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Biosynthetic Copper Oxide Nanoparticles by Lacto Bacillusssp and its Anti-Cancer Activity against Human MCF-7 and PC3 Cell Lines

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Abstract . Recent development in nanotechnology Metal and metal oxides nanoparticles have a wide range of applications in a variety of fields, research institutes, and industries. Copper oxide nanoparticles are the most common metal oxides. (CuONPs) Thegoodcapabilityofcopper-tolerantprobioticof*Lactobacillusssp*toleratinghighconcentrationsof copper^{+2} andproducingCopperOxideNanoparticles, Because of its unique properties and applications, these bacteria have gained more interest as a natural microbial cell nano-factory for a more effective and environmentally friendly method of biosynthesis of these nanoparticles.The morphological and structural properties of CuO NPs weredeterminedbyX-raydiffraction,FieldEmissionScanningElectronMicroscope andTransmission Electron Microscopy (TEM) showedthatthesynthesizednanoparticleswerecrystalline,moderatelystable,roughlyspherical andpure , (CuONPs) biosynthesized by *Lactobacillus plantarum* show activity against human cancerous cell line MCF-7 and PC3 cell lines discerp moreactivity in less concenteration CuONPs on cancer MCF-7 compare PC3 be affected in highconcenteration of 6 CuONPs , The cytotoxic effect of CuO nanoparticles from *Lactobacillus planetarium*on normal cell line (WRL) was small on highconcenteration CuONPs.

Keywords:CuONPs,Lactobacillusplantarum,CuONPs Anti-cancer activity against human MCF-7 , CuONPsAnti-cancer activity against PC3 cell lines

1- Introduction

Nanotechnology is the creation,Materials that are produced, exploited, and synthesized on a scale smaller than 1 µm [1]. The term "nano" comes from a Greek word that means "dwarf" or "extremely thin." Nanoparticles are metal particles with a diameter of 1–100 nm and a variety of shapes such as pherical, triangular, rod, and others Sau and Rogach (2010).. They are used in biotechnology, nanotechnology, physics, chemistry, and other fields Rai et al., (2008).Lactic acid bacteria are Gram-positive, non-spore-forming Rods, Cocci, and Cocco-bacilli that produce lactic acid as one of the main fermentation products by using carbohydrates during fermentation. They are non-respiring but aerotolerant, fastidious, acid resistant, and catalase negative without cytochromes. to the retail [2].Lactic acid bacteria are one of the most important microbes in the fermentation and processing of food. Lactic acid bacteria are commonly used in the production of fermented foods such as kefir, cheese, butter, yogurt, and beverages, and they help to speed up the fermentation process .During sugar fermentation, LAB produces lactic acid and other organic acids, which lowers the pH of the atmosphere and thus inhibits the growth of unwanted microbial agents. They also help to keep food fresh by developing secondary metabolites such as lactic acid, fatty acid, and bacteriocin, which inhibit the growth of spoilage and pathogenic bacteria [3]. NanoCuO is cheaper than silver, easy to combine with polymers, and relatively stable in terms of chemical and physical properties, according to the limited knowledge available on its potential antimicrobial activity. CuO as well as other highly ionic nanoparticulate metal oxides, which can be prepared with extremely large surface areas and peculiar crystal morphologies, can be especially useful antimicrobial agent [4].CuO, TiO2, Fe3O4, ZnO, and NiO NPs are transitional metal oXides that have shown to be useful as advanced nano-substances in the fields of energy, biomedicine, and the environment.These NPs' high adsorption potential increases their performance and applications significantly[5]. Lactobacillus plantarum is used as an effective reduction and capping agent in the current research, which results in a low-cost, previously unreported, and simple method for the biosynthesis of CuO NPs. Lactobacillus plantarum is a gram-positive facultative anaerobic bacteria that is nonpathogenic. It has the largest genome among the lactic acid bacteria. Lactobacillus plantarum is

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electrokinetically negativeCuO NPs are biosynthesised using a potential that absorbs cations easily and acts as a catalyst[6].In addition to the previously listed applications, CuONPs have shown exclusive anticancer, antimicrobial, and antioXidant efficacy, making them a promising biomedical process[7,8].

