Original Article

Calcium Peroxide Nanoparticles from Capsicum against Colon Cancer

Diagnosis and Effectiveness of Calcium Peroxide Nanoparticles Prepared from Capsicum Plant Extract against Colon Cancer

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ABSTRACT

Objective: To diagnose the function of natural biomolecules in the biological reduction of metal salts during nanoparticle synthesis.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the College of Education for Pure Sciences/Ibn Al-Haitham at the University of Baghdad from 1st January 2024 to 31st March 2025.

Methods: Capsicum plant extract was used and treated with a readily available inorganic salt ($CaSO_4\,2H_2O$). It was used as a basic material to obtain particles.

Results: Calcium peroxide nanoparticles in the form of a yellowish-white powder were confirmed by using, UV, XRD, SEM, TEM, AFM, and EDX, confirmed that the compound is calcium peroxide nanoparticles with an average nano size of 31.288 nm. The effectiveness of these particles against colon cancer (HT-29) was clearly and reliably demonstrated by diagnostic tests and examinations of infected laboratory cells cultured.

Conclusion: The use of calcium peroxide nanoparticles represents a novel approach to cancer treatment, focusing on developing imaging and therapeutic methodologies.

Key Words: Green chemistry, Plant extract, Calcium peroxide nanoparticles, Cancer resistance

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INTRODUCTION

Capsicum, a globally prevalent vegetable crop, is utilized in nutrition either in its fresh or processed forms, as well as in numerous specialties, and extracts. The extract is abundant in proteins, phenolic, oils, aromatic molecules, lipids, vitamins, minerals, ascorbic acid compounds, and other chemicals.[1] The components, carotenoids and capsaicinoids, elucidate the significance of red capsicum cultivars and their fat extracts in the food and medicine sectors.[2]

Pepper's spiciness is controlled by capsaicinoids, which are made up of seven similar branched-chain alkyl vanillyl amides.

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The main compounds are capsaicin and dihydrocapsaicin, nordihydrocapsaicin, homo-dihydrocapsaicin, and homocapsaicin are found in smaller amounts.[3] In the last twenty years, there has been a huge rise in interest in figuring out and proving the effects of capsaicin. Researchers have looked at how capsaicin can help with analgesia, preventing cancer, controlling body mass via adipose thermogenesis, improving heart and digestive health, lowering blood sugar levels, and treating blood in the urine.[4] Capsaicinoids are very useful because they can be used as natural food ingredients, antimicrobials, and parts of self-defence products.[5]

The compounds that contribute to the profound red hues are capsanthin and capsanthin-5, 6-epoxide. Carotenoids exhibit various significant biological functions. They are powerful antioxidants that function as scavengers of singlet molecular oxygen, reactive nitrogen species, and peroxyl radicals.[6] Consuming foods rich in carotenoids diminishes the prevalence of various disorders, including malignancies, cardiovascular diseases, macular degeneration due to age, cataracts, immunological dysfunction-related diseases, and other degenerative conditions.[7]

Nano calcium peroxide is in the form of a white to yellow crystalline powder belonging to the group of superoxides. It is considered a strong oxidizer. Calcium peroxide exists as ($CaO_2.8H_2O$) and begins to lose water of crystallization on heating above 130°C.[8]

Tumor-induced hypoxia can lead to high cancer mortality rates. Furthermore, most cancer therapies require oxygen to function properly. Nanomaterials have demonstrated significant antitumor activity and low toxicity, due to the combination of chemotherapeutic effects.[9]

The primary global problem in the management and treatment of HT-29 is the premature identification and provision of effective therapeutic techniques. Numerous advancements have effectively utilized nanotechnology in conjunction with radiopharmaceuticals, rendering individualized treatment for HT-29 a feasible future trajectory.[10]

Nanoparticles exhibit distinctive characteristics, including diminutive size, varied forms, heightened sensibility, and tailored chemical formulations based on their nano-formula. These qualities enable their function as contrasting and coating materials in cancer imaging and therapeutics for the delivery of nanodrugs. Furthermore, they augment the biochemical and physiological comprehension of HT-29, rendering them attractive, innovative instruments forHT-29 care. Nonetheless, various hurdles impede the advancement of nanoparticles owing to their intricate pharmacokinetics.[11]

