Comparative Cytotoxic Effect of Zinc Oxide Nanoparticles Assisted Lodhra (Symplocos Racemosa) and Cinnamon Bark (Cinnamomum Cassia) Formulation

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Abstract

Aim: The aim is to analyze cytotoxic effects Lodhra and cinnamon bark formulation and its assisted zinc oxide nanoparticles

Materials and Methods: 200 ml of distilled water was used to dissolve 2g of iodine-free salt. 10–12 ml of saline water were added to 6 well ELISA plates. That was followed by the progressive addition of 10 nauplii (5µL,10µL,20µL,40µL,80µL, and control) to each well. The nanoparticles were then introduced in the appropriate concentrations. For 24 hours, the plates were incubated. The ELISA plates were examined after 24 hours to count the live nauplii that were present and to determine their number using the formula.

number of dead nauplii/number of dead nauplii+number of live nauplii×100.

Results: Cytotoxic effects of lodhra and cinnamon bark and its mediated ZnO Nanoparticles show an incremental pattern with increase in concentration, where LD 50 concentration was found to be 80µl.

Conclusion: With the above results and discussion we come to the conclusion that lodhra and cinnamon bark and its mediated ZnO nanoparticles have potent cytotoxic activity. It's activity increases with increase in dosage. Further study to be used against a range of human cancer cell lines in cancer Therapy.

Keywords: Zinc oxide nanoparticles, Lodhra and Cinnamon.

1. Introduction

Cinnamon is a native spice from the Lauraceae family that is present in practically every home. It has long been a significant ingredient in our food, mostly used as a flavour agent. Our forefathers have used it as a treatment for digestive and respiratory conditions for a very long time. Less is understood, however, about its advantageous effects as an anti-inflammatory, antilipemic, antidiabetic, anti-microbial, and anticancer drug (Kawatra and

Rajagopalan, 2015). Currently, sources used in traditional and folk medicine such as vegetables, plants, spices, and herbs have the ability to fight cancer. (Abdullaev, 2002). Epidemiological and animal model research have suggested that dietary factors have an impact on cancer risk (Wattenberg, 1992). Numerous naturally occurring components of plant foods, such as spices, have demonstrated the ability to provide protection from carcinogenic exposure. Cinnamaldehyde, the primary compound in cinnamon, and its derivatives have been shown to possess a range of biological activities, including immunomodulatory and anti-angiogenic activity (ElKady and Ramadan, 2016). It has been shown that cinnamaldehyde inhibits the proliferation of human cancer cell lines, leukemia, ovarian, breast, and lung tumor cells. (Jeong *et al.*, 2003)

The Lodhra tree is a member of the Symplocos genus. There are numerous metabolites in Symplocos and its various species that have potential medical benefit against a variety of ailments. The most important component in the creation of traditional Ayurvedic and Unani medicines is the stem bark (Acharya *et al.*, 2016). Lodhra, also known by its botanical name Symplocos racemosa roxb, is an ancient therapeutic plant used in Ayurvedic medicine. It is a member of the Symplocaceae family. Different regions of the world have different names for Lodhra. In English, it is primarily referred to as the Lodh tree or Symplocos bark (Mehjabeen *et al.*, 2014). This amazing plant demonstrates the existence of bioactive elements as Symposide, Betulinic Acid, Acetyl Oleanolic Acid, Oleanolic Acid, Colloturidine, and Loturine. This powerful plant is widely used to prepare a variety of compositions designed to treat a variety of conditions, including piles, indigestion, diabetes, gum disease, and piles in addition to skin and blood disorders (Sunil *et al.*, 2012).

The phrase "cytotoxic" is frequently used to refer to chemotherapy treatments that kill cancer cells, but it can also refer to toxins like venom. We contain cytotoxic immune cells in our bodies, such as T cells that eliminate germs, viruses, and cancerous cells. Cytotoxic substances have a variety of ways to harm cells (Miura and Shinohara, 2009). Evaluation of the phytochemical components and antioxidant properties of by lodhra and cinnamon bark inhibition of DPPH radical was used to assess their free radical scavenging activity. They can injure the cell by weakening its cell membrane and cause it to blow up (lyse), or they might obstruct cell division, stopping the cell from developing and dividing (Devaraj *et al.*, 2020). Because of their unique physical and synthetic qualities, silver nanoparticles (AgNPs) are increasingly being used in a variety of industries, including clinical, food, medical care, buyer, and modern applications (Kumar, Kumar and Pathak, 2021). These have unusual properties that have led to a variety of uses, including as antibacterial specialists, in modern, family, and medical services related items, in consumer goods, clinical gadget coatings, optical sensors, and beauty care products, in the drug and food industries, in diagnostics, muscular health, drug delivery, and as anticancer substances (Ilaria and Kenny, 2013).

