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Research Article

Cytoprotection Alternatives for Cancer Treatment: *In-vitro* Evaluation of Alpinia Zerumbet as a Radioprotective Agent

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ABSTRACT

Cancer radiotherapy and modern synthetic medicine are critical for the treatment of cancer patients, but on the other hand they may have tremendous harmful side effect from the point of view of a weakened immune system. The focus of current research efforts in the industry and academia is not only to find affordable treatment methodologies but also to discover sources that mitigates or prevents the negative impact of these treatments on the patient's immune system. This review will provide details of optimal methodology involving *In-vitro* application of techniques such as phytochemical analysis, thin layer chromatography, cytogenetic analysis that were adopted to assess the efficacy of Alpinia Zerumbet extracts as a radioprotector. These methodologies provide a holistic evaluation technique for assessing any potential cytoprotective agent, especially if it's from the herbal domain. As a case study, analytical and inferential results summary of the research conducted to assess radioprotective property of Alpinia Zerumbet, a herbal and readily available derivative, will be presented. The key findings from the research indicate that the Alpinia Zerumbet extract has potential to be an effective radioprotector, with minimal side effects as compared to synthetic chemical cytoprotective agents.

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1 INTRODUCTION

Cancer is known medically as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. Global estimates for 2018, which cover 36 different cancers in 185 countries, report 18.1 million new cancer cases and approximately 9.6 million cancer-related deaths.^[1] Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world. There are many types of cancer treatment such as surgery, transplantation, gene, hormonal, chemo and radio therapy. Radiation therapy is one of the most common treatments for cancer. It is a local treatment which affects cancer cells only in the treated area. It is the medical use of ionizing radiation, generally as part of cancer treatment to control or kill cancerous cells using high-energy particles or waves, such as x-rays, gamma rays, electron beams, or protons, to destroy or damage cancer cells. It is also called radiotherapy, irradiation, or x-ray therapy. Radioprotective agents are commonly used to minimize the harmful side-effects of radiotherapy treatment. Most of the currently used radioprotective agents are derived synthetically and significantly increase the cost of cancer treatment. The

driver for the presented research is to discover the potential of Alpinia Zerumbet, a herbal source, which is affordable, safe and effective in minimizing the side of effects of commonly used cancer treatments of radiotherapy and also demonstrate the optimal evaluation technique that may be applied to evaluate the radioprotective properties of any chemical compound.

1.1 Radiotherapy Mode of Action

Radiotherapy is commonly applied to the cancerous tumor because of its ability to control cell growth. Ionizing radiation works by damaging the DNA of exposed tissue leading to cellular death. It is also common to combine radiation therapy with surgery, chemotherapy, hormone therapy, immunotherapy or some mixture of the four. Radiation involves the use of ionizing radiation to either cure or improve the symptoms of cancer. It is used in about half of all cases and the radiation can be from either internal sources in the form of brachytherapy or external sources. Radiation is typically used in addition to surgery and or chemotherapy and found effective for painful bone metastasis in about 70% of people. With the increasing technical power of radiotherapy to safely increase local tumor control for many solid tumors, it is an opportune time to rigorously explore

the potential benefits of combining radiotherapy with molecular targeted agents and immunotherapies to increase cancer survival outcomes [2].

The basic mechanism of action of radiotherapy is by damaging the DNA of cancerous cells. This DNA damage is caused by one of two types of energy, photon or charged particle. This damage is either direct or indirect ionization of the atoms which make up the DNA chain. Indirect ionization happens as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA.

Radiation therapy uses a special kind of high-energy beam to damage cancer cells. (Other types of energy beams include light and x-rays.) These high-energy beams, which are invisible to the human eye, damage a cell's DNA. Cancer cells are less organized than healthy cells, it's harder for them to repair the damage done by radiation. Therefore, cancer cells are more easily destroyed by radiation, while healthy, normal cells are better able to repair themselves and survive the treatment. Radiation can come from a machine (external radiation). It can also come from an implant (a small container of radioactive material) placed directly into or near the tumor (internal radiation). Conformal radiotherapy and Intensity modulated radiotherapy (IMRT) are example of external source. Radioactive liquids, Radioactive implants (brachytherapy) are examples of internal radiation source. Radiotherapy is an important modality and provides benefit to cancer patients, particularly in cases where surgical intervention is advisable, for example, lung, head, and neck cancers. On the other hand, high dose of radiation results in severe side effect that often leads to discontinuation of treatment cycle [3]. In the work presented, multiple level of radiation exposures were used against which the cytoprotective evaluation was done.