CaO_2 nanoparticles possess capability to function as an in real-time switch for independent thermoelectric dynamics treatment, attributed to their tumour environment (low pH)-specific thermal impact resulting from water release, a phenomenon that remains unexamined. The tumor-specific self-activated thermoelectric nano heterojunction, in conjunction with catalytic therapy, ion interference therapy, and immunotherapy, demonstrates superior anticancer efficacy in female mice. Overcoming these constraints necessitates additional study and exploration into nanoparticle design and the methodologies for radiolabeling in therapeutic applications.[12]

Cancer nanotechnology has revolutionized contemporary treatment modalities by offering enhanced cancer detection and therapies. It can be utilized for early-stage cancer detection and for the targeted delivery of chemotherapeutic agents to malignant cells.[13]

METHODS

This experimental study was carried out at the College of Education for Pure Sciences/Ibn Al-Haitham at the University of Baghdad from 1st January 2024 to 31st March 2025 vide letter No. 8360 date 10-11-2023. Ethyl alcohol (C_2H_5OH, ≥99.8%) was purchased from Honeywell (Germany), sodium hydroxide (NaOH) and calcium sulfate ($CaSO_4.2H_2O$) from Romil pure chemistry (United Kingdom), and Sodium borohydrate (H_4BNa) from Sigma-Aldrich, (USA). The solutions were prepared with distilled water and chili pepper (capsicum) plant extract after several preparatory procedures. A digital pH meter was utilised to assess pH. All compounds were of analytical quality.

Capsicum extract was obtained after the plant was chopped and washed with water to remove any dirt and impurities. The extract was transferred to a volumetric flask, and 250 mL of distilled water was added. The mixture was heated to 70°C with continuous stirring for 30 minutes, resulting in a green color. The mixture was filtered while hot, and 100 mL of the filtrate was taken and 50 mL of 0.1 M calcium sulfate dihydrate ($CaSO_4.2H_2O$) at 1:2 was added. The solution was stirred continuously and heated at 25°C. The color of the solution was light green. 0.1 M sodium hydroxide (NaOH) was added, followed by H_4BNa powder, with continuous stirring, resulting in a yellow precipitate. The precipitate was separated in a centrifuge for 10 min at 4000 rpm. The solution was filtered through filter paper. The precipitate was washed with hot deionized water and hot ethanol. The precipitate was allowed to dry for 24 h and then transferred to a dry oven at 285°C for 48 h. The final product was white. The data was entered and analyzed through SPSS-24.

RESULTS

The electronic spectrum showed an absorption peak for CaO_2 at (λ max= 371.7 nm) inthe UV region. This indicates that the color of the nano powder is white, yellowish within the visible area (Fig. 1).

In this context, the wavelength is implemented, where k denotes the form factor (0.9), θ signifies the diffraction angles, and β indicates the full width at half maximum. The phase structure of the sample was ascertained by X-ray diffraction (XRD). The XRD pattern exhibited peaks at 25.18°, 31.06°, 36.15°, 40.58°, 42.96°, 48.44°, and 52.00° corresponding to the cubic CaO_2 planes (110), (002), (200), (112), (211), (202), and (220) respectively. These results were compared with JCPDS file 01–085–0514, revealing no detectable impurity phases. The crystallite size of CaO_2 nanoparticles corresponding to the peak of greatest intensity was ascertained using (220) plane. Size of crystallite was determined using the Scherrer equation, resulting in a measurement of 20 nm. Structural characteristics of the synthesized CaO_2 nanoparticles were determined using equations derived from XRD data (Table 1). Ideal parameters were determined by the minimal size of particles (25.18 nm) and maximal crystallinity (distinct XRD peaks).

Techniques were used to examine the microstructure and surface characteristics of CaO2 nanoparticles. The scanned images obtained via SEM, illustrating a highly homogeneous and fine particle distribution with minimal agglomeration. The dimensions of the CaO_2 nanoparticles align with TEM images, which depict spherical particles. Nonetheless, aggregated particles canbe observed in the micrographs. Intergranular gaps represent an additional characteristic observable, signifying greater connectivity among the grains.

