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RESEARCH ARTICLE OPEN ACCESS

Evaluation and effectiveness of sulfur nanoparticles against colon cancer prepared from capsicum plant extract

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Abstract
Objective: Using green chemistry, an effective, inexpensive, and environmentally safe method, sulfur nanoparticles with specific properties can be prepared and used in nanotechnology. This research aimed to prepare sulfur nanoparticles from chilli pepper extract and determine their effectiveness against colon cancer.
Method: Chilli pepper extract obtained from local markets was treated with aqueous sodium thiosulfate ($Na_2S_2O_7.5H_2O$). After mixing, it was continuously stirred, heated, and filtered. $NaBH_4$ was then added, resulting in a yellow precipitate. The precipitate was centrifuged, purified, and dried at 250°C.
Results: Standardised tests such as UV-Vis, XRD, SEM, TEM, AFM, and EDX were used, resulting in sulfur nanoparticles with an average nanosize of 38.7 nm that were effective against HT-29 colon cancer cells.
Conclusion: Sulfur nanoparticles prepared from chilli pepper extract using green chemistry have proven highly effective against colon cancer cells, following cell culture and clinical testing. Tests revealed that these nanoparticles exhibited strong resistance at this site, due to their uniquely small nanoscale size.

Keywords: Green chemistry, plant extract, Sulfur, nanoparticles, Cancer resistance

Plain English summary

The current study aims to identify modern methods for preparing unique nanoparticles using readily available, inexpensive, and safe plant extracts, which lead to the production of nanoparticles with numerous modern applications and significant properties. This is achieved through (studying the plant extract suitable for preparing nanoparticles using green chemistry ,studying the most suitable and least expensive inorganic materials that lead to reactions with important results in the preparation of nanoparticles ,studying the resulting nanoparticles, comparing them with previous literature, and determining their type and nanoscale size, and studying the anti-colon cancer applications of the particles prepared through this study). These studies aim to develop a rapid, safe, and environmentally friendly method with useful nanotechnological applications and scientific value that can be utilised in various medical, scientific, and other important fields.

Background

Single-nucleotide nanoparticles (SNPs) are gaining significant interest in the field of nanomaterials due to their multiple applications such as heavy metal adsorbents, antioxidants, antibacterial agents, and anticancer agents (1). They have also been utilised in numerous industrial applications, owing to their unique particle size distribution and surface properties, which are crucial for their potential applications (2).

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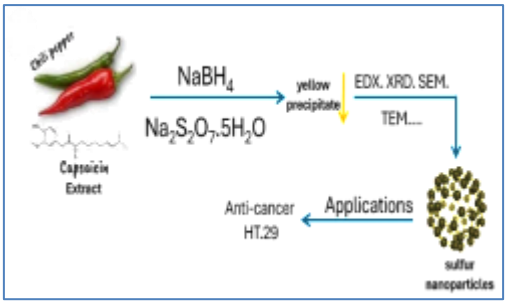

Scheme 1: the preparation method of Sulfur NPs

The complex disease known as cancer, which has long presented difficult treatment problems, requires innovative methods that can overcome the drawbacks of traditional therapies such as chemotherapy and radiation therapy, which sometimes cause serious side effects and produce unsatisfactory results (3). A viable paradigm for cancer diagnosis and treatment has emerged in this era of medical urgency: nanotechnology (4, 5). Engineering and manipulating materials on the nanoscale, usually between 1 and 100 nm, is made possible by nanotechnology (6). These nano materials' special physicochemical characteristics allow for unique interactions with cells and tissues, opening the way for new nanoscale targeting strategies that could revolutionise cancer diagnostics and treatment (7). The present cancer treatment environment highlights the urgent need for innovative approaches, and this study explores how nanotechnology may be able to meet this vital requirement (8).

