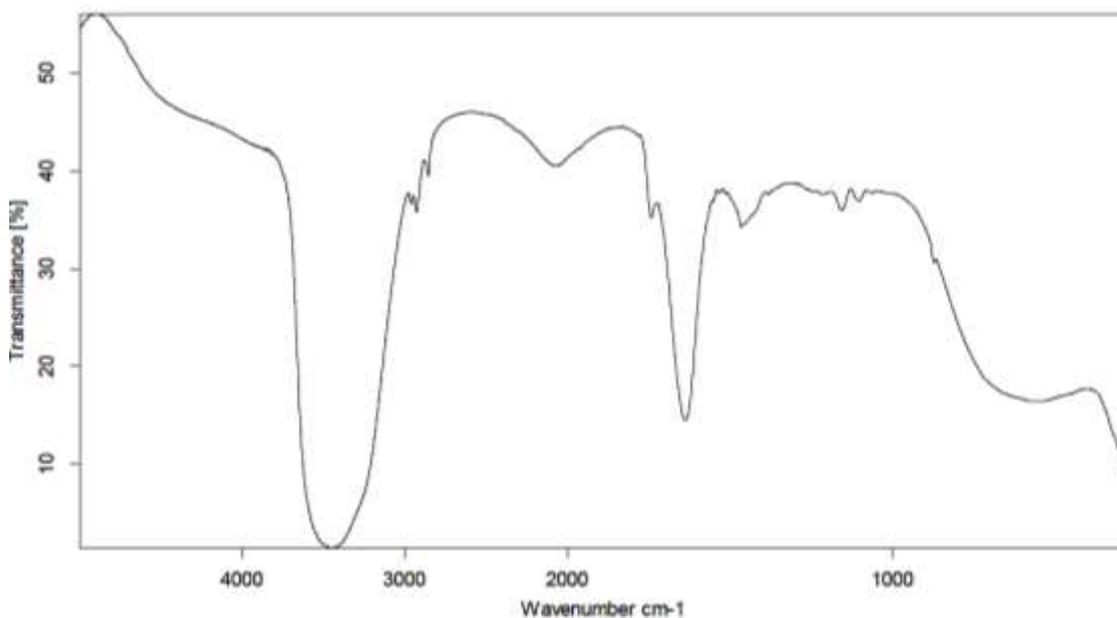


Fig. (3): The FTIR absorbance spectra of the prepared curcumin Nanoemulsion

Assessment of *in vitro* anti-tumor effects on HEPG2 cells following a 24-hour exposure using the MTT assay

Figure (4) illustrates the *in vitro* cytotoxic effect resulting from the exposure of the *HepG2* cell line to both curcumin and curcumin nanoemulsion, as well as a photodynamic system involving curcumin nanoemulsion stimulated by a laser beam with a wavelength of 640 nm⁻¹. The

concentrations tested ranged from 0 to 1000 ng/mL of curcumin nanoemulsion, and the incubation period was 24 hours. The figure presents the dose-dependent response of the cells under these experimental conditions. The effectiveness of curcumin nanoemulsion and curcumin nanoemulsion combined with laser stimulation was evaluated by assessing *HepG2* cell viability

after a 24-hour incubation period. The nanoemulsion significantly reduced cell viability in a dose-dependent manner. Specifically, the IC₅₀ was determined to be 600 ng/mL for cells treated with curcumin nanoemulsion and laser stimulation. This concentration was significantly lower than that required for cells treated with curcumin nanoemulsion alone (850 ng/mL), indicating the enhanced cytotoxic effect of laser stimulation. Importantly, no

significant cell death was observed in cells treated with curcumin nanoemulsion alone until the dose reached 200 ng/mL, demonstrating the concentration-dependent cytotoxicity of the nanoemulsion. The addition of laser stimulation further enhanced this cytotoxic effect, leading to a more pronounced reduction in cell viability at lower concentrations of the nanoemulsion.