Using EDX analysis, the production of calcium peroxide nanoparticles, and different regions were identified during the analysis. The presence of calcium peroxide in the produced nanostructure was confirmed by EDX spectroscopy, with atomic ratios of 40% calcium and 55% peroxide. Although the synthesized samples contained impurities of hydrogen, oxygen, and carbon, and 5% plant extract residues (Fig. 2).

This technique is used to examine the size, shape, and structure of nanomaterials, as well as their dispersion and aggregation, the AFM histogram and statistical particle analysis of CaO_2NPs powder.

The nanoparticles have a cytotoxic effect against HT-29 cells, and can control untreated HT-289 cells and monitor the morphological changes in HT-29 cells after been treated with CaO2 NPs. Magnification power 10x (Figs. 3-4. These partic1es associate with cancer cells by producing reactive oxygen species, which influence protein expression and trigger cell cyc1e arrest and death. Moreover, they can augment the immune response, rendering them a va1uable asset in cancer therapy methodo1ogies.

Table No. 1: XRD analysis results of CaO_2 powder

2 Theta (degree)	Hkl	FWHM (deg)	2 Theta (Rad)	FWHM (Rad)	D (nm)	Matched by
25.1802	110	0.2539	0.219738	0.004	32.051	01-085-0514
31.0689	002	0.2962	0.271127	0.005	27.828	
36.157	200	0.3385	0.315529	0.006	24.769	
40.5866	112	0.3385	0.354185	0.006	25.013	
42.9608	211	0.2116	0.374903	0.004	40.338	
48.4496	202	0.2539	0.422802	0.004	34.301	
52.0052	220	0.2539	0.453831	0.004	34.803	

