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A Study on Evaluation of Anticancer Activity (Liver Cancer) of TiO_2 Nanoparticles Synthesized Using Gigantea Leaf Extract

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Abstract: ***Aim and Objectives: This study looks at how titanium dioxide (TiO_2) nanoparticles, made using the leaves of a plant called* Gigantea*, can fight liver cancer using (HepG2) Cells.***
Materials and Methods: The nanoparticles were created using a green, eco-friendly method with the plant's leaf extract. We checked the shape, size, and nature of the nanoparticles using lab tools. The results showed that the TiO_2 nanoparticles made with* Gigantea *leaf extract were very effective against liver cancer cells. We reduced the growth of these cancer cells without harming normal cells. This means it can stop the cancer cells from multiplying. The characterization recorded from scanning electron microscope, SEM and XRD. It also supports environmental protection. The study shows a strong link between natural plant extracts and modern cancer treatments. This could be a new way to treat liver cancer in the future.
Result: The result recorded from MTT assay, ROS Production and DNA Fragmentation.
Keywords: Gigantea, TiO_2 nanoparticles, MTT, ROS, DNA.

I. INTRODUCTION

Liver cancer is one of the major causes of death worldwide. Current treatments like chemotherapy have many side effects and are often expensive and Physiological cell Death**[1]**. Scientists are now exploring new, safer ways to treat cancer using nanotechnology **[2]** and natural plants. Titanium dioxide (TiO_2) nanoparticles are tiny particles that can be used to kill cancer cells. Using plants to make these nanoparticles is a green and eco-friendly method. Gigantea is a medicinal plant known for its healing properties. In this study, Gigantea leaf extract was used to make TiO_2 nanoparticles. The aim was to check if these nanoparticles can fight liver cancer cells. This method could provide a natural and low-cost way to treat liver cancer. It also supports the use of herbal medicine in modern healthcare.

GIGANTEA LEAF

A. Plant Profile

Scientific Name: Colocasia gigantea
Family Name: Araceae (the arum family)
Synonyms: Alocasia gigantean, Colocasia esculenta var. gigantean
Common Names: Giant Elephant Ear,Giant Taro.

B. Classification

Kingdom: Plantae
Subkingdom: Tracheobionta (vascular plants)
Superdivision: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida (dicotyledons)
Subclass: Asteridae
Order: Gentianales
Family: Apocynaceae
Subfamily: Asclepiadoideae
Genus: Calotropis
Species: Calotropis gigantean

II. MATERIALS AND METHODS:

1) Collection and Authentication of Plant: Gigantea leaf was collected from Mallasamudram, Namakkal district, Tamil Nadu, India in the month of January 2025. The plant material was identified and authenticated by Dr.D.Vijayakumar Assistant Professor, Mahendra Institue of Technology Namakkal, TamilNadu, India.
2) Preparation of Plant Extract: Fresh leaves of Gigantea leaf was collected, cleaned, and used for extract preparation. Remove dust and other impurities. The clean leaves were then shade-dried at room temperature for about 7–10 days until all moisture was removed. After drying, the leaves were ground into a fine powder using a blender or grinder. About 10 grams of the leaf powder was mixed with 100 mL of distilled water[3] in a beaker. This mixture was then boiled at 60–80°C for 30–45 minutes to allow the active compounds to dissolve into the water. After boiling, the mixture was cooled to room temperature and filtered using Whatman No.1 filter paper or a muslin cloth to remove solid particles. The clear extract obtained was stored in a clean container and kept in a refrigerator at 4°C for further use in the synthesis of TiO_2 nanoparticles[4].
3) Synthesis of Tio_2 Using Gigantea leaf: 10ml of Gigantea leaf extract was mixed with Tio_2 Solution (90ml) in dark condition for 24hours. A change in the color of the solution from white to light yellow indicated the synthesis of Tio_2
4) Characterization of synsthesized Tio2 Nanoparticles: The synthesized TiO_2 nanoparticles were characterized using SEM and XRD techniques. SEM analysis revealed that the nanoparticles were mostly spherical or irregular in shape and ranged in size from 20 to 80 nm, with good dispersion and minimal clumping. XRD analysis confirmed that the nanoparticles were crystalline in nature. The diffraction peaks matched well with the standard pattern of the anatase phase of TiO_2. This phase is known for its high stability and biological activity. The average crystallite size calculated using the Scherrer formula**[5]** was found to be between 30 and 60 nm.

