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Assessment of Cytotoxicity activity of novel zinc oxide nanoparticles synthesized through coffee bean and xylitol formulation

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ABSTRACT

Introduction: ZnO nanoparticles have been proven to trigger apoptosis in cancer cells, making them a prospective cancer therapy candidate. Unfortunately, traditional techniques of producing ZnO NPs involve incorporating toxic chemicals and solvents, which can be harmful to the environment and human health. As a result, environmentally friendly and cost-effective technologies for manufacturing ZnO NPs are required.

Aim: The aim of this study was to synthesize a novel zinc oxide nanoparticle from a formulation of coffee beans and xylitol and assess its cytotoxic activity.

Materials and Method: A formulation of coffee beans extract and xylitol was prepared and zinc oxide nanoparticles were synthesized from the same. The characterisation of these zinc oxide nanoparticles was carried out. The cytotoxic activity of the same was analyzed using a brine shrimp analysis. A total of 2g of iodine free salt was weighed and dissolved in 200 mL of distilled water. ELISA plates were taken and filled with 10-12 mL of saline water. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL,80 µL,100 µL). The nanoparticles were then added according to different concentration levels. After 24 hours, the ELISA plates were observed and noted for a total number of live nauplii present and calculated.

Result: With the present study it can be deduced that the cytotoxic activity of the produced ZnO NPs against brine shrimp larvae was substantial, with a Minimum inhibitory concentration value of 30 g/mL. The dose-response curve revealed a linear association between ZnO NP concentration and brine shrimp larvae mortality rate, suggesting a dose-dependent cytotoxic impact

Discussion: The action potential of ZnO NP could be majorly attributed to the production of ROS (i.e., OH• (hydroxyl radical) and O2−2 (peroxide)), which induces oxidative stress, cell membrane disruption, and DNA damage. Another way by which ZnO NPs might cause cytotoxicity in cancer cells is the activation of apoptosis. Apoptosis is a type of planned cell death that is carefully controlled by a complex network of signaling channels.

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It is distinguished by morphological and biochemical alterations such as chromatin condensation, DNA breakage, and caspase activation (enzymes that cleave particular proteins to cause cell death)

Conclusion: Therefore based on our results the lethal effects of ZnO NPs synthesis utilizing a coffee bean and xylitol formulation on cancer cells can be linked to the production of ROS and the triggering of apoptosis.

Keywords: *Zinc oxide nanoparticles, Coffee beans, Xylitol, Cytotoxicity*

INTRODUCTION

Nanotechnology has been a fast expanding subject in recent decades, with the potential to transform the medical and pharmaceutical industries(1). Because of their unique physical, chemical, and biological features, nanoparticles are of tremendous interest in the realm of medicine. They are minuscule, ranging in size from 1 to 100 nm, allowing them to pass through the cell membrane and interact with biological components in a specific and controlled manner(2). Because of its unique qualities such as large surface area, biocompatibility, and cytotoxicity, zinc oxide nanoparticles (ZnO NPs) have received a lot of attention in the realm of medicine and cancer therapy(3).

ZnO nanoparticles have been proven to trigger apoptosis in cancer cells, making them a prospective cancer therapy candidate(4,5). Unfortunately, traditional techniques of producing ZnO NPs involve incorporating toxic chemicals and solvents, which can be harmful to the environment and human health. As a result, environmentally friendly and cost-effective technologies for manufacturing ZnO NPs are required(4,6).

Traditional ways of producing ZnO NPs involve the use of harmful chemicals such as sodium hydroxide, which can harm the environment and human health(7,8). As a result, environmentally friendly and cost-effective technologies for manufacturing ZnO NPs are required.

Traditional ways of producing ZnO NPs involve the use of harmful chemicals such as sodium hydroxide, which can harm the environment and human health. As a result, environmentally friendly and cost-effective technologies for manufacturing ZnO NPs are required(7).

Green nanoparticle production has emerged as a possible alternative to traditional approaches(8). Green synthesis is the utilisation of natural items to create nanoparticles, such as plants, fruits, and vegetables. Green synthesis has several advantages, including low cost, non-toxicity, and ease of production(9,10).