Particles that range in size from one nanometer (nm) to one hundred nanometers (nm) are referred to as nanoparticles or ultrafine particles. Nanoparticles' small size and high surface area frequently contribute to their distinctive properties (9). The periodic limits of a crystalline particle become inconsequential when its size approaches the nanoscale and its characteristic length scale approaches or falls below the wavelength of light (10). Because of this, nanoparticles have different physical characteristics from bulk materials, opening a wide range of special applications (11, 12). SNPs have attracted particular attention from researchers in the fields of clinical therapies and the environment (13). When compared to bigger particles, SNPs' distinct and enhanced physico-chemical characteristics are responsible for these innovative uses. SNPs are in high demand among semiconductor nanomaterials because of their many uses as heavy metal adsorption material (14), antioxidant (15), antibacterial agent, and anticancer agent (16). The use of SNP doping in the modification of carbon nanotubes, lithium/sulfur battery cathodes, temperature sensing probes, infrared thermal imaging technology, and self-healing materials has also resurfaced (17, 18). Therefore, the need for precise synthesis of SNPs in terms of particle size distribution and surface characteristics is necessary for their possible use (19).

Nanomaterials were synthesised using an eco-friendly biosynthetic technique. Capsicum annuum extract was used in this procedure to decrease the sodium thiosulfate solution. Among the techniques used in the methodology were X-ray dispersion, energy-dispersive X-ray fluorescent spectrometry (EDXRF), field-emitted scanning electron microscopy (FESEM), the Fourier transform of infrared spectroscopy (IRS), reverberating sample magnetometry (VSM), and transmission electron microscopy for transmission. Using X-ray diffraction analysis, the nanoparticles' cubic shape and crystalline structure were identified, and their average particle diameter was found to be 38.7 nm (20, 21).

The human colorectal cancer cell line HT-29 has an epithelial appearance. The chemotherapy medications, 5-fluorouracil and oxaliplatin, which are common treatments for colorectal cancer, affect these cells. The HT-29 cell line is utilised as an in vitro model to investigate intestinal cell absorption, transport, and secretion, in addition to being a xenograft tumour model for colorectal cancer. These cells develop as an undifferentiated, nonpolarized multilayer under typical culture conditions (22). However, a differentiated and polarised morphology, marked by the redistribution of membrane antigens and the formation of an apical brush-border membrane, is the outcome of changing the culture conditions or administering different inducers to the cells (23).

Because of its capacity to display traits of mature intestinal cells, the human colon adenocarcinoma cell line HT-29 is gaining particular attention in research pertaining to food digestion and bioavailability, in addition to being utilised to investigate the biology of human colon malignancies. They can produce a monolayer with tight cell connections and a characteristic apical brush boundary in the differentiated phenotype (24). Although the enzymatic activity

is less than that observed in vivo, these differentiated cells also exhibit brush-border-associated hydrolases that are typical of the small intestine. Their resemblance to small intestinal enterocytes makes them a useful model, but this chapter also examines their drawbacks and applicability to in vivo setting (25). This cell line's use in transport studies of medications and food substances is demonstrated, particularly when the mucus layer's impact is taken into account or when it is co-cultured with $CaCO_2$ cells. They have also been widely employed to investigate the immunological response of the digestive tract to bacterial infection as well as the invasion, adhesion, and survival of microorganisms. Lastly, a summary of how these cells are used to assess the impact of various dietary ingredients and mucin secretion is provided (26).

Because cytotoxicity depends on several variables, including size, capping materials, nanoparticle dosage, and surface chemistry, our current understanding of it is limited. Before using these nanoparticles in therapeutic settings, a careful and comprehensive analysis is needed (27).

Materials and Methods

Synthesis and characterisation of Sulfur NPs (Figure 1).

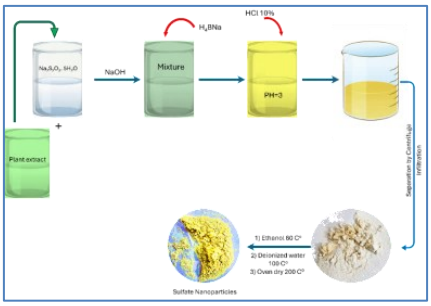

Figure 1: The preparation steps of powder sulfur NPs

Preparation of Capsicum plant extract

The Capsicum plant was obtained from local markets and washed with water to remove any dirt and impurities. Take (20 g) of the plant after spraying it and place it in a (500 mL) beaker. Add (250 mL) of deionised water. Then heat the mixture to (80C°) with continuous stirring for 30 min until the green colour of the mixture is obtained. The mixture was filtered while hot to get rid of impurities, then the filtrate was taken and placed in a centrifuge at 4000 rpm for 15 min to ensure the removal of the remnants of suspended fine materials.