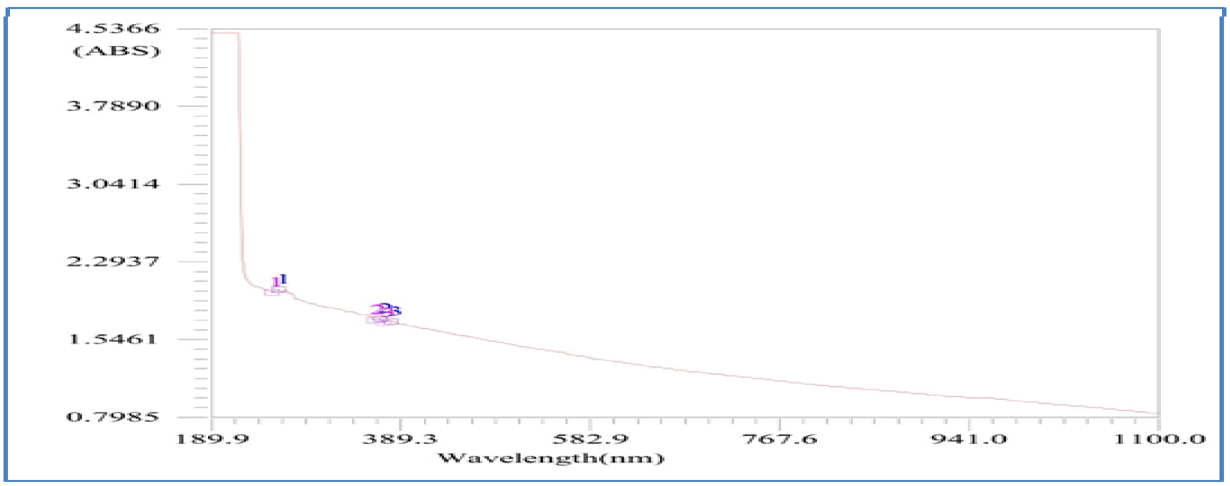


Figure No. 1: UV – Vis absorption spectrum of CaO_2NPs powder

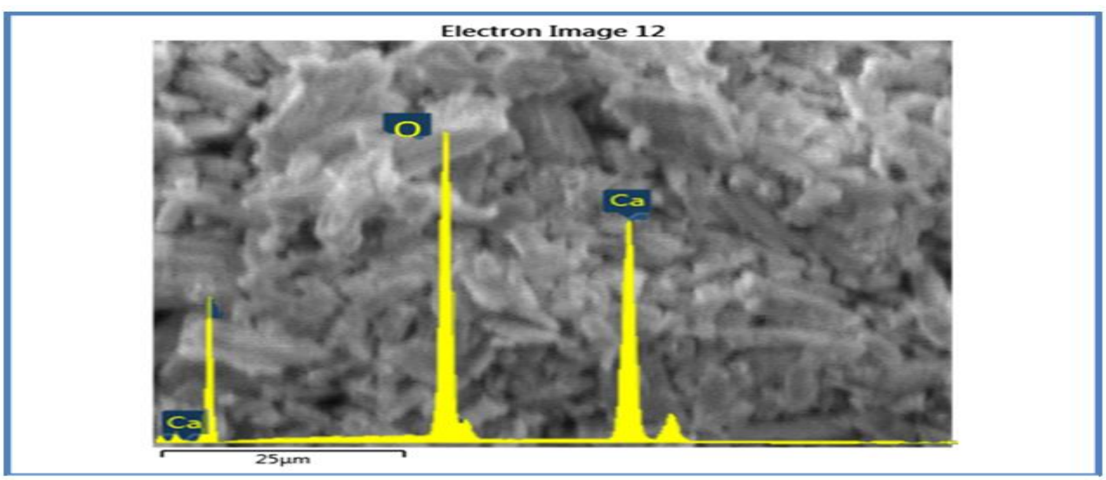


Figure No. 2: EDX analysis of CaO_2NPs powder

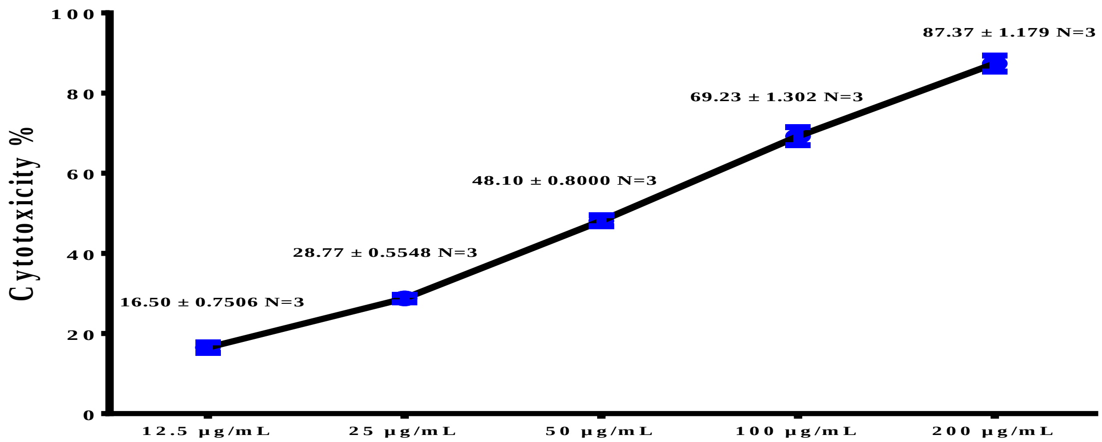
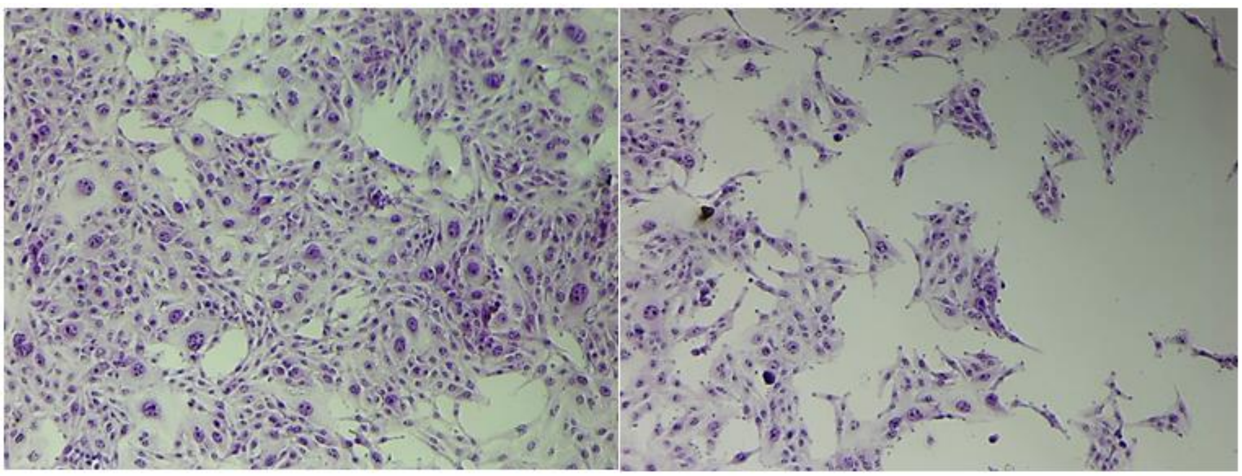


