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Genotoxicity Analysis of *Nigella sativa* (black cumin seed) Extract on Oral Cancer Cell Line by DNA Fragmentation

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Abstract:

This study is aimed to analyze the genotoxicity property of *Nigella sativa* (Black Cumin Seed) extract on KB oral cancer cell line by DNA Fragmentation. Genotoxicity is the property of chemical agents to cause a destructive effect on the genetic material of a cell that causes mutations, which may lead to cancer. *Nigella sativa* Linneaus (N.*sativa*) (Family Ranunculaceae) is a herbal plant commonly referred to as black cumin seed and Karun-jeeragam (in Tamil). *Nigella sativa* has many beneficial pharmacological properties including immune stimulating, hypotensive, anti- inflammatory, anti-cancer, antioxidant, hypoglycemic, spasmolytic and bronchodilator properties. The genotoxic substance in N. sativa invades the nucleus and causes damage to the nucleic acid. This changes caused can be viewed by DNA fragmentation. Oral cancer is a common concern in South India. Herbs possessing genotoxic properties can be explored in using as an anti-cancer drug to replace the synthetic counterparts that have harmful side effects.

Keywords: Anti-cancer drug, Black Cumin Seed, Genotoxicity, *Nigella sativa*, Oral Cancer, Oral Carcinoma

INTRODUCTION:

Nigella sativa L. (Family: Ranunculaceae) is an indigenous herb of Southwest Asia including regions of Iran, India, and Pakistan. Growing to a maximum height of about 40–70 cm, this plant has finely divided foliage and pale blue and white flowers. From the fruit capsules, many small caraway-type black seeds are produced (length: 2.5 to 3.5 mm and width: 1.5 to 2 mm).[1] The plant is known by various names in different languages; black cumin, black seed, black-caraway (English), Karun-jeeragam (Tamil), Kalonji (Hindi), Habbah Al-Sauda; seed of blessing (Arabic), Chernushka (Russian), çörek otu (Turkish), and Cyah-daneh in Persian.

For a long span of time, the seeds of this plant have been used as a spice and additive in bread, cookies, and other dishes in many Asian and Eastern countries. Therapeutic benefits of this miraculous spice and its active ingredients are being explored.[2] *N. sativa* has been extensively studied for its biological activities and therapeutic potential and shown to possess a wide spectrum of activities *viz.* as diuretic, antihistamic[3], hypoglycemic[4], antihypertensive, antidiabetic, anticancer and immunomodulatory[5], analgesic, antimicrobial, anthelmintic, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil.[6]

Genotoxicity, in genetics is defined as a destructive effect on the cell's genetic material affecting its integrity. DNA damage can be induced by diverse factors such as radiation and chemical substances. DNA-damaging chemicals are considered genotoxic.[7] These genotoxic substances can induce mutations in somatic cells which may lead to chromosomal alterations, insertions, deletions, or translocations. There are three primary effects that genotoxins can have; they can be carcinogens, or cancer-causing agents, mutagens, or mutation-causing agents, or teratogens, birth defect-causing agents. In most cases, genotoxicity leads to mutations in various cells and other bodily systems. Mutations can lead to a host of other problems, from cancer to a wide variety of different diseases. Mutations can come in many different forms; genetic information can be duplicated, deleted, or inserted.[8] The genotoxic substance invades the nucleus and causes damage to the nucleic acid. These changes can be observed by DNA fragmentation. Genotoxicity is a property possessed by some substances that makes them harmful to the genetic information contained in organisms. Agarose gel electrophoresis is a method that is very suitable for clinical routine analyses of proteins in plasma and other body fluids since a good resolution is obtained with patterns which are easy to interpret.[9]

Oral cancer is the eleventh most common form of cancer worldwide. Incidence and mortality rates are higher in men than women. Differences across countries particularly relate to distinct risk profiles and availability and accessibility of health services. Tobacco use, including smokeless tobacco, and excessive alcohol consumption are

estimated to account for about 90% of oral cancers.[10] Approximately 90% of oral cancers are squamous cell carcinoma (SCC), which is seen typically on the lip or lateral part of the tongue usually as a lump or ulcer that is white, red, or mixed white and red.[11]

Plant derived compounds have been an important source of several clinically useful anticancer agents.[12] India is a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. There is thus an urgency to explore the potency of herbs to develop much effective and less toxic drugs.[13] Some studies suggest that herbs could increase the anti-cancer activity in several cancer cell lines including KB.

MATERIALS AND METHODS

Extract Preparation

Ethanol extract of *Nigella sativa* was prepared. (Figure 1)

Maintenance of cell lines

The KB Oral Cancer Cell Lines were acquired from NCCS. Oral cancer cells were seeded in 24 well plate and kept in CO2 incubator. Cells were treated with the black cumin seed extract in two different concentrations (75µg and 125µg) for 24 h. Treated cells were subjected to DNA fragmentation assay.[14]

Isolation of DNA

Reagents

The cell lysis buffer comprising of 40ml 1M tris, 40ml of 0.5M EDTA, 20ml of 10% SDS was made upto a final volume of 200 ml. 3.5 M ammonium acetate, Tris saturated phenol, Chloroform: isoamylalcohol (24:1), Ice cold isopropanol, 70% ethanol was also used.