$Na_2S_2O_3.5H_2O$

(M_w= 248.17 g/mol) CDH Central Drug House (P) Ltd. (India)

Preparation of Sulfur Nanoparticles (S-NPs)

4 g of NaOH was added to the 0.1 M solution by dissolving it in 100 mL of deionised water in drops. The pH value reached 12, and the colour changed to yellow. $NaBH_4$ was added to the solution with continuous stirring, followed by a few drops of 40% HCl. The yellow colour changed to colourless, then to yellow, forming a precipitate at pH = 3. The precipitate of the two solutions was separated into a centrifuge for 15 min at 4000 rpm. The precipitate was washed with hot deionised water and hot ethanol. The precipitate was left to dry for 24 hr. The precipitate was dried in a drying oven at 200 °C for 48 hr.

Results

The electronic spectrum showed an absorption peak for sulfur at (λ max 361 nm) in the UV region. This indicates that the colour of the nano powder is yellow within the visible area, as shown in Fig. 2 (28).

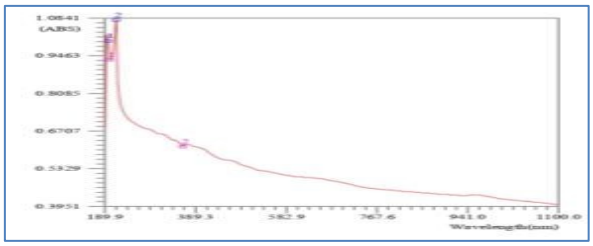

Figure 2: UV – vis absorption spectrum of sulfur NPs powder

XRD for the phase formation and crystallite size. As shown in the XRD pattern (Fig. 3), diffraction peaks can be indexed associated with (21°, 23°, 24°, 25°, 26°, 27° and 28°). They correspond to the crystal planes (220, 222, 133, 026, 311, 040 and 313) respectively.

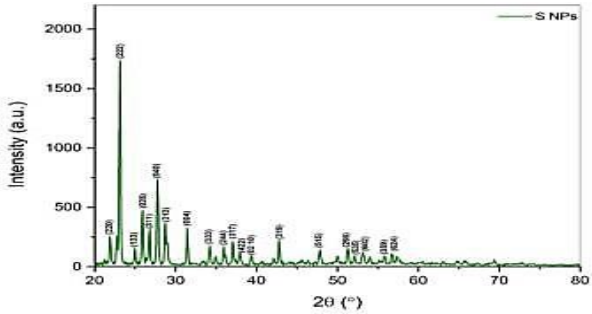

Figure 3: XRD of Sulfur NPs powder

The crystallite size is determined using Debye-Scherrer's method, d = K λ / β cos θ (29, 30). d: represented, Perpendicular to the average crystallographic dimension of the reflective phases, (λ: wavelength of x-ray), and (K: Scherer's constant) (0.92), to calculate the full width at half maximum (FWHM) (β) of the diffraction peaks. When instrumental broadening is excluded, a Bragg reflection's (FWHM: full width at half maximum) where (θ is the Bragg angle). The products' calculated average crystallite size was discovered in the range (38.73 nm. The sample's rhombohedral structure is revealed by the obtained diffraction to be crystalline, matching reference pattern JCPDS files:00 – 0010478 as shown in Table 1 and (Fig.4) (31).