2-Methodology

BacterialIsolate:.Lactobacillus ssp was preferred as a biological model for CuO NP synthesis because it is more effective. This isolate was collected from dairy products stored at the University of Babylon's Advanced Microbiology Lab. We cultured the isolate for 24 hours on MRS agar at 38°C, and it was diagnosed using the Vitek2 system

Copper oxide Nanoparticles' Biosynthesisby*Lactobacillusssp:* The *Lactobacillus ssp* pure culture was inoculated in a flask containing MRS broth that had been autoclave sterilized , at 37° incubated C for 24 hours at 100rpm. After the incubation time, we centrifuged the supernatant at 5000 rpm for 25 minutes. To delay the transformation process, the pH of the supernatant was controlled with 0.4 M NaOH (the pH of the supernatant is acidic 4.7 to be neutralweaddedtheNaOHtoreachpH7toeliminatetheinfluenceoforganicacids).20 g. 0.4MC(uNO33) $_2$H2O dissolved in 1000 ml distilled water, was added to 250 ml supernatant, and then heated for 5-10 minutes in a water bath at 85 ° C. The transformation process is indicated by the appearance of a black precipitate at the lowest of flask. The flask was then incubated at 37° C for 12 hours, during which time all of the particles collected at the bottom of the flask. To separate the black precipitate, the produce was centrifuged at 10000 rpm for 20 minutes and washed with D.W. The procedure was then repeated three times to obtain pure products, after which the product was dried at 60 ° C in a hot air oven for four hours [9].

The instruments that used in determining thepropertiesofCuOnanoparticlesbiosyntheticby*lactobacillusplantarum*:-

- X-raydiffractionanalysis(XRD).
- FieldEmission-ScanningElectronMicroscopy(FESEM).
- Transmission Electron Microscopy (TEM)

On Tumor Cell Lines, the Cytotoxic Effect of Copper Oxide Nanoparticles Synthesized *from L. plantarum* on (MCF-7& PC3) cancer human cell and WRL 68normal cell lineThis method was used to investigate the possible cytotoxicity of various concentrations of Cuo NPs nanoparticles synthesized from *L plantarum*. Maintaining Cell Lines [10].

A- After aspirating the growth medium, the cell sheet was washed with PBS.

B- A two to three mL trypsin/EDTA solution was applied to the cell. The vessel was turned over with gentle rocking to fully cover the monolayer. Before being removed from the vessel, the cells were allowed to incubate for 1 to 2 minutes at 37°C.

C- A fresh complete RPMI medium (15-20 ml) was added to the growth medium, and cells from the wedding surface were pipetted in.

D- Cells were redistributed to the target concentration in culture vessels, flasks, or plates, and incubated for 24 hours at 37°C in a 5% CO2 incubator. A haemocytometer was used to count the cells and assess their concentration.applying the formula:Total Cell Count/ml: cell count x dilution factor (sample volume) x104

MTT Protocol

Copper Oxide and Cuo NPs were tested for cytotoxicity using an MTT prepared to use kit (Intron Biotech):

(MTT solution 1 mL x 10 vials, Solubilization solution 50 mL x 2 bottles) are included in the package.

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Tumor cells (1x104–1x106 cells/ml) were grown in a final volume of 200ml complete culture medium for 24 hours per well in 96 flat well micro-titer plates. After being wrapped in sterilized parafilm, the microplates were gently shacked and set at 37°C with 5% CO2. After incubation, the medium was removed and the wells were filled with two-fold serial dilutions (25, 50, 100, 200, and 400 mg/ml) of the desired compound.The controls, as well as triplicates of each concentration, were used (cells treated with serum free medium). For the duration of the exposure time, plates were incubated at 37°C with 5% CO2 (24 hrs). After exposure, 10 ml of MTT solution was added to each well.. Plates were then incubated for another 4 hours at 37°C with 5% CO2. The media were carefully extracted, and each well received 100ml of solubilization solution for 5 minutes. At a wavelength of 575 nm, an ELISA reader was used to calculate the absorbance.The optical density data was statistically analyzed in order to determine the compound concentration needed .Each cell line's viability has been reduced by 50%.[31,32] through following equation:

$$Y= D+ A-D / 1+10 (x-logC)B$$

The data were processed and analyzed using one way ANOVA in the social science statistical program (SPSS 19), and the results were expressed as (Mean $\pm$S.D)[33,34] The log (Inhibitor) versus response curve is plotted in Graphpad Prism 6 using data analysis of MTT assays carried out in g/ml and log values of g/ml. For the most important IC 50 values, the best values were chosen.