Procedure

$1*10^6$ cells were incubated with 100µl of cell lysis buffer at room temperature for one hour. This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: isoamylalcohol mixture was added to the supernatant and mixed well. This was centrifuged at 5000 rpm for 15min and the supernatant was transferred to new tube and the above mentioned step was repeated once again. Then to the final aqueous phase 40µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. It was incubated at -20°C for 1hour, followed by the centrifugation at 10000 rpm for 15min. The pellet was retained and washed with 70% ethanol and stored in 20-50µl of TE buffer. The samples were analyzed in 2% agarose gel stained with Ethidium bromide. [14]

Analysis of DNA fragmentation by Agarose gel electrophoresis method

Principle

For the majority of DNA samples, electrophoretic separation is carried out in agarose gels. This is because DNA molecules and their fragments are considerably larger than proteins; therefore larger size agarose gels are required. Under an electric field, any given fragment of DNA should move towards the anode with the same mobility. This is due to the charge per unit length owing to the phosphate groups. Separation on agarose gels is achieved because of resistance to their movement caused by the gel matrix. Thus, the largest molecules will have difficulty moving, whereas the smallest molecules will be relatively unhindered. Consequently, the mobility of DNA molecules during gel electrophoresis will depend on size. Gel concentrations must be chosen based on the molecules to be separated such as for plasmid molecules – 1%; genomic DNA – 0.8% and RNA – 1.5%, mitochondrial DNA – 0.8% and amplified samples at 1.5% was used.

Reagents

TAE buffer (stock solution 50X) comprising Tris base – 242g, Acetic acid glacial – 57.1ml, EDTA 0.5M. The Working concentration being 1X. The gel loading buffer comprising of Bromophenol blue, Xylene cynol and Sucrose was used including Agarose, Ethidium bromide 20mg/ml and Gel loading buffer.

Procedure

The agarose gel was prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. The % of agarose depended upon the molecule to be separated. The samples were loaded with loading dye (2µl of loading dye was used). Electrophoresis of DNA fragments was carried out at 50 volts. DNA fragments were visualized under the UV trans-illuminator. [14]

Figure 1: Ethanol Extract Preparation

RESULTS AND DISCUSSION

The gel picture (Figure 2) depicts the DNA fragmentation of the KB cells incubated with black cumin seed extract. The first lane being the 1 kb ladder, the second lane being the DNA from untreated cells and the third and fourth lane containing DNA from cells treated with 75µg sample and 125µg sample respectively. DNA from the cells treated with the extract showed more disintegration with increase in the concentration of extract.

DNA fragmentation was observed with both the concentrations of black cumin seed extract on oral cancer cell lines by agarose gel electrophoresis method. Apoptosis has been distinguished biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 base pairs and can be visualized as an 'oligosomal ladder' by standard agarose gel

electrophoresis.[14] This proves that black cumin seed extract shows genotoxicity on the oral cancer cells by degrading its DNA. Hence, *Nigella sativa* has the potential to be an anti-cancerous drug.[15]

Genotoxicity analysis was done on pineapple extract using DNA fragmentation method and found to possess genotoxic property. DNA fragmentation was seen in cells treated with the extract.[9]

Genotoxic potential of wheatgrass on oral cancer cell line by DNA fragmentation method was done where treatment with the herb extract caused DNA fragmentation showing its efficacy as a potential anti cancer drug.[16]

Genotoxicity analysis of jojoba oil showed DNA degradation in oral cancer cells treated with jojoba oil. Thus, proving it to be a possibly effective anti cancer drug.[17]

In this study, treatment of oral cancer cells with black cumin seed extract caused DNA fragmentation. The genotoxic property of black cumin seed has been analysed and the herb is found to possess genotoxic property. This property can further be explored to study its anti cancer property more effectively.

Unlike the synthetic counterparts, the genotoxic properties of herbs can be used to target the mutated cells sans affecting other cells, hence proving to be a better anti-carcinoma alternative.

Figure 2: Gel showing DNA Fragmentation in lane 3 and 4
Lane 1 – 1 kb ladder
Lane 2 – DNA from untreated cells
Lane 3 – DNA from cells treated with 75 µg sample
Lane 4 – DNA from cells treated with 125 µg sample

CONCLUSION

In spite of all the significant advancements in modern medicine, traditional herbal medical profession has given rise to many important drugs that are used even today. Many plants used today are being studied for their potential mechanism of action and medicinal properties. Toxicological studies and safety evaluation are further being explored.[18]

The pharmacological research of the black cumin seed extracts reveal a wide spectrum of activities including immunopotentiation[5] and antihistaminic[3], antidiabetic[4], anti-hypertensive[19], anti-inflammatory[20] , and antimicrobial activities[21] . Many of these properties have been attributed to the quinone constituents of the seed.[22]

From this study, it is observed that the black cumin seed extract has a destructive effect on the DNA of the cancer cells. Thus, it can be inferred that the extract possesses genotoxicity and has a genotoxic effect in the oral cancer cell lines. Further advanced research with black cumin seed extract can be highly advantageous for patients suffering from oral cancer. Black cumin seed extract can thus undeniably be used as an anticancer drug to drastically reduce the horrifying consequences of malignancy. The genotoxic property can further be employed to treat cancers of other regions as well as in the synthesis of more advanced, site specific anticancer drugs.

Exploring avenues in pharmacological benefits of herbs and spices can pave way for safer, easier and cost effective advancements in synthesis of drugs for oral carcinoma and other health conditions. Further research may be needed to explore the mechanism of action of *Nigella sativa* extract on cancer cell lines.

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