A. MTT Assay

Principle

MTT assay is based on the principle that metabolically active (living) cells can reduce a yellow-colored compound called MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into an insoluble purple-colored formazan product. This reduction occurs in the mitochondria of live cells through the action of specific enzymes. Dead or inactive cells cannot perform this reaction. The amount of purple formazan formed is directly proportional to the number of viable cells. After incubation, the formazan crystals are dissolved using a suitable solvent, and the absorbance is measured using a spectrophotometer. Lower absorbance indicates fewer living cells, showing cytotoxic or anticancer effects of the tested sample.

Materials required:

The MTT assay, liver cancer cells (e.g., HepG2) are cultured in DMEM medium with FBS and antibiotics. TiO_2 nanoparticles synthesized from Gigantea leaf extract are added to the cells. MTT reagent**[6]** is used to assess cell viability, and formazan crystals are dissolved in DMSO.

The absorbance is measured at 570 nm using a spectrophotometer to evaluate cytotoxicity. Sterile pipettes, PBS, and personal protective equipment are required to maintain a sterile environment.

B. Procedure

Cell Culture

Change liver cell linewere purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37°C.

MTT Assay

The Test sample was tested for in vitro cytotoxicity, usingChange liver cells by MTT assay. Briefly, the cultured Change liver cells were harvested by trypsinization and pooled in a 15 ml tube[11]. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into the 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with the Test sample in a serum-free DMEM medium[12]. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (10 µL of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope[13]. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min[14]. The absorbance for each well was measured at 570 nm using a microplate**[7]** reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

Formula Cell viability % = Test OD/Control OD X 100

C. ROS Production

Principle

The principle of ROS (Reactive Oxygen Species) production is based on the ability of TiO_2 nanoparticles to generate harmful oxygen molecules inside cells. When these nanoparticles enter liver cancer cells, they produce ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. These reactive molecules cause oxidative stress by damaging important cell parts like DNA, proteins, and membranes**[8]**. This damage disrupts normal cell functions and activates apoptosis, or programmed cell death. The increased ROS levels play a major role in the anticancer effect of TiO_2 nanoparticles.

D. Project Requirement

- To measure the DCFDA expression study on Hep-G2 cell lines by Fluorescence Microscopy.
- The sample named as follows:

Sl. No.	Sample Code	Concentration	Cell line
1	CG-TINPs	(45 µg/ml)	Hep-G2

Materials required

- Cell lines: Hep-G2- cell lines (NCCS, Pune)
- MEM medium (Himedia)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- 2',7'-dichlorofluorescein diacetate (H2DCFDA)
- 12 well culture Plate (Thermo Scientific)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 µl tips (TORSON)
- 96 Well Black/Clear Bottom Plate (#165305, Thermo)

.Equipments

- Centrifuge (Remi: R-8 oC).
- Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
- Ice bucket with cover. Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.
- 37°C incubator with humidified atmosphere of 5% CO2 (Healforce, China)
- Fluorescence Microscope (ICX41, China)
- Software: Image J

E. Steps Followed

- Culture cells in a 96-well glass bottom plate at a density of 10,000 cells/200 µl and incubate in a CO2 incubator at 37°C for 24 hours.
- Treat the cells with CG-TINPs as given in table and along with H2DCFDA (1µl in 1 ml media (10 µM) incubate the cells again for 24 hours.
- At the end of the treatment, remove the medium from all the wells and give a PBS wash. Remove the PBS completely.
- Observe cells under Fluorescence Microscopy with blue filter for excitation and detection at 535nm for green fluorescence.
- Images were analysed by Image J Software.

F. DNA Fragmentation

Principle

The principle of DNA fragmentation is based on the process of apoptosis, or programmed cell death, where the cell's DNA is broken into small pieces. When liver cancer cells are treated with TiO_2 nanoparticles synthesized using Gigantea leaf extract, the nanoparticles may trigger apoptosis. During this process, specific enzymes called endonucleases cut the DNA at regular intervals, creating fragments. These fragments can be observed as a "ladder" pattern when run on an agarose gel during electrophoresis. The appearance of this DNA ladder confirms that the cells are undergoing apoptosis**[9]**, indicating the anticancer activity of the TiO_2 nanoparticles.