3-ResultsandDiscussion

Copper Oxide Nanoparticlesbiosynthesisby *Lactobacillusssp:* Thebiosynthesisofnanoparticles from *Lactobacillus ssp*.The reduction of CuO into CuO NPs during exposure to bacterial extract, accompanied by a shift in color from brown to black over the course of 24 hours, has been confirmed by observing a change in solution color during the synthesis of CuO NPs.the black color is deposited at the bottom of the flaskcombining Lactobacillus spp. (*L.plantarium ,L.casi ,L.acidophilus and,L.bulgarius*) with C(uNO $_2$ 33)H2O dna done biosynthesis of CuO NPs by all these The color of the solution changes during the reaction, from light blue to green to dark brown to black at the end [11]strian show hange in color from brown to black and precipitate black color show the ability of all these strains to biosynthesis CuO NPs and completed CuO NPs is characterized by their optical and structural properties. Different ratios of CuO NPs were biosynthesised, but L plantarium was the most active species, according to the findings. The biosynthesis of CuO NPs in L plantarium is agreed with the results described on [12,13].*Lactobacillusssp*hasamajorrolein theCuONPs generatation . It's possible thatThe negative electrokinetic ability of bacteria, which readily absorbs cations and causes nanoparticle synthesis. Lactobacillus can also expand even when oxygen is present, allowing for greater metabolic growth potentialFurthermore, the presence of glucose in the MRS media used to synthesize CUONPs tends to reduce oxidation-reduction potential. All of these factors, including energy-producing glucose, work together to negotiate CuO NPs synthesis in the presence of Lactobacillus (which controls the value of rH2). medium pH ionic status, and full oxidation reduction potential (rH2) partially mediated tthrough the use of sodium hydroxide he final stage of CuO NPs biosynthesis by Lactobacillus plantarumdryingthesenanoparticlesintheoven[14].

Morphological & Structural properties of CUONPsbiosynthesizedby*Lactobacillusplantarum*:

X-Raydiffractionanalysis(hexagonalphase)show.synthesized nanoparticles were crystallineandpureinnature.respection lines of spherical CUONPs respectively, figure(1)The Scherrer equation was used to calculate the average particle size of CuO NPs [15]. using the full width at half maximum FWHM{16, }D=0.9λ/ $\Delta(2\theta)$.cos (θ).....Thus D is the size of the crystallite, k is the X-ray source's wavelength, and cos 16= 0.968583 The FWHM is beta, and the angle of diffraction is h. The average size of copper oxide nanoparticles has been determined using the formula to be the maximum diameter measured.for particles CuO NPs biosynthesized by L.plantarum is 18.84nm . , CuO NPs biosynthesized by L. acidophilus.17.4 nm and CuO NPs biosynthesized by L. casi is 17nm.Line extension of peaks is ana representation that the synthesized materials fall within the nanometer range(figure)1.*L. bulgarius* do not biosynthesized CuO NPs with out peak. The obtained nanoparticles

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(NPs) have a too small synthesis, which may be due to the biological synthesis method used to prepare them. demonstrating the biosynthesis of copper oxide nanoparticles on pure copper oxide planes The average crystallite size of biosynthesized copper oxide nanoparticles was calculated using the Scherer formula [16].

The Lactobacillus plantarum strain was used to make moderately stable CuO NPs. In the nanoparticles, the impact of response time is important. The shape, average size, and particle sizedistribution of copper nanoparticles were determined using the field-emission scanning electron microscopy characteristics of CuO. [20,21]. Some nanopolymers have been confirmed to be damaged by SEM. As a result, the Nanoparticlesneedto be ablewithstand the effects of vacuum pressure formorphological research with efficiency SEM.Microscopyimage of the CuONanoparticles is**Ambiguous**shows(ni figure.)3

The descriptionsin the number of functional groups involved in biosynthesis of CuO Nanoparticles are accessed using FTIR spectra. CuO NPs from L. plantarium, which were collected had prominent peaks in their FTIRspectral data3365 cm refer to Aliphatic primary amine ,1622.16 cm, Alkenyl c=c stretch , 1570 cm Aromatic ring , 1404.22 ,carbonyl compound , 1082 cm CN Stretch and 613 cm Alphatic iodo c-1stretch show fig3[17,18,19]

X-Transmission Electron Microscopy (TEM)

