Copper nanoparticles synthesize using ginger and lemongrass and its anti-cancer activity against lung cancer cell line

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ABSTRACT

Introduction: Lung cancer is the leading cause of cancer deaths worldwide. People who smoke have the greatest risk of lung cancer, though lung cancer can also occur in people who have never smoked. The risk of lung cancer increases with the length of time and number of cigarettes you've smoked. If you quit smoking, even after smoking for many years, you can significantly reduce your chances of developing lung cancer. One of the most popular technologies in translational research is nanotechnology. Significant emphasis has been paid to the eco-friendly development of metallic nanoparticles using biological resources. Nanotechnology is concerned with the synthesis method and manipulation of particles with a size range of 1 to 100 nm. The mechanism of action for an anticancer medication involves triggering apoptosis.

Aim: To Evaluate copper nanoparticles synthesis using ginger and lemon grass and its anti-cancer activity against lung cancer cell line.

Materials and Methods: A-549 cell lines were purchased from NCCS, Pune and grown in T25 culture flasks. 30 mM of sodium selenite was dissolved in 50 mL of distilled water and 50 mL of ginger and lemongrass extract was added. Cell viability assay determined using MTT assay. Morphology analysis by phase contrast microscope. Detection of cell death were also determined by DAPI staining.

Results: Ginger and lemongrass copper nanoparticle treatment inhibits lung cancer cell proliferation. and we observe inhibitory concentration (IC-50) is 20µg/ml represents the statistically significant $p<0.05$ btw control and treatment group. The number of cells decreased after treatment and they exhibit shrinkage and cytoplasmic

membrane blebbing. Under DAPI staining treated cells show chromatin condensation and nuclear fragmentation. On further studies they can be formulated as an alternative with minimal side effects.
Conclusion: The present study demonstrates that extracts of ginger and lemongrass shows cytotoxic effect and induces apoptosis with further studies they can be formulated as an effective drug against lung cancer.

Keywords: Copper, Nanoparticles, lemongrass, lung cancer, Cytotoxicity.

INTRODUCTION

Lung cancer is the leading cause of cancer deaths worldwide. People who smoke have the greatest risk of lung cancer, though lung cancer can also occur in people who have never smoked (1). The risk of lung cancer increases with the length of time and number of cigarettes you've smoked (2). If you quit smoking, even after smoking for many years, you can significantly reduce your chances of developing lung cancer. One of the most popular technologies in translational research is nanotechnology (3). Significant emphasis has been paid to the eco-friendly development of metallic nanoparticles using biological resources. Nanotechnology is concerned with the synthesis method and manipulation of particles with a size range of 1 to 100 nm. The mechanism of action for an anticancer medication involves triggering apoptosis (3,4). According to a study on the antibacterial effects of copper nanoparticles employing E. coli and Bacillus subtilis, the antibacterial activity of copper nanoparticles was superior to that of silver nanoparticles (5) Cu NPs showed strong fungicidal influence on Penicillium spp. microorganisms and strong antibacterial influence on Bacillus spp. Compared to Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Propionibacterium acnes, Escherichia coli was more inhibited by Cu NPs. Cu NPs have been produced utilising extracts of numerous plants that can be found worldwide (6). However, no studies have been done on the use of Ethiopian medicinal plant extracts in the green synthesis of Cu NPs. Therefore, it was suggested that the current research effort investigate the synthesis of green Cu NPs (g-Cu NPs) using extracts of Ethiopian medicinal plants (7). More than 95% of India's traditional medicines are made from plants (8–13). Hagenia abyssinica is a type of Ethiopian medicinal plant used in the biogenic synthesis of g-Cu NPs in aqueous medium.

The most common reason for tumour-associated mortality is lung cancer (14). Fascaplysin, a marine sponge bis-indole, is being researched as a medication to combat chemoresistance following the failure of targeted medications or immunotherapy. It exhibits broad anticancer action as a particular CDK4 inhibitor among other mechanisms (14). Lung cancer cell lines, primary Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) cells, as well as SCLC circulating tumour cell lines were used to investigate the cytotoxic action of fascaplysin (CTCs) (15,16). The aqueous leaf extract of Alternanthera sessilis Linn was used in the current investigation to characterise the biogenesis of Cu NPs (A.S). The recent rise in popularity of biological nanoparticle synthesis can be attributed to its ease, low cost, lack of hazards, and ability to be carried out in difficult circumstances (17). There are enough terpenoids, carbohydrates, and flavonoids in the

Alternanthera sessilis leaf aqueous extract to change metal ions into metal and stabilise the resultant nanoparticles (17,18).