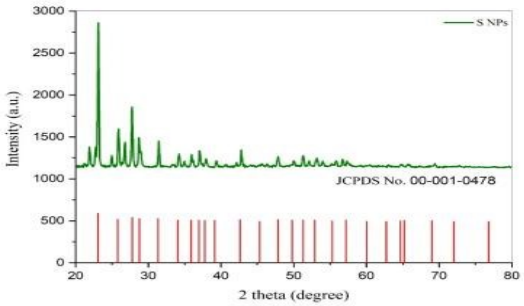

Figure 4: XRD of Sulfur NPs powder

Table (1) XRD analysis results of Sulfur powder

No.	2 theta (degree)	hkl	FWHM (deg)	2 theta (Rad.)	FWHM (Rad.)	D (nm)	Matched by
1	21.9144	220	0.1968	0.191239	0.003	41.101	00-008-0248
2	23.1191	222	0.1968	0.201753	0.003	41.187	
3	24.9717	133	0.1968	0.21792	0.003	41.329	
4	25.8852	026	0.246	0.225891	0.004	33.123	
5	26.7792	311	0.1968	0.233693	0.003	41.479	
6	27.7617	040	0.1968	0.242267	0.003	41.565	
7	28.7053	313	0.1476	0.250501	0.003	55.535	
8	31.444	004	0.1968	0.274401	0.003	41.920	
9	34.9547	333	0.1968	0.305038	0.003	42.305	
10	35.3901	244	0.1968	0.313549	0.003	42.420	
11	37.078	317	0.1968	0.323566	0.003	42.560	
12	39.3508	422	0.2952	0.3434	0.005	28.569	
13	42.7998	0210	0.1476	0.373499	0.003	57.786	
14	47.799	319	0.2952	0.417125	0.005	29.424	
15	51.2668	515	0.246	0.447387	0.004	35.805	
16	52.0826	266	0.1968	0.454506	0.003	44.911	
17	53.2212	535	0.3936	0.464443	0.007	22.566	
18	55.7805	602	0.2952	0.486777	0.005	30.436	
19	56.7142	359	0.246	0.494925	0.004	36.683	
20	64.8283	624	0.3936	0.565733	0.007	23.899	

Morphological study

SEM and TEM analysis showed (in Figures 5 and 6) that the size and the morphology of the synthesised Sulfur) was shaped like a sand area, it was a non-spherical shape and had a uniform size and distribution, with a particle size (22.566 – 57.786 nm). The average particle size is (38.73 nm) (32).

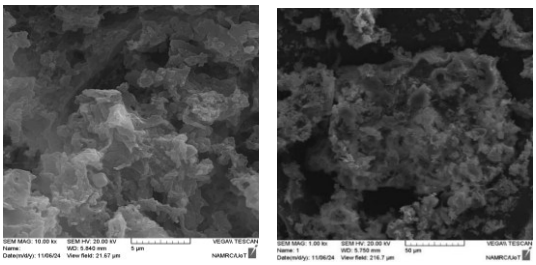

Figure 5: SEM for sulfur NPs powder

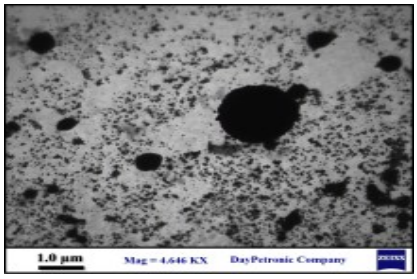

Figure 6: TEM for sulfur NPs powder

For EDX analysis (Figures 7 and 8), the appearance of a single peak is characteristic of nano-sulfur powder. The remaining peaks (C, O, N, H) are considered impurities because they are components of the plant extract used in the preparation process (33).