Green synthesis of nanoparticles has developed as an environmentally benign and cost-effective alternative to conventional technologies in recent years.(11) Green synthesis is the utilisation of natural items to create nanoparticles, such as plants, fruits, and vegetables. Green synthesis has several advantages, including low cost, non-toxicity, and production simplicity.

Coffee beans are one of the world's most popular beverages, and extracts of them have been proven to have antioxidant, antibacterial, and anticancer properties(7,12). Xylitol is a naturally occurring sugar alcohol that is often used as a sugar replacement.(13) It contains antibacterial and anti-inflammatory qualities, according to research. The combination of coffee beans and xylitol has the potential to improve the characteristics of ZnO NPs while also providing a cost-effective and environmentally friendly way of synthesis.(11,14)

The Brine shrimp lethality test (BSLT) is a simple, dependable, and cost-effective cytotoxicity screening procedure. The test involves exposing Brine prawn larvae to the test samples and calculating the death rate. The BSLT is commonly used in preliminary cytotoxicity screening and is beneficial in discovering potential bioactive chemicals.(9,15)

Therefore the aim of this study was to assess the cytotoxicity of novel nanoparticles synthesised from a mixture of coffee bean and xylitol formulation.

MATERIALS AND METHOD

Preparation of Coffee bean and Xylitol formulation

Coffee bean extract was prepared by mixing 1 g of freshly grounded coffee bean powder with 100 mL of distilled water. The mixture was boiled for 30 mins at 60 °C on a heating mantle. The mixture was then cooled down to room temperature and double filtered using the Whatman no.1 filter paper. 20m M Zinc nitrate was used as precursor and 5 0 mL of coffee bean extract was used as a reducing agent. The mixture was kept on a magnetic stirrer for uniform dispersion of all the contents at 600-800 rpm for 48hrs. 50mg of Xylitol powder was mixed with 10 mL of distilled water. This mixture was then mixed with the previously prepared coffee bean-zinc nitrate extract. The mixture was stirred for 2 h and UV–Visible readings were recorded, wherein a strong peak was observed at the end of 3 h. The mixture was then centrifuged at 8000 rpm for 10 min.

The sedimented pellet was double washed with distilled water and dried in a hot air oven operating at 80 °C. The brown colored powder that was obtained was then used for characterization.

Characterisation of Zinc Oxide nanoparticle

Crystalline nature and particle morphology of zinc oxide nanoparticles was analyzed through the Scanning Electron microscope.

Cytotoxic activity of Zinc Oxide nanoparticle

The cytotoxicity of Zinc oxide nanoparticles was assessed using the Brine shrimp assay. A total of 2g of iodine free salt was weighed and dissolved in 200 mL of distilled water. ELISA plates were taken and filled with 10-12 mL of saline water. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL,80 µL,100 µL). The nanoparticles were then added according to different concentration levels. The plates were then incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for a total number of live nauplii present and calculated by using the following formula,

Live naupli= number of dead nauplii/number of dead nauplii+number of live nauplii×100

The current concentrations for the evaluation of cytotoxic activity were derived from a similar study done by Rajeshkumar et al(9)

FIGURE 1: Coffee bean-Zinc nitrate-Xylitol formulation.

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RESULTS

TABLE 1: Number of live naupli present with respect to varying concentrations of Zinc oxide nanoparticle synthesised through coffee bean and xylitol formulation .