The TEM images CuO NPs nanoparticles shown in Figure (4). If the doping amount of metals increases, the particle size decreases. The size and crystallinity of the synthesized CuO NPs were described using Transmission Electron Microscopy, Dynamic Light Scattering was used to determine the zeta potential of the biosynthesized CuONPs (DLS). DLS was used to determine the average size of the nanoparticles, which was found to be between 20 and 30 nanometers. The average size of biosynthesized CuO NPs was discovered to be nm [22] .The best among other electron microscopy techniques for determining the morphological identities of CuONPs and other metal nanoparticles is transmission electron microscopy (TEM) [23]. In the TEM technique, a beam of energetic electrons is transmitted through an ultra-thin sample and interfaces, resulting in an image. [24].HRTEM (high resolution transmission electron microscopy) is a type of TEM that has a higher resolution and can image crystallographic structures at the nuclear scale. [25].

elbaT (1): X-Ray diffraction analysis biosynthesis CuO NPs from Lactobacillus spp. (*L.plantarium* ,*L.casi* ,*L.acidophilus and*,*L.bulgarius*)

Sample	Pos. 2θ(deg.)	FWHM	I/I_o	No. of peak	Average Crystal size (nm)
CuO NPs from	**44.0682**	**0.48880**	**79**	**32**	
L. plantarium	**64.4091**	**0.48040**	**99**	**45**	**18.84±1.28A**
	77.5108	**0.56940**	**100**	**48**	
CuO NPs from	**44.0625**	**0.50540**	**85**	**36**	
L.acidophilus	**64.4106**	**0.48650**	**84**	**47**	**17.4±0.96B**
	77.5353	**0.56570**	**100**	**51**	
CuO NPs from	**44.0610**	**0.53180**	**74**	**32**	
L.casi	**64.4173**	**0.51050**	**88**	**47**	**17±1.02B**
	77.4960	**0.53830**	**100**	**55**	**Average Crystal size (nm)**
$LSD_{0.05}$					**1.08**

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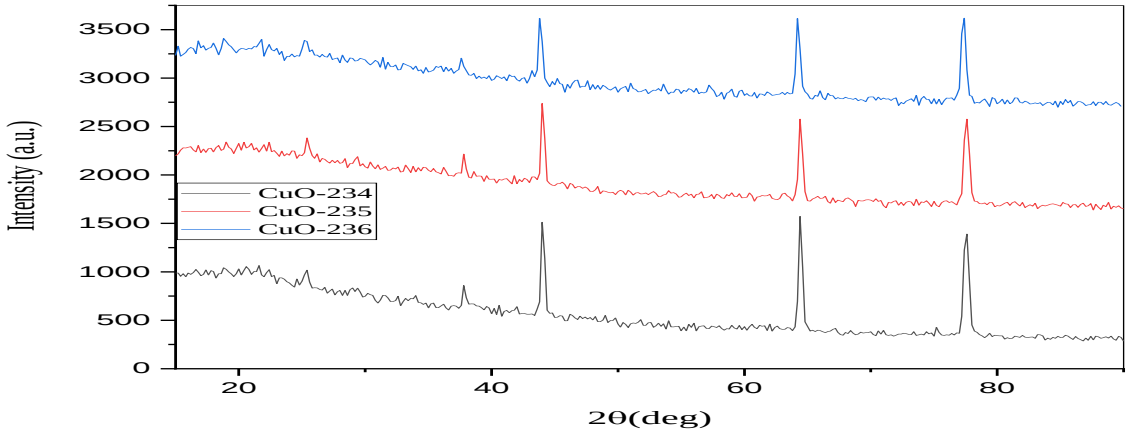

Figure1. XRD analysis for a. CuO NPs biosynthesized by *L. plantarum*. b. CuO NPsbiosynthesized by *L. acidophilus*. c. CuO NPsbiosynthesized by *L. casi*

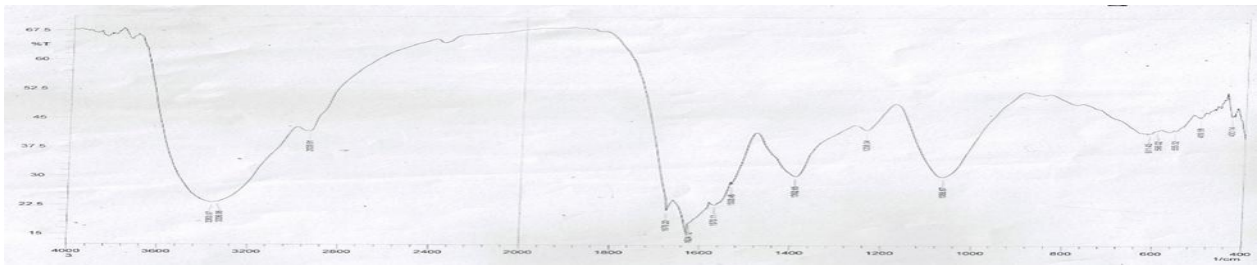

Figure (3) FTIR results biosynthesized CuO NPs From *L. planetarium*.