Figure No. 3: Cytotoxicity effect of CaO_2NPs in HT-29 cells



a b

Figure No. 4: (a) Control uninfected HT-29 cells. (b) Morphologica1 alterations in HT-29 cells following treatment with CaO_2 nanoparticles. Magnification power of 10x

DISCUSSION

The findings of the present study showed that HT-299 ranks among the leading causes of cancer-related mortality globally. The diagnosis and management of this condition encounter multiple hurdles. Traditional diagnostic techniques exhibit diminished accuracy in assessing tumor growth or metastasis, and the resultant treatments sometimes entail adverse side effects stemming from this inaccuracy. The application of calcium peroxide nanoparticles has introduced a novel paradigm in cancer therapy, focused on advancing imaging and treatment methodologies. The biomolecules present in capsicum plant extract of enable eco-friendly procedure and green synthesis for synthesis of CaO_2NPs.Capsium plant contains various bioactive compounds

The HT-29 cell line was cultured in RPMI-1640 enriched with 10% fetal calf serum, 100 units/mL of penicillin, and 100 µg/mL of streptomycin. Cells were transmitted with Trypsin-EDTA, reseeded at 80% concentration biweekly, and incubated at 37°C.[14] HT -29 is a form of cancer that impacts the colon (large intestine) or rectum. It is among the most prevalent forms of cancer globally and can result in significant injury and mortality. The likelihood of getting HT-29 grows with age, predominantly affecting individuals over fifty years old. One of the most important symptoms of this disease is low iron levels in the body.[15] While it may manifest at any age, it typically initiates as diminutive, benign cellular aggregates known as polyps that develop within the colon. Over time, some polyps may progress to colon cancer. In most cases, colon cancer begins as a small clump called an adenomatous polyp.[16] The cytotoxic effect of nanoparticles was assessed using the MTT assay conducted in 96-well plates. HT-29 cells were inoculated at a density of 1×10^4 cells per well. Subsequent to 24 h. Upon achieving a confluent monolayer, HT-29 cells were treated with nanoparticles at varying concentrations. Cell viability was assessed after 72 h and treatment by removing the medium, adding 100 µL of a 2 mg/mL MTT solution, and incubating the cells for 2.5 h at 37°C.[17] Subsequent to the removal of the MTT solution, the residual crystals in the holes were solubilized by the addition of

130 µL of DMSO (Dimethyl Sulphoxide), followed by incubation at 37 °C for 15 min with agitation. The absorbance was measured using a microplate reader at 492 nm; the test was conducted in triplicate.[18]

The use of calcium peroxide nanoparticles in colon cancer therapy is a promising research domain. This study's results emphasize the detection and efficacy of calcium peroxide nanoparticles derived from capsicum plant extract in combating colon cancer.Future research should concentrate on the advancement of more efficient calcium peroxide nanoparticles, specifically for the targeting of colorectal cancer biomarkers, alongside an examination of their safety and efficacy in clinical trials. The application of calcium peroxide nanoparticles in colon cancer therapy may enhance therapeutic efficacy and patient quality of life

CONCLUSION

The use of calcium peroxide nanoparticles represents a novel approach to cancer treatment, focusing on developing imaging and therapeutic methodologies. Bioactive molecules found in chili pepper extract enable environmentally friendly and green synthesis procedures for the production of calcium peroxide nanoparticles (CaO_2NPs). Chili peppers contain a variety of bioactive compounds.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hassan MA. Al-Redha, Waleed K. Mahdi
Drafting or Revising Critically:	Hassan MA. Al-Redha, Waleed K. Mahdi
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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