Although various properties were estimated, previous studies were done on these plant extracts based on their phytochemical and other quantitative assays, Studies on cytotoxic properties exhibited by the lodhra and cinnamon bark were done insufficiently. Our team has extensive knowledge and research experience that has translate into high quality publications(Panda *et al.*, 2014; Nambi *et al.*, 2018; Venkatesan *et al.*, 2018; Vadivel *et al.*,

2019; Kamath *et al.*, 2020; Li *et al.*, 2020; Mehta *et al.*, 2020; Paramasivam and Priyadharsini, 2020; Bhansali *et al.*, 2021; Deepanraj *et al.*, 2022). In this present investigation, we have prepared the plant extract of lodhra and cinnamon bark and observed cytotoxic property using Brine shrimp lethality assay.

2. Materials and Methods

Plant material and Extraction

For extraction and isolation purposes, cinnamon and lodhra bark plant extract were collected, shade-dried, and powdered. After being diluted with 100ml of distilled water, the dried powdered plant material—1g of lodhra bark and 1.017g of cinnamon powder—was cooked for 9 minutes at 60–80°C under vacuum. The plant extract is then made after it has been filtered using Whatman's filter paper.

Synthesis of Zinc Oxide Nanoparticles

0.0169 g of zinc nitrate [Zn(NO3)2] solution is prepared for the production of zinc oxide nanoparticles. A 90 ml portion of distilled water is added to the produced solution. The 10 ml of plant extract solution is now added to it. This results in the production of a 100 ml mixture of zinc nitrate and Symplocos racemosa, cinnamon solution. In order to prevent silver nitrate from being contaminated and photo inactivated, the flask is incubated at 37 degrees Celsius. The resulting silver nanoparticles are then further purified using centrifugation for 15 minutes at 10,000 rpm. For roughly 72 hours, preliminary readings were taken every two hours. Fill the six centrifuge tubes with 12 ml of ZnNp plant extract after 72 hours, and centrifuge for approximately 10 minutes.

Characterization of Synthesized Nanoparticles

Utilizing double beam UV vis spectroscopy, the produced zinc oxide nanoparticles were measured optically. It speaks of visible-range absorption spectroscopy and directly influences the colour of the compounds present. The majority of its applications in analytical chemistry are for the quantitative analysis of various ions, chemicals, and biological macromolecules at various wavelengths. At various wavelengths starting at 540 nm, optical measurements of the produced Zn(NO3)2 were made.

Brine Shrimp Lethality Assay

200 ml of distilled water was used to dissolve 2g of iodine-free salt. 10–12 ml of saline water were added to 6 well ELISA plates. 10 nauplii were gradually added in batches of 5µL, 10µL, 20µL, 40µL, and 80µL to each well. The nanoparticles were then introduced in the appropriate concentrations. For 24 hours, the plates were incubated. The number of dead nauplii/number of dead nauplii+number of living nauplii+number of 100 was used to calculate the number of live nauplii present in the ELISA plates after 24 hours.

3. Results

One nauplii died at a concentration of 5 µL, two died at a concentration of 10 µL, two died at a concentration of 20 µL, and three died at a concentration of 40 µL. This demonstrates that an increase in extract concentration leads to a rise in lethality as a percentage. At an 80 µL concentration, nauplii died at the highest rate.

4. Discussion

The study of Senaratne et al shows HT29 cell line showed a strong cytotoxic effect from EESR, MCF7 cell line showed a moderate cytotoxic effect, and HepG2 cell line showed a less cytotoxic effect. The XIT assay was used to compare the effects of Symplocos bark extract (test) and cyclophosphamide (control) on the proliferation of Hela and HL60 cell lines. The HeLa cell line, which is more potent than cyclophosphamide, was the target of the extract's maximum cytotoxicity, demonstrating that the extract was more effective against the HeLa than cyclophosphamide (Senaratne and Pathirana, 2021). The study of shah et al on KB and L1210 cells, preparations from Ceylon cinnamon had cytotoxic effects. In the first and second trials using these tumour cells, the average ED50 for the petroleum ether extract was 60 and 24 pg/ml, whereas it was 58 and 20 pg/ml for the chloroform extract. Using the KB technique, both
extracts showed positive results for anticancer properties (Shah *et al.*, 1998). The study of singh et al has shown cytotoxic effects by radiolabeled urea breath tests. It was used to measure H. pylori levels both before and after the administration of cinnamon alcohol extract. Although it was only partially successful in eliminating H. pylori, it did significantly lessen colonisation. Consequently, it was proposed that at an 80 mg/day concentration (Singh *et al.*, 2022). Numerous studies have been conducted to determine how cinnamon affects melanoma cells. It has been discovered to suppress the activity of pro-angiogenic factors, which is a crucial requirement for the proliferation of tumour cells, and to stimulate CD8(+) T cell activity at the same time (Kwon *et al.*, 2009). According to research, polyphenols found in plants may inhibit the growth of several malignancies (Park and Pezzuto, 2002), perhaps as a result of their capacity to act as antioxidants. Recent studies have shown that the water-soluble polymeric polyphenols in cinnamon can interfere with phosphorylation/dephosphorylation signaling processes to influence the cell cycle and restrict proliferation (Schoene *et al.*, 2005).