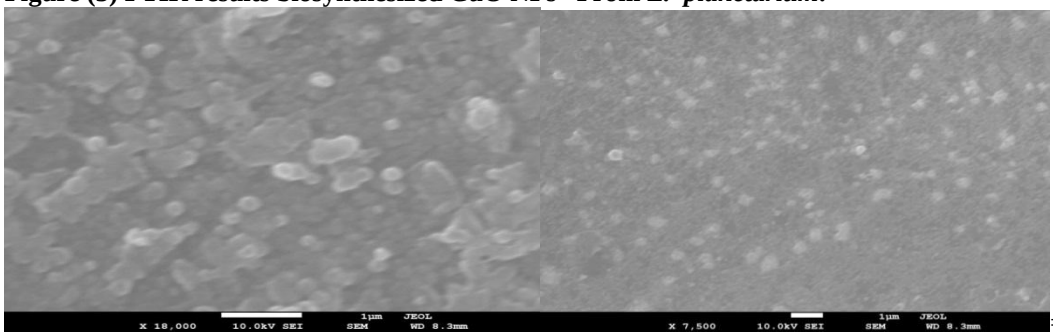

Figure2.FieldEmissionScanningElectronCuO NPs biosynthesized *by L. plantarum*

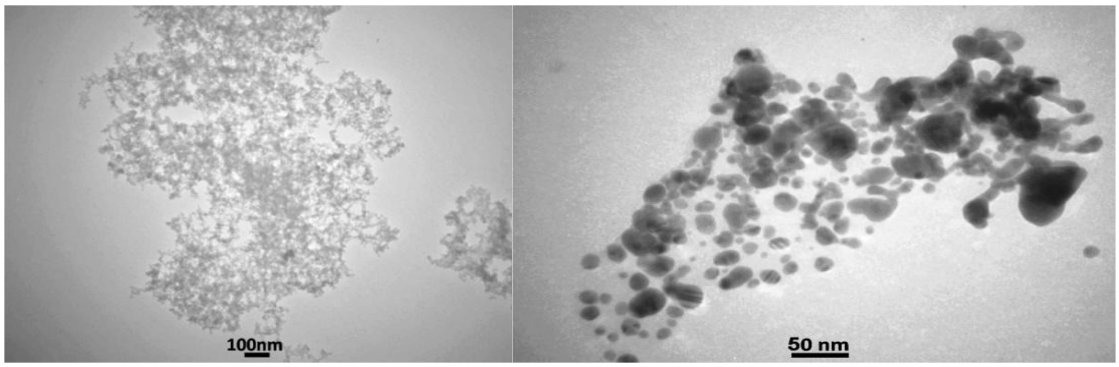

Figure4.TEM micrographs of Cuo NPsbio-synthesized *by L.plantarium*

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Anticancer activity of Cuo NPsby using MTT analyze