The FTIR and EDX confirmed that phyto-chemicals were responsible for the reduction and stabilisation of Cu NPs, and the UV-visible spectrophotometer confirmed the formation of Cu NPs with the formation of a characteristic peak at 580 nm (19). The XRD determined the Crystalline FCC nature of biogenic Cu NPs (-29.1 mv)confirmed that stable Cu NPs had formed (20). TEM investigation confirmed the object's spherical form and dimensions of 3–12 nm. The biogenic Cu NPs showed remarkable dose-dependent antioxidant activity, with an EC50% of 78.83 g/ml and a maximum activity of 68.36 at 100 g/ml, as well as considerable photocatalytic activity against Congo red dye, which was entirely broken down after 26 minutes. The tests also showed that Gram negative bacteria were more resistant to the antibacterial effects of Cu NPs than were Gram positive bacteria. The present study aimed to evaluate the copper nanoparticles synthesis using ginger and lemon grass and its anti-cancer activity against lung cancer cell line

MATERIALS AND METHOD

Reagents

DMEM (Dulbecco's Modified Eagle Medium), Phosphate Buffered Saline (PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. Acridine orange (AO), ethidium bromide (EtBr), Dimethyl sulfoxide (DMSO), [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), DAPI, AO/EtBr were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from SRL, India.

Cell line maintenance

Lung cancer cell lines (A-549) were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37◦C in a humidified atmosphere containing 5% CO2. Upon reaching confluency, the cells were trypsinized and passaged.

Ginger and lemongrass-Copper nanoparticle preparation:

1g of ginger and 1g of lemongrass was added to 100 mL distilled water. The mixture was boiled for 15-20 minutes using a heating mantle at 70^{o} C for 15-20 minutes. The boiled extract was filtered through Whatman No: 1 filter paper. The filtered extract was stored and used for nanoparticle synthesis. 30 mM of copper sulphate was dissolved in 50 mL of distilled water. To that, 50 mL of Ginger and lemongrass extract was added. The reaction mixture was kept in an orbital shaker for 48-72 hours. UV- visible readings were recorded to preliminarily confirm the synthesis of nanoparticles at specific intervals of time. After that, centrifugation was done to collect the pellect the nanoparticle solution. The centrifugation was done at 8000 rpm for 10 minutes and the pellet was washed thrice with distilled water and supernatant was discarded.

Cell viability (MTT) assay

The cell viability of plant extract treated A549 cells was assessed by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically

active cells. A549 cells were plated in 96 well plates at a concentration of $5x10^3$ cells/well 24 hours after plating, cells were washed twice with 100µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in different concentrations for 24 hours. At the end of treatment, the medium from control and drug Name treated cells were discarded and 100µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4 h at 37ºC in the CO_2 incubator.

The MTT containing medium was then discarded and the cells were washed with 1x PBS. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (100µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells]×100.

Morphology study

Based on MTT assay we selected the optimal doses (IC-50: 20µg/ml) for further studies. Analysis of cell morphology changes by a phase contrast microscope. $2×10^5$ cells were seeded in 6 well plates and treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (20µg/ml for A-549 cells) for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope.

Determination of nuclear morphological changes of cells (DAPI staining)

For the nuclear morphological analysis, the monolayer of cells was washed with PBS and fixed with 3% paraformaldehyde for 10 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature and incubated with 0.5µg/ml of DAPI for 5 min. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were viewed under a fluorescent microscope.

Statistical analysis

All data obtained were analyzed by One way ANOVA followed by Students-t-test using SPSS, represented as mean ± SD for triplicates. The level of statistical significance was set at $p<0.05$

RESULTS

Effect of ginger and lemongrass extract on cell viability of lung cancer cell line

The cytotoxic potential of ginger and lemongrass extract in lung cancer cells was assessed by an MTT assay. The cells were treated with different concentrations (5-60µg/ml) of ginger and lemongrass extract for 24 h. Ginger and lemongrass extract treatment significantly decreased the viability of A-549 cancer cells compared to control at 24 h time point (Fig. 1). The percentage of cell viability reduced gradually with increase in the concentration. We observed the 50% growth inhibition at (20µg/ml)

concentration. Hence, IC-50 dose (20µg/ml) was considered for the further experiments.