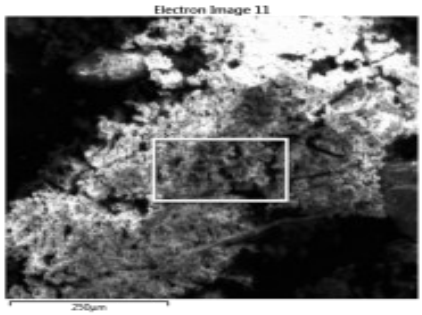

Figure 7: electronic image of EDX spectrum for Sulfur NPs powder

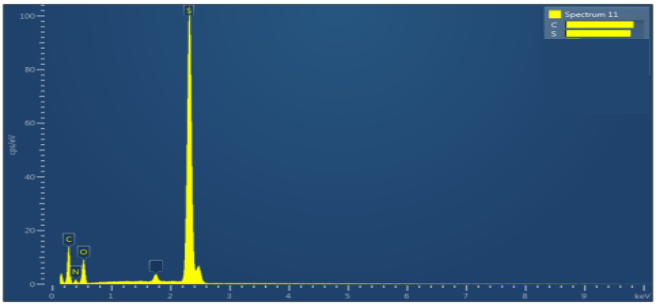

Figure 8: EDX result for Sulfur NPs Powder

AFM is a technique used to examine the size, shape, absorption and structure of nanomaterials as well as their dispersion and aggregation. Figure 9 shows the detection of particle analysis threshold and information with individual results and global statistics for nano sulfur powder. Table 2 and Figure 10 show AFM analysis results. Figure 11 shows the graph of AFM (34). AFM images are shown in Figures 12 and 13.

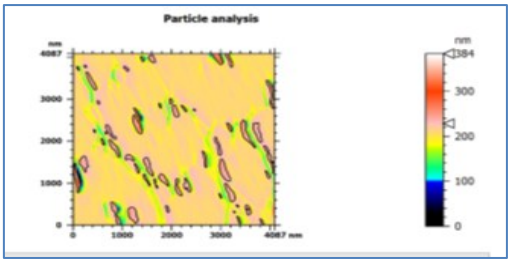

Figure 9: AFM particle analysis and threshold detection of Sulfur NPS powder

Table 2: AFM information and particular results with statistics of Sulfur NPS powder

Information				
Method	Threshold detection			
Threshold 1	228.5	nm		
Number of particles	71			
Coverage	5.576	%		
Density	4251587	Particles/mm²		
Individual results				
Parameters	**Projected Area**	Area	Mean diameter	Z-maxim...
Unit		nm²	nm	nm
Particle #1	Medium	33611	190.4	311.9
Particle #2	Small	18742	117.4	266.5
Particle #3	Small	15979	131.1	245.0
Particle #4	Small	4322	63.81	253.4
Particle #5	Small	2006	44.49	229.8
Particle #6	Small	2038	39.98	236.8
Particle #7	Small	5578	67.91	229.9
Particle #8	Small	72.23	5.938	230.8
Global statistics				
Mean	*****	**13217**	**84.27**	**244.7**
Min	*****	72.23	**5.938**	228.5
Max	*****	57042	**229.7**	384.0