Cuo NP was tested for cytotoxicity using the 3-(dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) stain on two cell lines: MCF-7 breast cancer cell line and WRL-68 human hepatic cell lineUsing different concentrations of Cuo NP on tumor cell lines, this assay was used to determine cell viability and inhibition rate. MTT colorimetric assays were used to assess cell viability at each time point .Cuo NPs synthesis by L.plantarium at various concentrations (25, 50, 100, 200, and 400)g/ml resulted in a dose-dependent reduction in cell viability on MCF-7 and WRL-68 cells, as shown in table (2) and Fig (5). LSD0.05 (Fig. 6). The data was analyzed in g/ml, and the log values of g/ml were plotted in Graphpad Prism 5 using the log (Inhibitor) versus response curve. For the most important IC50 values, the best values were chosen. MTT colorimetric assays were used to assess cell viability at each time point . On a regular cell line, graveolens (WRL) The results showed that incubating WRL cells with CuO NPs and acontrol for 24 hours at different concentrations ranging from 50 to 400g/ml showed Low reduction in cell viability as compared to cancer cells, with a dose-dependent pattern in which cell viability decreases as CuO NP concentration rises.[26] Extract CuO NPs at a concentration of 400g/ml had the lowest WRL cell viability (66.86 percent). The concentration 400 g/ml reduced MCF-7 cell viability by 34.07 percent on average, while WRL-68 cell viability was 66.86 percent, and MCF-7 cell viability was (82.45, 63.58, 54.48, and 36.06 percent) respectively.and the viability of the same CuO NPs on WRL-68 are (94.25,96.18,87.11 and72.92%), respectively. The IC 50 for WRL-68 are (153.0), while IC50 of MCF7 cell lines was 63.64 µg/ml.Table(2): CuO NPssynthesis from L.plantarium on control human liver cell line (WRL-68) and (MCF-7) cell line toxicity increases with increasing CuO concentration, reaching a limit at 50 g/mL. (85.9%). CuONPs biosynthesized from black bean extract were tested for anticancer activity using the sulforhodamine B analyze, which showedWhen incubated with CuONPs, there are a few variations in mitochondrial structure. CuONPs also stopped cervical carcinoma cells from growing[27]. According to a study, CuONPs derived from Ficus religiosa inhibit the growth of A549 adenocarcinomic cells basal epithelial cells. [28] Based on the mortality data obtained from this study, we were able to predict their capacity as cytotoxic agentsAntitumor activity has been demonstrated for flavonoid compounds, especially quercetin and genistein; these compounds were toxic to cancer cells but had not effect on normal cells [29].

Table(2): Represents the activities Cuo NPssynthesis from *L.plantarium* . on control human liver cell line (WRL-68) and breast cancer cell line (MCF-7)

CuO NPs Conc. µg/ml	Viability % of MFC-7 mean± SD	IC_{50} of MFC-7 µg/ml	Viability % of WRL-68 mean± SD	IC_{50} of WRL-68 µg/ml
25	82.45±2.53A	63.64	94.25±1.36A	153
50	63.58±3.22B		96.18±1.25B	
100	54.48±3.83C		87.11±3.65C	
200	36.06±4.41D		72.92±2.20D	
400	34.07±2.49D		66.86±3.35E	
$LSD_{0.05}$	2.78		1.65	

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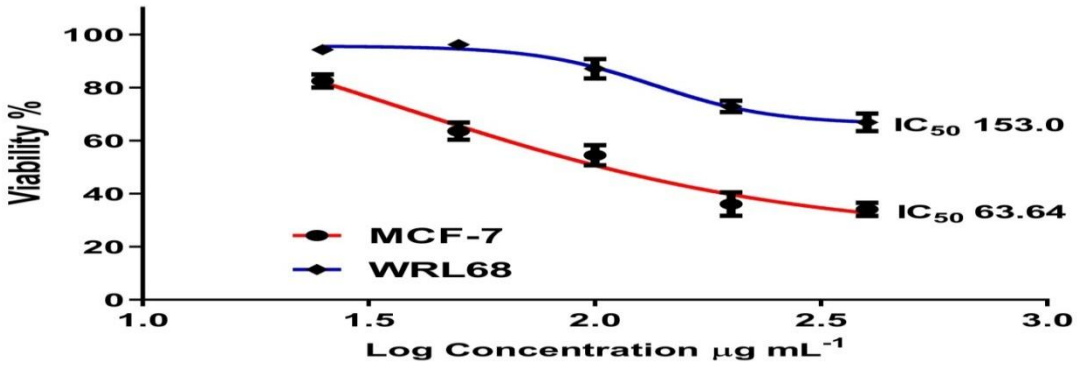

Figure (5): displays the IC50 of Cuo NPssynthesis from activities on control WRL-68 and breast cancer cell line MCF-7.