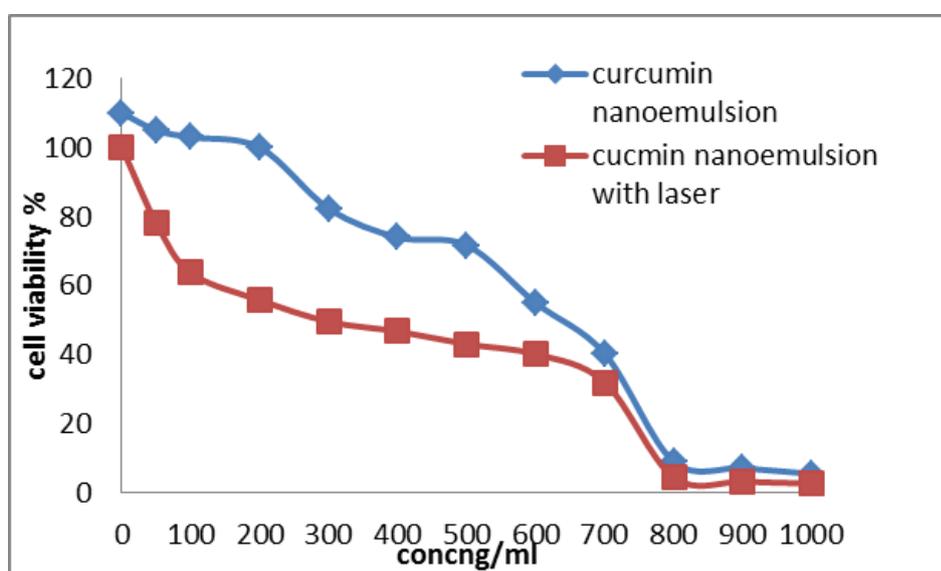


Fig. (4): The cell viability % of *HepG2* cell line treated with different concentration of curcumin nanoemulsion and curcumin nanoemulsion with laser.

Discussion

Cancer is an upsetting and perilous illness that causes serious fatalities all over the planet. The most well-known therapy for malignant growth is chemotherapy, however it has a few disadvantages, like non-particular dispersion of medications, multidrug obstruction, improved drug harmfulness, unwanted secondary effects on ordinary

tissue, and innate absence of helpful reaction to cytotoxic anticancer medications (Sahoo et al., 2007). A state of the art strategy for treating bosom malignant growth is Photodynamic treatment (PDT). In this methodology, a non-harmful medication or a photosensitizer (PS) is regulated to the patient, either foundationally, locally, or topically, contingent upon the case. The

PS focuses on the injury, which is frequently, however not consistently, carcinogenic, and is initiated by light to deliver reactive oxygen revolutionaries that obliterate the malignant cells (**Li et al., 2017; Strunk et al., 2018**). PDT is an astonishing disease treatment choice that has acquired notoriety because of its exceptional accuracy and explicitness (**Ko and Moon, 2015**). This is on the grounds that the PS is explicitly designated to the harmful tissue, so when light is coordinated towards it, ROS are created, bringing about the obliteration of the disease cells. Lately, PDT has been widely investigated as a possible therapy for different sorts of disease (**Brown et al., 2004; De Rosa and Bentley, 2000; Schuitmaker et al., 1996**). The point of this study was to evaluate the viability of curcumin nanoemulsion as a photosensitizing agent when presented to a laser beam with a frequency of 620 nm for treating the hepatocellular carcinoma cell line. The advancement of nanoemulsion curcumin has been pointed toward beating huge obstacles in the medication conveyance process, like unfortunate dissolvability, fast debasement, and low bioavailability. Following fruitful definition, cautious thought was given to the physicochemical portrayal of the medication conveyance framework, as it assumes a vital part in deciding the

actual soundness, cell take-up, bio-distribution, and arrival of the epitomized drug (**Sanidad et al., 2019**). In such manner, the size circulation and surface charge of NPs were taken for perception. As the little size of particles are profitable for inactive focusing to growth tissue by improved penetrability and maintenance impact (**Alexiades-Armenakas, 2006**) and higher zeta potential impact the molecule dependability, cell take-up and intracellular dealing (**Sahoo et al., 2002**). In view of the little size and high surface charge of our formed nanoparticulate curcumin, we can guess that it will have altogether upgraded dissemination half-lives and stay away from the reticulo-endothelial framework (RES). Our outcomes from TEM assessment of the arranged curcumin nanoemulsion showed a mean molecule size of 17.47 nm, a zeta capability of -76.8 mV, a typical hydrodynamic capability of 173.7 d.nm, and a round shape. In one more review the circumstances utilized for the improvement of the curcumin nanoemulsions prompted a last mean bead measurement of 80.0 ± 0.9 nm, being especially proficient for the advancement of little drop sizes (< 100 nm). PdI and Zp values were 0.177 ± 0.009 and -65.8 ± 5.8 mV, individually. A comparative report committed that at