G. Project Requirement

- To measure the DNA fragmentation study on Hep-G2 cell lines by Fluorescence Microscopy.
- The sample named as follows:

SI.NO.	Sample Code	Concentration	Cell line
1	CG-TINPs	(45 µg/ml)	Hep-G2

Materials Required

- Cell lines: Hep-G2- cell lines (NCCS, Pune)
- MEM medium (Himedia)
- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- Hoechst 33342 Cayman Chemical, USA
- 12 well culture Plate (Thermo Scientific)
- 50 ml centrifuge tubes (# 546043 TORSON
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000 µl tips (TORSON)
- 96 Well Black/Clear Bottom Plate (#165305, Thermo).

Equipments

- Centrifuge (Remi: R-8 oC).
- Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
- Ice bucket with cover. Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.
- 37°C incubator with humidified**[10]** atmosphere of 5% CO2 (Healforce, China)
- Fluorescence Microscope (ICX41, China)
- Software: Image J

H. Steps Follwed

Hoechst Dye Staining Protocol (DNA Fragmentation Assay)

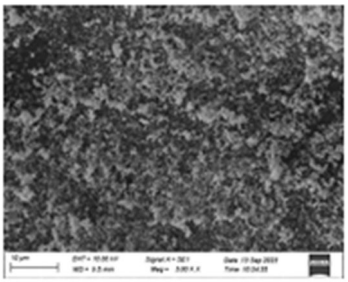
To prepare the Hoechst dye stock solution, dissolve 1 mg of dye in 10 mL of deionized water to achieve a concentration**[11]** of 100 µg/mL (1.23 mM). Due to its limited solubility, sonication may be required to ensure complete dissolution. The stock solution can be stored at 2–6°C for up to six months or at –20°C for extended storage. For staining, dilute the stock solution 1:2,000 in phosphate-buffered saline (PBS). After removing the culture medium, add a sufficient volume of the staining solution to cover the cells and incubate in the dark for 5–10 minutes. Following incubation, cells can be observed under a fluorescence microscope either directly in the staining solution or after rinsing three times with PBS (Sakayanathan et al., 2024a).

III. RESULTS & DISCUSSION

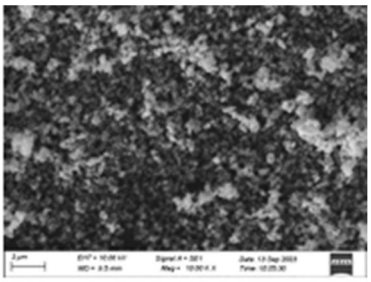
A. Characterization of synsthesized Tio2 Nanoparticles

1) Scanning Electron Microscopy (SEM)

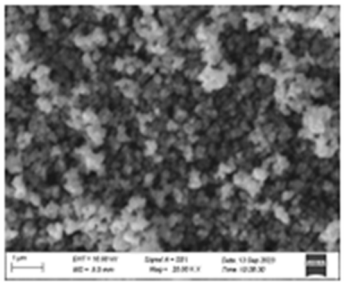
Scanning Electron Microscopy (SEM) is a technique used to study the surface structure and morphology of the synthesized TiO_2 nanoparticles. In this study, SEM was used to observe the shape, size, and distribution of nanoparticles prepared using *Gigantea* leaf extract. The images obtained from SEM showed that the nanoparticles were mostly spherical or irregular in shape and well dispersed, with sizes ranging from 20 to 80 nanometers. This confirms that the green synthesis method produced nanosized particles. SEM helps in understanding how the particle size and shape may influence their biological activity, especially in cancer treatment.