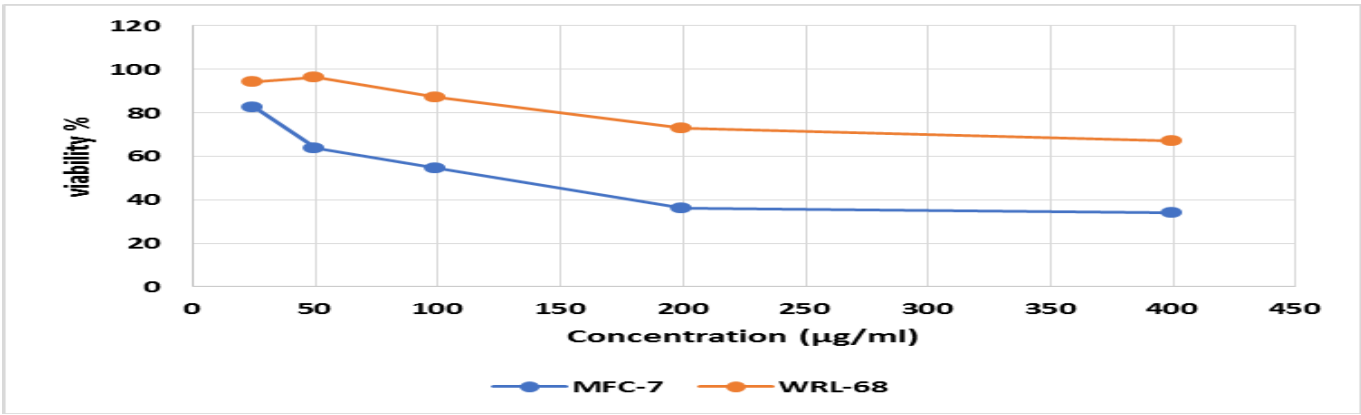

Figure (6) viability cell percentage of different concentrations of CuO NPs on MFC-7 and WRL-68 cell line.

Cuo NP was tested for cytotoxicity using the 3-(dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) stain on two categories of cell lines: prostate cancer cell line (PC3) &human hepatic cell line WRL-68. By applying different concentrations of Cuo NPs to tumor cell lines, this assay was used to determine cell viability and reserve rate. Cuo NPssynthesis *by L.plantarium* used in different concentration (25, 50, 100, 200 and 400)µg/ml .In a dose-dependent manner, induced a decrease in cell viability on PC3 and WRL-68 to showed the viability and cytotoxicity of Cuo NPs as in table (3) and Fig.(7) Fig (8)$LSD_{0.05}$. The results showed that incubating WRL cells with CuO NPs and acontrol at different concentrations ranging from 50 to 400g/ml for 24 hours resulted in a low reduction in cell viability as compared to cancer cells in a dose-dependent fashion, with cell viability decreasing as the concentration of CuO NPs increased. Extract CuO NPs at a concentration of 400g/ml had the lowest WRL cell viability (66.86 percent). The viability of the PC3 cell line is 51.94 percent, while the viability of the WRL-68 cell line is 72.34 percent, and the viability of the other concentrations is unknown. (25, 50, 100 and 200) µg/ml on MCF-7 cell line is (93.52,85.69,77,28 and 61.92 %) respectively, and the viability of the same CuO NPs on WRL-68 are (94.44,93.79,85.49 and 80.80 %), respectively. The IC 50 for WRL-68 are (221.2), while IC50 of PC3 cell lines was 143.3 µg/ml ,The antiproliferative profile was improved by increasing the CuNPs concentration[30] .The antitumor activity of Cu NPs was studied in the A549 cell line, and it was found to have excellent antiproliferative activity with an IC50 of 120 g/mL CuNPs concentration. Biological and nonbiologically synthesized NPsIC50 values for cervical HeLa human cancer cells were 78.9, 66.4, 71.8, and 85.5 g/mL, respectively, and 88.6, 82.9, 77.5, and 91.7 g/mL for PC3 human prostate cancer cells were 88.6, 82.9, 77.5, and 91.7 g/mL, respectively. were found for nonbiologicallyNanoparticles of Ag, Au, Co, and Cu were synthesized[29].

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Table(3): Represents the activities Cuo NPssynthesis from L.plantarium . on control human liver cell line (WRL-68) and prostate cancer cell line (PC3).

CuO NPs Conc. µg/m	Viability % of PC3 mean± SD	IC_{50} of PC3 µg/ml	Viability % of WRL-68 mean± SD	IC_{50} of WRL-68 µg/ml
25	93.52±1.1A	221.2	94.44±0.31A	143.3
50	85.69±3.94B		93.79±0.93A	
100	77.28±2.49C		85.49±3.37B	
200	61.92±2.61D		80.8±4.47C	
400	51.94±0.85E		72.34±0.69D	
$LSD_{0.05}$	2.78		1.65	