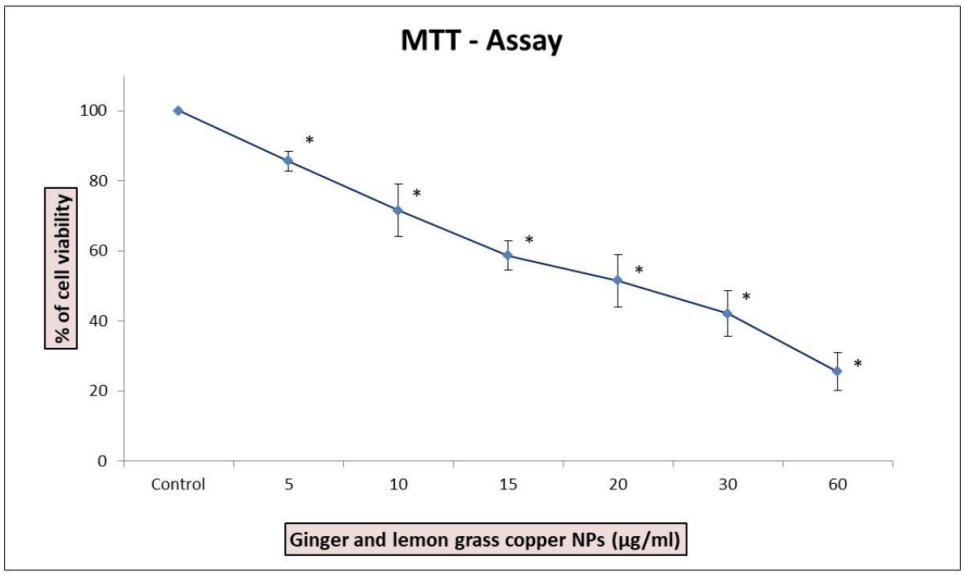

Figure 1: The cytotoxic effects of Ginger and lemon grass copper NPs on lung cancer cells. Cells were treated with different concentrations of Ginger and lemon grass copper NPs (5, 10, 15, 20, 30, 60µg/ml) for 24 h, and cell viability was evaluated by MTT assay. Data are shown as means ± SD (n = 3). * compared with the control blank group, $p < 0.05$.

Effect of ginger and lemongrass on cell morphology

The cell morphological analysis of ginger and lemongrass extract treated lung cancer cells was observed in an inverted phase contrast microscope. The A-549 cells were treated with ginger and lemongrass (20 µg/ml) for 24 h, compared with the untreated cells, treated cells showed significant morphological changes, which are characteristic of apoptotic cells, such as cell shrinkage and reduced cell density were observed in the ginger and lemongrass extract treated cells (Figs. 2). Cells undergoing apoptosis also displayed other types of morphological changes such as rounded up cells that shrink and lose contact with neighbouring cells. Some sensitive cells were even detached from the surface of the plates.

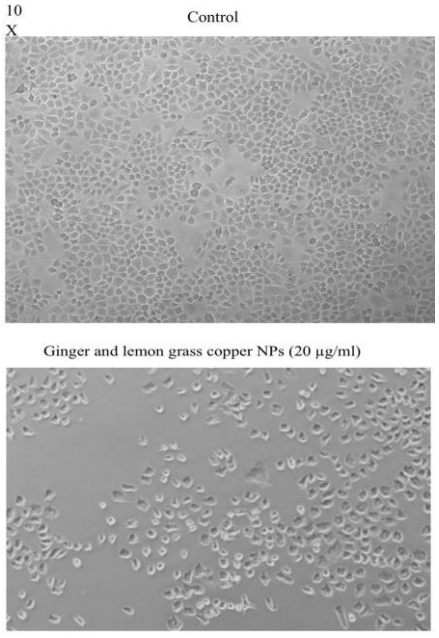

Figure 2: Cell morphological analysis. Cells were treated with ginger and lemon grass copper NPs (20µg/ml) for 24 h along with the control group. Images were obtained using an inverted phase contrast microscope.