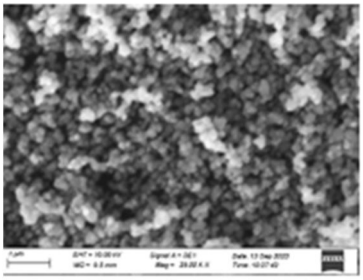
a) ×3,000 magnification



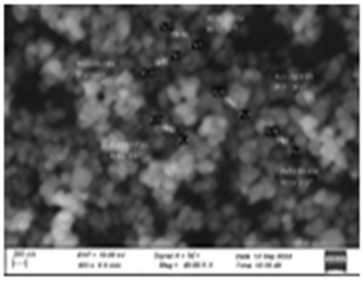
b) ×10,000 magnification



c) ×20,000 magnification



d) ×25,000 magnification



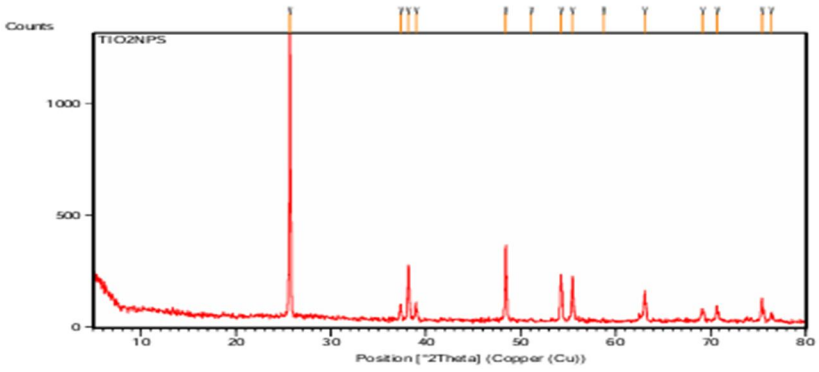
e) ×40,000 magnification

2) X-ray diffraction (XRD):

X-ray diffraction is used most frequently to investigate the structure of biocomposites with embedded nanostructure.

Measurement Conditions:

Dataset Name	TiO_2NPS
Raw Data Origin	BRUKER-binary V4 (.RAW)
Scan Axis	Gonio
Start Position [°2Th.]	5.0000
End Position [°2Th.]	80.1600
Step Size [°2Th.]	0.0400
Scan Step Time [s]	13.4400
Scan Type	Pre-set time
Offset [°2Th.]	0.0000
Divergence Slit Type	Fixed
Divergence Slit Size [°]	9999.0000
Specimen Length [mm]	10.00
Receiving Slit Size [mm]	0.1000
Measurement Temperature[c]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	30 mA, 40 kV
Diffractometer Type	Theta/Theta
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	91.00
Incident Beam Monochromator	No
Spinning	No



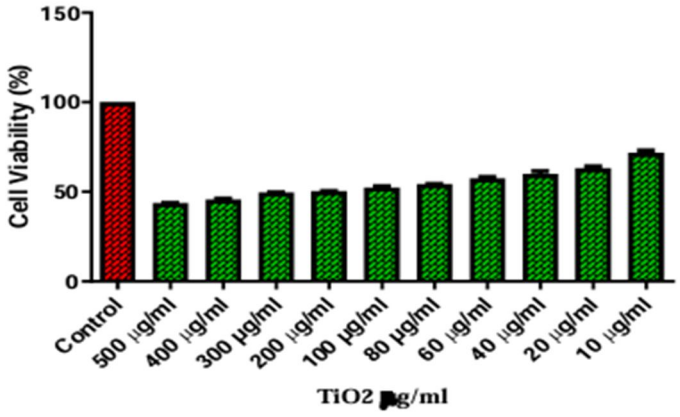
Peak List of XRD:

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
25.7039	1180.84	0.1181	3.46594	100.00
37.3352	68.06	0.1574	2.40859	5.76
38.1740	252.27	0.1574	2.35758	21.36
38.9627	79.99	0.1968	2.31165	6.77
48.4084	333.81	0.1968	1.88038	28.27
51.0752	10.33	0.4723	1.78829	0.87
54.2616	219.02	0.1574	1.69056	18.55
55.4423	202.34	0.1968	1.65733	17.14
58.7508	11.95	0.2362	1.57164	1.01
63.0669	139.36	0.1181	1.47408	11.80
69.1823	51.24	0.3149	1.35796	4.34
70.6650	75.06	0.1181	1.33306	6.36
75.4360	107.32	0.1181	1.26016	9.09
76.4292	40.18	0.3149	1.24624	3.40