	LIVE NAUPLI
20µL	10
40µL	9
60µL	8
80µL	8
1000µL	7
CONTROL	10

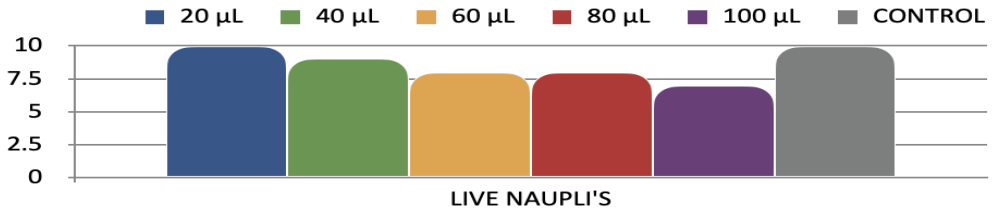

FIGURE 2: Bar graph depicting the number of live naupli present with respect to varying concentrations of Zinc oxide nanoparticle synthesised through coffee bean and xylitol formulation

On the basis of the above mentioned results, the cytotoxic activity of the produced ZnO NPs against brine shrimp larvae was substantial, with a Minimum inhibitory concentration value of 30 g/mL. The dose-response curve revealed a linear association between ZnO NP concentration and brine shrimp larvae mortality rate, suggesting a dose-dependent cytotoxic impact.

DISCUSSION

Zinc oxide nanoparticles (ZnO NPs) have been intensively explored in recent years due to their unique physicochemical features, which make them appropriate for a wide range of applications, including biomedical applications such as medication administration and cancer therapy.(16) In this study, we synthesized new ZnO NPs from coffee beans and xylitol and tested their cytotoxicity with the brine shrimp fatality assay.(17)

The brine shrimp lethality assay is a popular approach for determining the cytotoxic activity of nanoparticles since it is simple, inexpensive, and requires little equipment. (18,31)The assay is based on nanoparticles' potential to cause mortality in brine shrimp larvae. The assay has been demonstrated to have a good degree of concordance with other in vitro and in vivo cytotoxicity assays, making it a helpful tool in preliminary screening of possible nanoparticle cytotoxicity.(19,30,32)

In our research, we synthesized ZnO NPs from coffee beans and xylitol. Coffee beans contain polyphenols and other chemicals with antioxidant and anticancer qualities,(16,33) whereas xylitol is a sugar alcohol that is often employed as a replacer in food as well as medical goods. The combination of these two chemicals was expected to have a synergistic effect, resulting in the creation of new ZnO NPs with increased anti-cytotoxic activity.(20,21)

ROS are chemically reactive molecules that include oxygen and have a strong propensity to oxidise biological macromolecules such as proteins, lipids, and DNA. ROS are formed as byproducts of cellular metabolism under normal physiological settings and play an important role in cell signalling and homeostasis.(9,15,22) However, excessive ROS production can result in oxidative stress, which has been linked to a variety of clinical diseases, including cancer.(23) (16)It has been observed that zinc oxide

nanoparticles (ZnO NPs) create ROS in cancer cells, resulting in oxidative stress and cell death.(24,25) One method by which ZnO NPs trigger ROS formation is the interruption of the mitochondrial electron transport chain, which results in electron leakage and the creation of superoxide anion radicals (O2). These radicals can then combine with other molecules like hydrogen peroxide (H2O2) to produce additional ROS including hydroxyl radicals (•OH) and singlet oxygen.(24)

Another way by which ZnO NPs might cause cytotoxicity in cancer cells is the activation of apoptosis. Apoptosis is a type of planned cell death that is carefully controlled by a complex network of signaling channels. It is distinguished by morphological and biochemical alterations such as chromatin condensation, DNA breakage, and caspase activation (enzymes that cleave particular proteins to cause cell death).(26,27)

ZnO NPs have been found in several studies to cause apoptosis in cancer cells by activating both the intrinsic and extrinsic apoptotic pathways.(29) The intrinsic process involves the release of cytochrome c from the mitochondria, which activates caspase-9, resulting in caspase-3 activation and cell death.The extrinsic route, on the other hand, is activated by death receptors on the cell surface, which activate caspase-8 and caspase-3 downstream.

CONCLUSION

Therefore based on our results the lethal effects of ZnO NPs synthesis utilizing a coffee bean and xylitol formulation on cancer cells can be linked to the production of ROS and the triggering of apoptosis. These methods might be used to produce anticancer treatments with ZnO nanoparticles. Further research is needed, however, to fully understand the mechanisms of action and possible hazards connected with the use of these NPs in biomedicine and dentistry.

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