5. Conclusion

With the above results and discussion, we conclude that lodhra and cinnamon bark and its mediated ZnO nanoparticles have potent cytotoxic activity. Its activity increases with an increase in dosage. Further study to be used against a range of human cancer cell lines in cancer therapy.

Conflict of Interest: The author declares that there is no conflict of interest in the present study.

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Royal hospital, thanjavur.

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Appendix

Figure 1 : synthesized ZnO nanoparticle

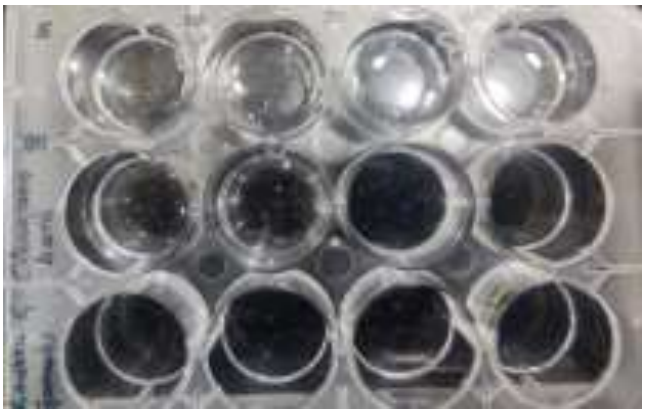

Figure 2: Cytotoxicity assay in ELISA plates with nauplii

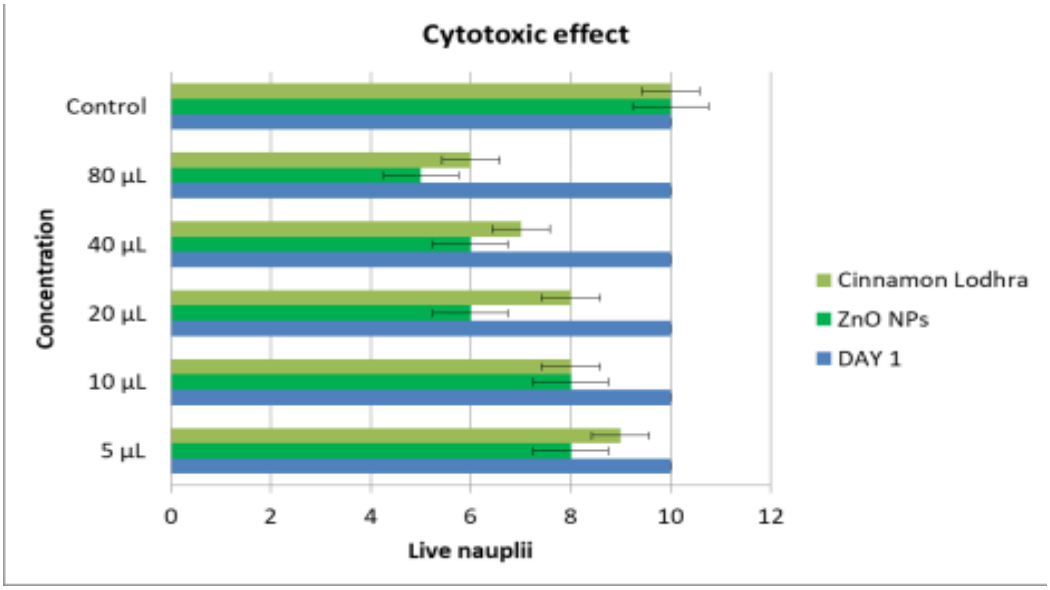

Figure 3: Graph representing the comparison of the cytotoxic effect of lodhra ((Symplocos racemosa) and cinnamon bark (Cinnamomum cassia) formulation and its assisted zinc oxide nanoparticles. X axis represents the number of live nauplii and Y axis represents concentration of sample. Light green colour represents cinnamon lodhra and dark green colour represents ZnO NPs.

Table 1: Table representing the comparison of the cytotoxic effect of lodhra ((Symplocos racemosa) and cinnamon bark (Cinnamomum cassia) formulation and its assisted zinc oxide nanoparticles.

Concentration	Cytotoxicity results
5µL	8
10µL	8
20µL	6
40µL	6
80µL	5
control	10