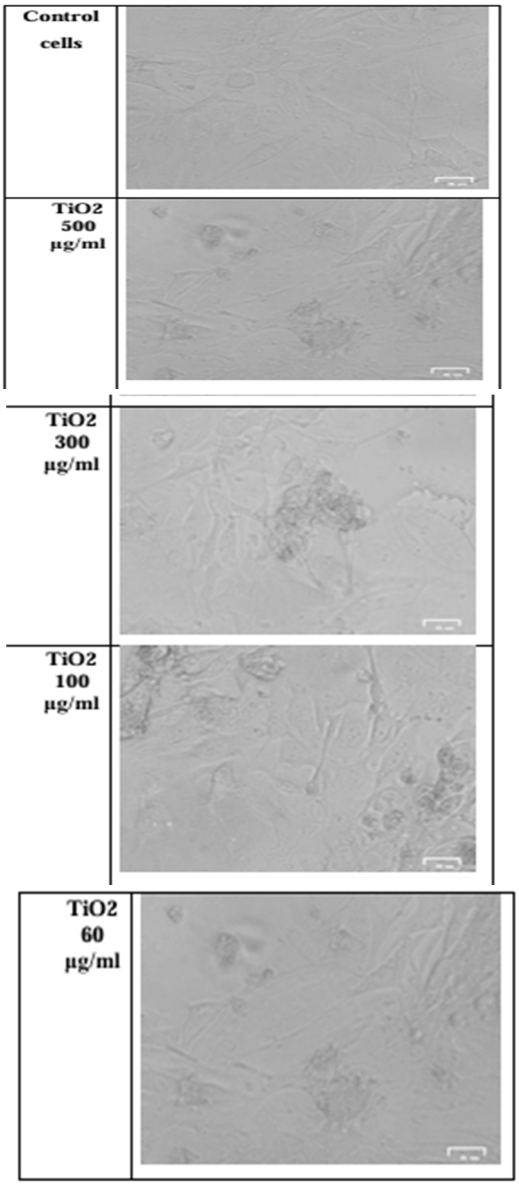
B. MTT Assay in HEPG2 Cells

1) OD Value at 570 nm

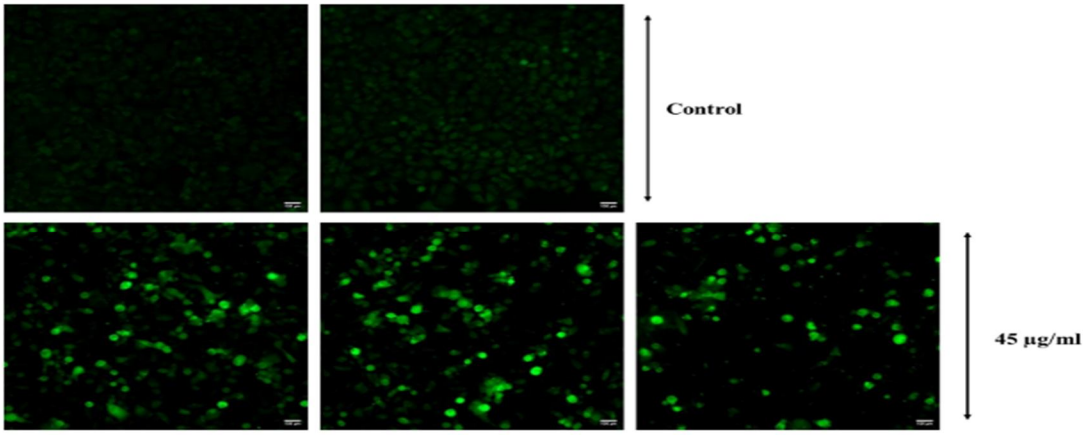
S. No.	Tested sample concentration (µg/ml)	OD value at 570 nm (in triplicates)		
1	Control	2.012	2.019	2.021
2	500 µg/ml	0.876	0.877	0.887
3	400 µg/ml	0.901	0.929	0.929
4	300 µg/ml	0.998	1.004	1.007
5	200 µg/ml	1.013	1.017	1.02
6	100 µg/ml	1.043	1.051	1.08
7	80 µg/ml	1.091	1.094	1.1
8	60 µg/ml	1.133	1.171	1.173
9	40 µg/ml	1.176	1.216	1.242
10	20 µg/ml	1.244	1.289	1.289
11	10 µg/ml	1.421	1.441	1.482



2) Images of control cells and treated cells.