Number of particles

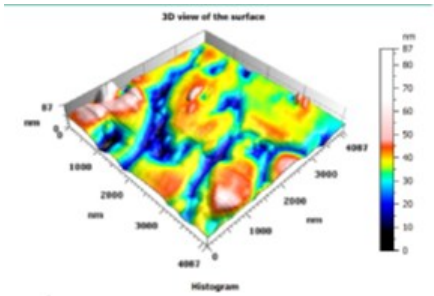

Figure 10: AFM image of particle threshold of Sulfur NPS powder

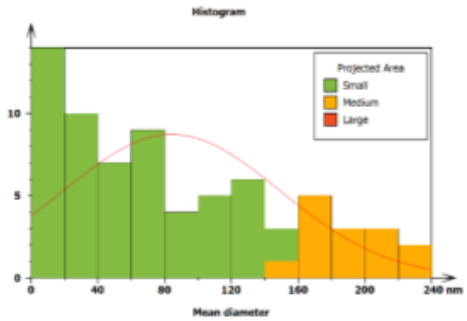

Figure 11: AFM histogram and statistical particle analysis of Sulfur NPS powder

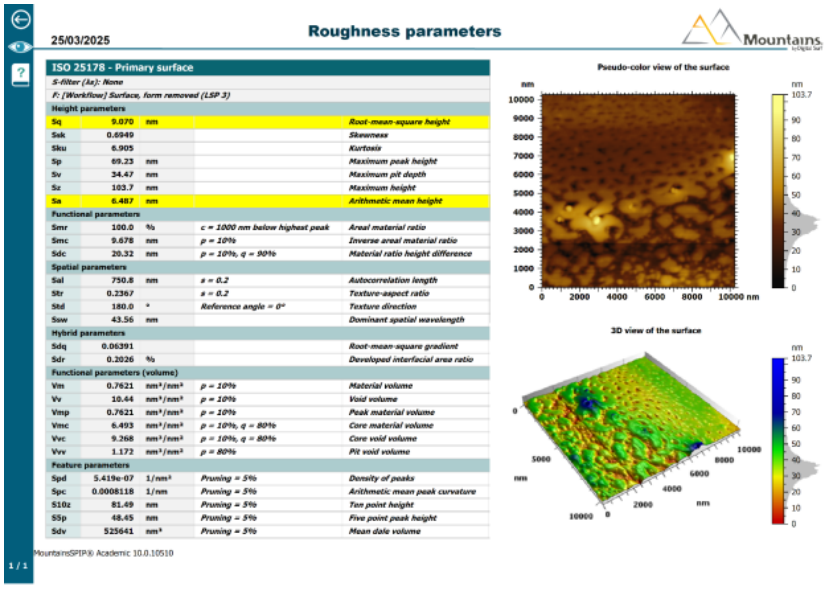

Figure 12: AFM images: (Roughness (S-L), and Primary surface) and their topography parameters of Sulfur NPs powder

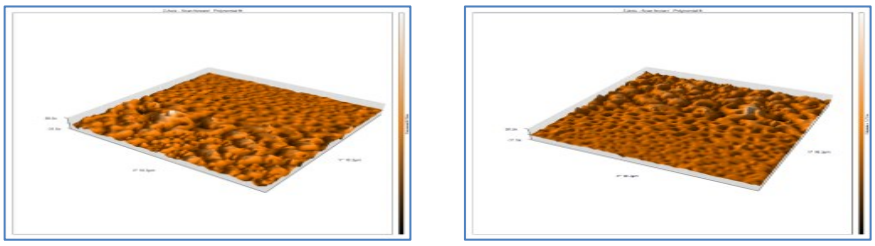

Figure 13: 3D images of the surface structure of Sulfur- NPs powder using AFM

Application

Tables 3 and 4 highlight the specific details relating to the materials, chemicals, reagents and instruments used.

Table 3: Materials, Chemicals and Reagents

No.	Items	Company	Country
1	Trypsin/EDTA	Capricorn	Germany
2	DMSO	Santacruz Biotechnology	USA
3	RPMI 1 640	Capricorn	Germany
4	MTT stain	Bio -World	USA
5	Fetal bovine serum	Capricorn	Germany

Table 4: Instruments

No.	Item	Company	Country
1	CO_2 incubator	Cypress Diagnostics	Belgium
2	Microtiter reader	Gennex Lab	USA
3	Laminar flow hood	K & K Scientific Supplier	Korea
4	Micropipette	Cypress Diagnostics	Belgium
5	Cell culture plates	Santa Cruz Biotechnology	USA

Maintenance of cell cultures

The HT-29 cell line was maintained in RPMI-1640 with 100 units/mL of penicillin, 100 µg/mL of streptomycin, and 10% fetal bovine serum added. Trypsin-EDTA was used to passage the cells, which were then reseeded twice a week at 80% confluence and incubated at 37°C (35, 36).

Cytotoxicity Assays

Using 96-well plates, the MTT test was performed to ascertain the cytotoxic effect of nanoparticles (37). The number of HT-29 cells seeded in each well was 1 × 104. 24 hr later. HT-29 cells were treated with nanoparticles at varying concentrations until a confluent monolayer was formed (38).

After 72 hr of treatment, the media was removed, 100 µL of a 2 mg/mL MTT solution was added, and the cells were incubated for 2.5 hr at 37 °C to determine the cell viability. Following the removal of the MTT solution, 130 µL of DMSO (dimethyl sulphoxide) was added to the wells to dissolve the crystals that remained, and the mixture was then shaken and incubated for 15 min at 37 °C while being shaken (39).

The absorbance was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation (40).