High amplification transmission electron microscopy (TEM) the size and morphology of the pre-arranged Nano-Mutt contain round shapes with uniform size circulation. With mean breadth of Nano-Dog, in light of estimations by Picture Tools® and SPSS programming on TEM results, is 15.7 ± 3.55 nm. Besides, the FTIR examination obviously exhibited the compound respectability of curcumin nanoemulsions. In this view, our spectroscopic outcomes exhibited The nanoemulsions development causes ghostly changes as a slight variety in the range, FTIR groups retention normal for nano emulsion were shown at 3452, 1637 and 1213 cm^{-1} , which are relegated to the ν (Gracious), ν (C=C), and [ν (C=O), δ CC), and δ (CC=O)], and δ (C-O-C), separately). As indicated by **Mangolim et al., (2014)**, curcumin was found to exist in the keto-enol tautomeric structure as there were no critical tops in the carbonyl locale (1800-1650 cm^{-1}) while analyzed utilizing ghashly examination. Other FTIR examination was taken in to thought show the FTIR spectra's of local curcumin and nanoparticulate curcumin. A band at 3490 cm^{-1} has been recently credited to eOH bunch extending vibration in local curcumin (**Bisht et al., 2007; Misra et al., 2009**). In nanoparticulate curcumin a shift from

3490 cm^{-1} to 3392 cm^{-1} is shown, and the pinnacle of 3392 cm^{-1} becomes more extensive, this demonstrates hydrogen holding is improved. The solid tops at 2925 cm^{-1} and 2855 cm^{-1} and a powerless top at 1375 cm^{-1} in all the case could be because of extending and distortion of methyl gatherings. Essentially, a feeble top at 1465 cm^{-1} has seen in every one of the three cases could be because of eCH₂ twisting vibration. The solid top at 1740 cm^{-1} in void NP and nanoparticulate curcumin is because of C=O adsorption. The mark tops at 1627 cm^{-1} and 1602 cm^{-1} are found in local curcumin and nanoparticulate curcumin were because of C=C twofold bonds and sweet-smelling C=C twofold bonds separately. As these marker tops were not tracked down in void NPs, proposing curcumin exists inside the nanoparticulate curcumin. Further, no moving of tops at 1627 cm^{-1} and 1602 cm^{-1} found in nanoparticulate curcumin contrast with local curcumin, crediting curcumin could be available in scattered condition if there should arise an occurrence of nanoparticulate curcumin plan. To survey the antitumor action of the pre-arranged PDT, HepG2 cells were treated with curcumin nanoemulsion and curcumin nanoemulsion with laser for 24 hours of hatching. The outcomes showed that the IC₅₀ focus was 600 ng/ml for cells treated with curcumin

nanoemulsion and laser, while those treated with curcumin nanoemulsion alone had an IC_{50} centralization of 850 ng/ml (Silva et al., 2018). A past examinations assessed by silva et al., 2018 that curcumin introduced no natural cytotoxicity ($>4.75\mu\text{g curcumin}\cdot\text{mL}^{-1}$), more over the outcomes showed that nanoemulsions were poisonous for Caco-2 cells after 4 h of brooding, inside the concentrated on focus range, with IC_{50} upsides of $1.125 \pm 0.028 \mu\text{g mL}^{-1}$. In this study The still up in the air for SDS was $34.30 \pm 2.18 \mu\text{gSDS}\cdot\text{mL}^{-1}$, which was 3.3-overlay lower than the IC_{50} values found for nanoemulsions and These outcomes recommend that the reduction in the cell feasibility is brought about by the presence of the SDS in the nanoemulsions and. It's fascinating to take note of that EFSA doesn't perceive SDS for use in food items (EFSA, 2010), though the FDA supports the utilization of SDS as a surfactant for organic product juice drinks under 25 ppm (CFR, 2014a, 2014b). Albeit the nanoemulsion utilized in this work is cytotoxic, the review recommends that the utilization of nano-scale beads didn't upgrade the cytotoxic impact of SDS. Truth be told, the curcumin nanoemulsion decreased the cytotoxicity of SDS by 3.3-overlay. This finding shows that in the size ranges considered,