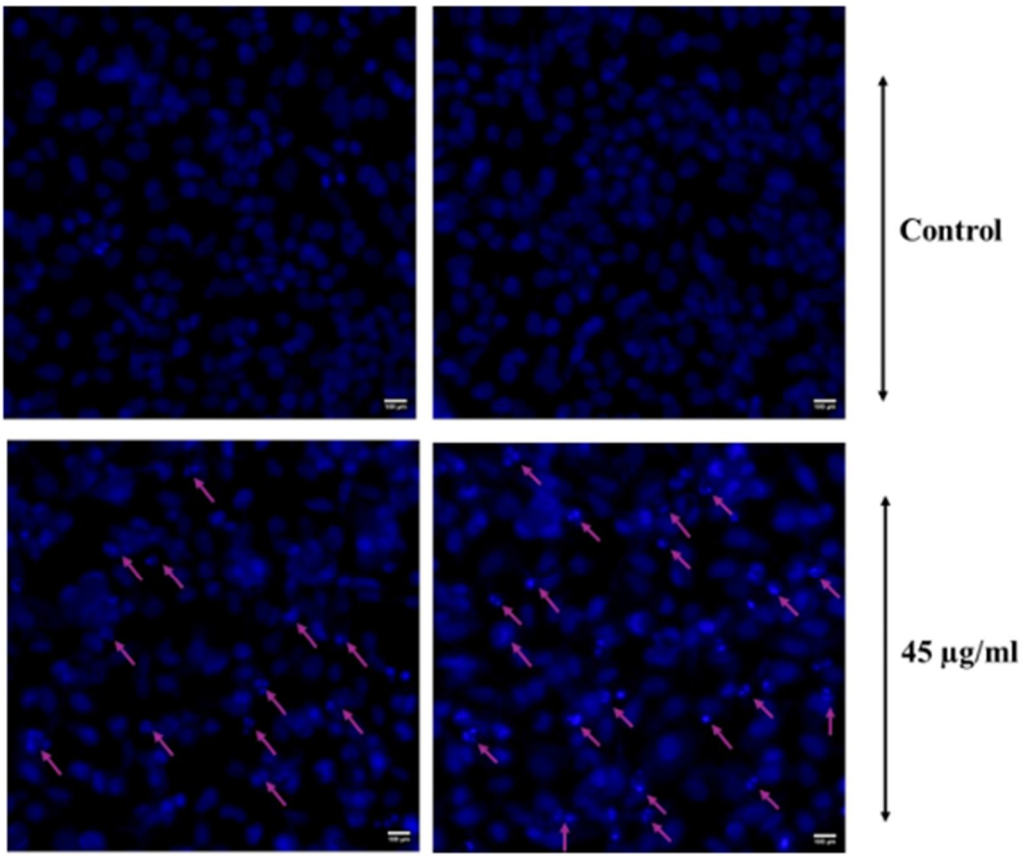
C. ROS Production



Interpretation

- The top row appears to show untreated or control cells. The fluorescence intensity is relatively low, indicating baseline ROS levels**[12]** under normal conditions.
- The bottom row shows cells treated with CG-TINPs. There is a notable increase in green fluorescence intensity compared to the control, indicating elevated ROS generation.
- The increased ROS generation indicates oxidative stress induced by the CG TINPs. This oxidative stress could potentially lead to cellular damage, apoptosis, or necrosis depending on the extent and duration of exposure.

D. DNA Fragmentation



Interpretation

- The image shows fluorescence microscopy images from a DNA fragmentation assay using Hoechst stain.
- The top row likely represents the untreated control cells. The nuclei appear uniformly stained with a consistent blue fluorescence, indicating intact nuclear morphology.
- The bottom row depicts cells treated with CG-TINPs. Notable morphological changes are evident, including condensed and fragmented**[13]** nuclei (indicated by the arrows).
- These fragmented nuclei display brighter and more intense fluorescence, characteristic of apoptotic cells undergoing chromatin condensation and DNA fragmentation.
- The observed nuclear condensation and fragmentation are hallmark indicators of apoptosis. The intensity and number of fragmented nuclei suggest that the CG-TINPs effectively induced apoptosis in HepG-2 cells**[14]**, with a dose-dependent or exposure time dependent increase in DNA fragmentation.