Inhibition rate = A- B/A*100

Where A is the optical density of the control, and B is the optical density of the samples

Statistical analysis

With GraphPad Prism 6, the acquired data were statistically examined using an unpaired t-test. The mean ±SD of three separate measurements was used to display the values (41).

Cytotoxicity of Nanoparticles against HT-29 cells

The MTT assay was used to examine the cytotoxic effect of nanoparticles on HT-29 cells. The findings showed that the nanoparticles were lethal to HT-29 cells, as illustrated in Figures 14, 15, and 16.

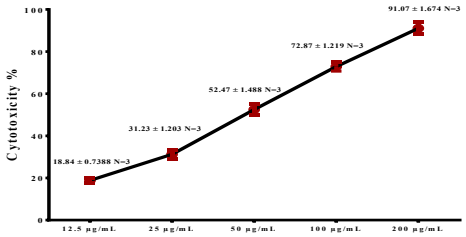

Figure 14: Cytotoxicity effect of (S NPs) in HT-29 cells

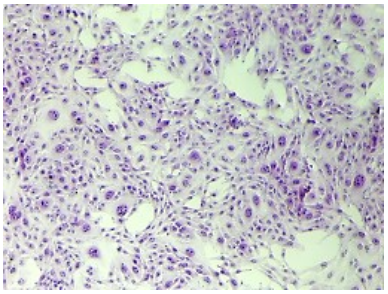

Figure 15: Control untreated HT-29 cells

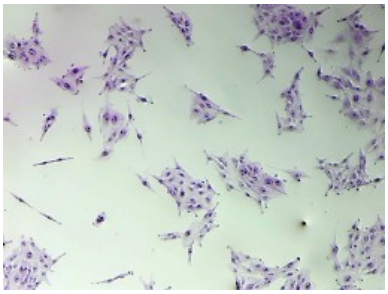

Figure 16: Morphological changes in HT-29 cells after being treated with S NPs, magnification power 10x

Discussion

The study highlights the pioneer approach for synthesising S-NPs using Capsicum annuum extract. According to U.V.-visible spectrum analysis -SNPs. XRD shows that the adsorption λ max at 361 nm, indicating successful formation of an average particle size of the synthesised S-NPs was found to be 38.73nm, which helps in bimedical applications of S-NPs.EDX spectrum analysis of Capsicum annuum extract contains a few phytochemicals that facilitate the reduction and stabilisation of S-NPs. AFM technique shows the detection of particle analysis threshold and information with individual results and global statistics for nano sulfur powder. The Cytotoxicity study of S-NPs against HT-29 cells showed that the nanoparticles were lethal to HT-29. Cells. Finally, this study suggests S-NPs as a potential biomaterial for biological, medical, eco-friendly, and safe processes.

Declarations

Ethical approval and consent to participate

The guidelines and instructions of postgraduate studies at Baghdad University were followed.

Consent for publication

All the author(s) gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this review will be made available by the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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There is no financial support for the submitted work, but rather the work was done through self-effort.

Author's contributions

Al-RHMA: Conceptualisation; Methodology; Supervision; Project administration; Resources; Writing – Review & Editing.

MWK: Investigation; Data curation; Formal analysis; Validation; Visualisation; Writing – Original Draft; Methodology.

Acknowledgments

When you intend to work on research, you have a lot of ideas. What type of research is most useful to me? Here is an explanation of the idea from Professor Dr Faleh Hassan Musa and Assistant Professor Dr Waleed K. Mahdi, which was the basis for this research, with encouragement from the professors close to me in the Department of Inorganic Chemistry at the College of Education for Pure Science - Ibn Al-Haytham, one of the members of the prestigious University of Baghdad.

List of Abbreviations

AFM: Atomic Force Microscope
CRC: Colorectal Cancer
EDS: Energy Dispersive Spectroscopy
HT-29: Human colon cancer cell line
NPs: Nanoparticles
SEM: Scanning Electron Microscopy
TEM: Transmission Electron Microscopy
UV-Vis: Ultraviolet–visible spectroscopy
XRD: X-Ray Diffraction

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