the cytotoxicity was not exclusively connected with size, yet rather to the presence of SDS, which features the synthetic nature's impact as opposed to actual size. As per a concentrate by Huang et al. in 2008, Curcumin was viewed as cytotoxic against CasKi, SiHa, and HaCaT not entirely settled by MTT measure. In any case, NE didn't show critical cytotoxicity, with most cell suitability rates above 80%. Strangely, the feasibility of cells was impacted by curcumin in a portion subordinate way (Huang et al., 2008). After treating the three cell lines with CNE at $20 \mu\text{M}$, the cell suitability stayed above 80% for every single dissected time. Along these lines, this focus was decided to play out extra investigations. Notwithstanding, it was seen that HaCaT cells were more impacted by the most noteworthy centralization of CNE ($40 \mu\text{M}$) than CasKi and SiHa cells, demonstrating higher cytotoxicity. Following 24 hours of brooding, all cell lines treated with free curcumin showed low suitability at the most elevated focus ($40 \mu\text{M}$), with just 7.8% of cell practicality for CasKi cells, 8.6% for SiHa cells, and 16.5% for HaCaT cells. Notwithstanding, during three reproduce tests, the centralization of free curcumin that kept up with cell feasibility above 80% was viewed as $5 \mu\text{M}$ for CasKi cells and $10 \mu\text{M}$ for both SiHa and HaCaT cells. To summarize,

the discoveries are predictable and show promising results, demonstrating that the created curcumin nanoemulsion has tremendous potential as a compelling anticancer agent.

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تقييم التأثير السام لمستحلب الكركمين النانوي على خلايا سرطان الخلايا الكبدية: كاستراتيجية علاجية محتملة

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يظل السرطان قضية صحية عالمية هامة، حيث يتسبب في عدد كبير من الوفيات ويفرض عبئًا على أنظمة الرعاية الصحية. وعلى الرغم من مختلف علاجات السرطان التقليدية، إلا أن انتشاره ومعدلات الوفيات تظل مرتفعة. وهذا يؤكد على ضرورة وجود أساليب علاج جديدة وأكثر فعالية.

يعد الكركمين، المركب الحيوي الطبيعي المستخلص من الكركم، قد اكتسب اهتمامًا كبيرًا بسبب خصائصه الدوائية الواسعة. حيث يظهر إمكانيات علاجية ضد السرطان ويمكن استخدامه كدواء محفز بالضوء في علاج الضوء الحساس للسرطان (PDT). ومع ذلك، فإن الفائدة السريرية للكركمين في علاج السرطان محدودة بسبب امتصاصه البيولوجي الضعيف والفاارماكوكينيتيكا المرتبطة به. وتركز هذه الدراسة على الخصائص الفيزيائية والكيميائية للكركمين، وكذلك امتصاصيته البيولوجية وسلامته. كما تستكشف الدراسة دور الكركمين كعامل في علاج السرطان كمحفز للضوء في علاج الضوء الحساس للسرطان. كما تسلط الدراسة الضوء أيضًا على الآليات المحتملة والأهداف الخلوية التي يتحقق بها تأثير الكركمين في علاج السرطان والضوء الحساس للسرطان. بشكل عام، تلقي هذه الدراسة الضوء على إمكانية الكركمين كعامل قيم في علاج السرطان والضوء الحساس للسرطان. وتؤكد على ضرورة التغلب على قيوده من خلال نهج مبتكر يعزز امتصاصيته البيولوجية. ومن خلال ذلك، يمكن للكركمين أن يقدم فوائد واعدة في مكافحة السرطان. لت تحقيق هذه الأهداف، تم إجراء المعايير التالية:

تم تحضير وتوصيف نانومستحلب الكركمين باستخدام تحليل TEM والمستوى البوتنشالي للأيونات وتحليل FTIR الدراسة المختبرية

تقييم التأثير المضاد للورم وامتصاص الخلايا وتأثير البروتينات البرمجة المحتمل لمستحلب النانو المحضر على مستوى الخلايا أن:

نانومستحلب CUR كروي الشكل بحجم جسيم متوسط قدره ١٧.٤٧ نانومتر بجهد زيتا -٧٦.٨ مللي فولت ومتوسط جهد هيدروديناميكي بحجم ١٧٣.٧ نانومتر.

له تأثير قاتل للخلايا في الدراسة -المختبرية ل خطوط الخلايا HepG2 لمستحلب نانو الكركمين ونظام حساس للضوء لنانومستحلب الكركمين المحفز بواسطة شعاع ليزر مما أدى إلى تركيز IC50 قدره ٦٠٠ نانوغرام/مل للخلايا المعالجة بنانومستحلب الكركمين والليزر، بينما أظهرت تلك المعالجة بنانومستحلب الكركمين وحده ٥٠٪ من الخلايا المعالجة