IV. CONCLUSION

The study demonstrated that TiO_2 nanoparticles synthesized using Gigantea leaf extract exhibit significant anticancer activity on HepG2 cellular lines. These findings suggest potential therapeutic**[15]** applications of the biosynthesized TiO_2 nanoparticles in liver cancer treatment.Its control the cancer cell growth**[16]**.

V. ACKNOWLEDGEMENT

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Conflict Of Interest

The authors declare that they have“ No Conflict of Interest”.

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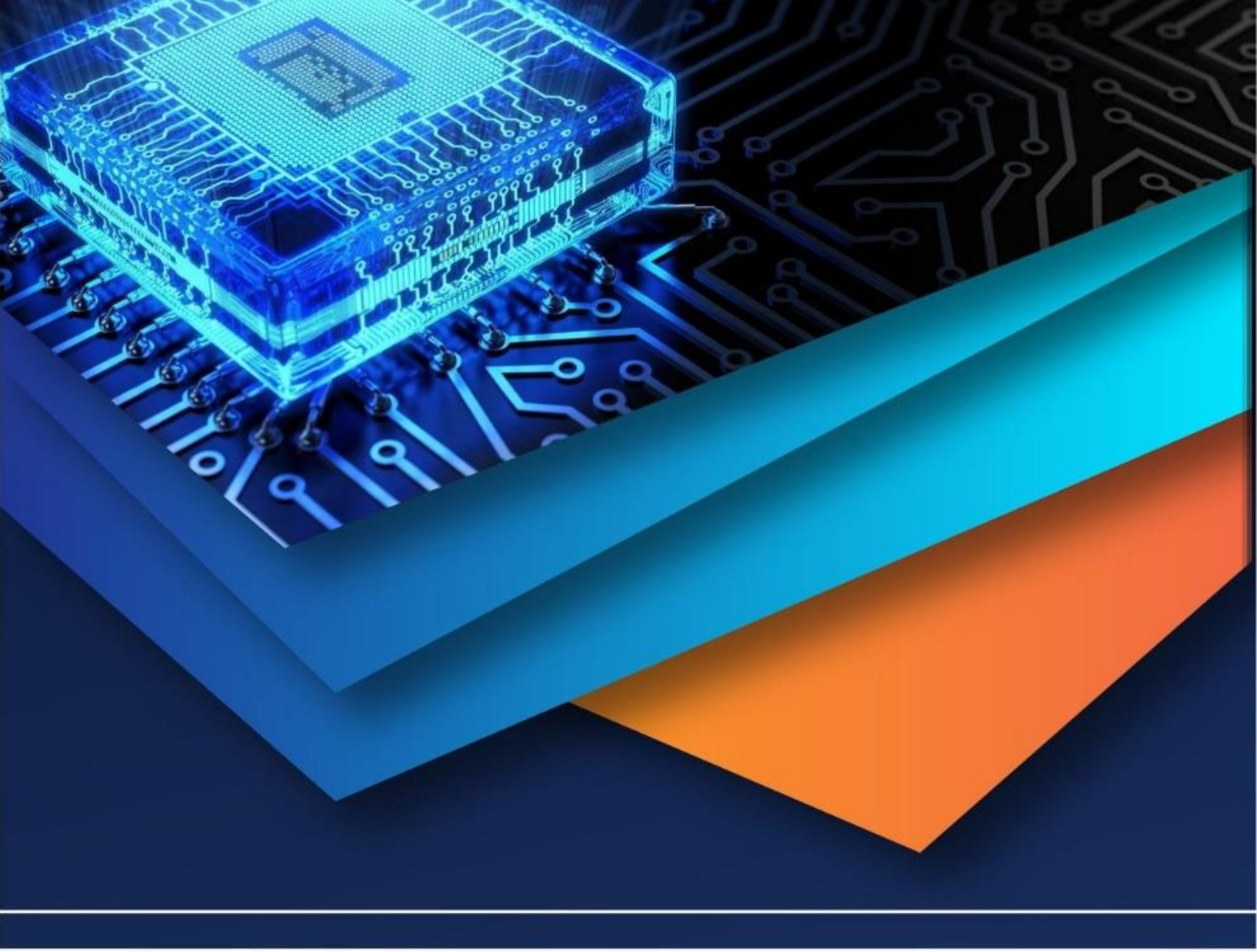

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