Pro-apoptotic effect of ginger and lemongrass

The induction of apoptosis **ginger and lemongrass** extract (20 µg/ml) treated cells was analysed by DAPI staining. After a 24 h treatment period, the cells were stained with nuclear staining (DAPI) and observed in fluorescence microscopy. The treated cells clearly showed condensed chromatin and nuclear fragmentation, which are characteristics of apoptosis compared to the control which showed clear round nuclei (Fig. 3).

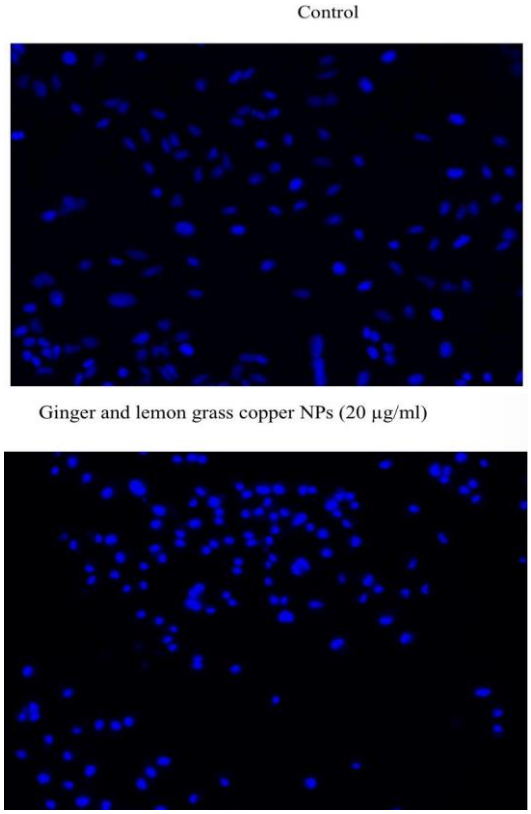

Figure 3: Detection of apoptotic cells by AO/EtBr dual staining in lung cancer cells. Cells were treated with Ginger and lemongrass copper NPs (20 µg/ml) for 24h along with the control group. Images were obtained using an inverted fluorescence microscope.

DISCUSSION

The use of medicinal plants as a source of healing dates back thousands of years. When compared to allopathic medications, it has no harmful consequences. Herbal medications are more widely available to patients, and their demand is rising. An essential member of the verbenaceae family that has therapeutic potential for a variety of disorders is ginger and lemongrass. Our team has extensive knowledge and research experience that has translate into high quality publications (21–30). It is regarded as both a traditional drug and a herbal product (31) in the previous studies the anticancer properties of ginger and lemongrass extract showed cytotoxci effects against lung cancer cells. The findings demonstrate that ginger and lemongrass leaf extract reduces lung cell line growth by triggering apoptosis and interfering with cell survival.It may serve both chemotherapeutic and chemoprevention objectives (32) Inducing apoptosis in cancer cells is a useful method for creating anticancer drugs (8,9,11,33,34). Many plant-based compounds have been investigated for their capacity to trigger apoptosis. Apoptosis is a physiological process that serves as a crucial component of tissue hemostasis and is thought to be a means of getting rid of unneeded cells (34–38)

The action of Cu NPs on bacteria has not yet been extensively investigated because previous researchers have already reported on the mechanisms of antibacterial activity (39) It is thought that the Cu NPs interact with the electronegative components of the bacterial cell membrane after becoming adsorbed on the cell wall of the bacteria (40) This causes unsuccessful metabolism,

which in turn causes interference with and disruption of transcription in bacteria, resulting in Cu NPs' antibacterial action (41) According to a recent study, it is also thought that the synergistic effect of Cu NPs with the bioactive components of the extract would have had a substantial impact on the ability to block the activity of harmful bacteria.

It is thought that the action of Cu NPs would have broken the helix structure of DNA molecules. Additionally, the interaction with the liberated Cu metal ion by the Cu NPs affects the integrity of the membrane by lowering the electrochemical potential across the cell membrane.

CONCLUSION

Overall, the present study results demonstrated that, the Anti-cancer effects using ginger and lemongrass and its anti-cancer activity against lung cancer cell line. Hence, its raises new hope for anti-cancer drug development from this plant. However, more research is needed to understand the molecular mechanisms of anti-cancer effects.

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