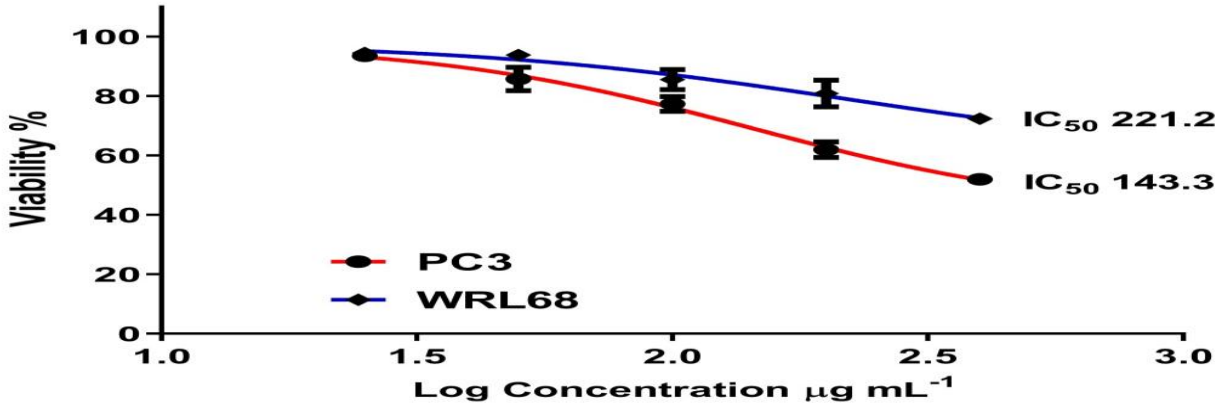

Figure (7): displays the IC50 of Cuo NPssynthesis from activities on control WRL-68 and prostate cancer cell line 3CP

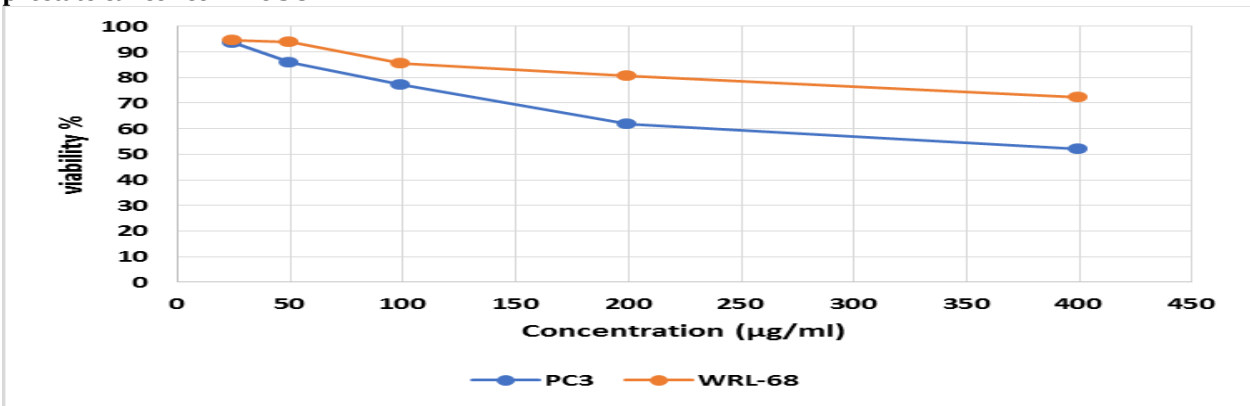

Figure (8) viability cell percentage of different concentrations of CuO NPs on PC3 and WRL-68 cell line.

4-Conclusion

The method used in the biological and catalytic applications of Lactobacillus ssp in the synthesis of CuONPs, which provides a great opportunity to medicinal institutes biological activity and mode of synthesis from Lactobacillus ssp,which provides medicinal institutes with a great opportunity Our actual situationresearch into the biosynthesis of CuO NPs by Lactobacillus Plantarum and the use of CuO NPs asanticancer has yielded excellent results with few complications and a low cost.It is well highlight the role of recent characterization techniques in evaluating the identities of CuONPs.CuO-NPs have a greater effect on MCF7 cell lines than PC3 cell lines, and CuONPs have more activity on MCF7 cancer cell lines. On high concenteration CuONPs, the cytotoxic effect of CuO nanoparticles from Lactobacillus planetarium on a normal cell line (WRL) was minimal. The synthesis and toxicity of the compound are

well explained. More research should be done to improve CuONPs' biological applications are being explored by focusing on ways to reduce CuO-NPs' toxicity while maintaining and improving their biological quality.

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