1.2 Radioprotectors

Any drug that can provide therapeutic differentiations between cancerous and normal tissue and protect the latter would be of great utility in the clinic. The development of radio protectors is important from the point of view of improving the effectiveness of cancer treatment multifold and also for strategic reasons (planned and unplanned radiation exposure).

A full range of research and development strategies is being employed currently in hunt for safe and effective radioprotectants. Restructuring or reformulating older protectants have proven efficacies but unwanted toxicities. Using nutraceuticals is only moderate protection but are essentially nontoxic. Using low dose combination of potentially toxic but effective agents, induce protection through different routes and fortifies radioprotective synergy [4]. The radio protector are agents or substances that provide protection against the toxic effect of ionizing radiation and reduce the toxicity, mutagenicity and other

adverse biological effects of the ionizing radiations in the living beings. The aminothiols was the first group of identified compounds as potential radio protectors. The main types of radio protector substances are the Sulfhydryl-groups compounds, amino acids, polyamines and other compounds, antioxidant and free radicals scavenger and lastly the phytochemicals compound [5].

In view of the significant side effects and associated high cost of these treatment, alternate radioprotectors which have similar efficacy and are more cost effective are needed to make cancer treatments more accessible and effective. Herbal derivatives is one such domain which may provide relevant alternatives for radioprotection. *Alpinia Zerumbet* was chosen for this evaluation.

2. OBJECTIVE: ALPINIA ZERUMBET AS A HERBAL ALTERNATIVE

Bio-prospecting and natural products drug development for cancer treatment has become an important area. Most of the cancer radiotherapeutic agents are associated with toxicity towards normal cells and tissues. Optimal dosing of cancer radiotherapeutic agents is often limited because of severe non-myelosuppressive and myelo-suppressive toxicities.

It is a continuing challenge to design therapy that is safer, effective and selective. Cytoprotective agents offer opportunities to reduce treatment related toxicity of anticancer therapy without diminution of efficacy. None of the available agents satisfy criteria for an ideal cytoprotection. This has stimulated research for discovering natural resources with immunomodulatory and cytoprotective activities.

Alpinia Zerumbet also known as shell ginger, or Pink Porcelain-lily, is commonly used as a perennial decorative garden plant. The plant has globose red fruit. Native of South-East Asia. *Alpinias* grew from thick fleshy roots called "rhizome", similar in appearance to the "ginger root". The plant flowers on old growth and individual flowers are reminiscent of small seashells, which accounts for the common name "shell ginger" [6].

The pharmacological action of *Alpinia Zerumbet* Roxb. are alterative, analgesic, anthelmintic, anti-atrabilius, blood purifier, carminative, demulcent, deobastruent, diaphoretic, diuretic, emmenagogue, expectorant, febrifuge, purgative, resolvent, sedative, and anti-inflammatory. *Alpinia Zerumbet* has various medicinal properties as it is an anti-inflammatory and analgesic. It contains rhizomes which are useful in rheumatism and catarrhal affections. In affections of gastrointestinal tract, the drugs can be used like other volatile oils. The rhizomes exhibit antiulcer activity. The rhizomes contain dihydro 5, 6-dehydrokawain which has been reported to inhibit the aggregation of ATP release from rabbit platelets induced arachidonic acid and collagen [6].

Table1: Botanical Classification of *Alpinia Zerumbet*

Kingdom	Plantae –Plants
Sub-kingdom	Tracheobionta – Vascular plants
Super-division	Spermatophyta – Seed plants
Division	Magnoliophyta
Class	Lilliopsida – Monocotelydons
Sub-class	Zingiberadeae
Order	Zingiberales
Family	Zingiberaceae-Ginger family
Genus	<i>Alpinia</i> Roxb - <i>Alpinia</i> (P)
Species	<i>Alpinia Zerumbet</i> (Pers) B.L.Burt & R.M.Sm. –shell plant (P)

Figure 3 : Botanical Classification of *Alpinia Zerumbet*

Alpinia Zerumbet is a perennial plant widely distributed in subtropical and tropical regions. Consider to native north eastern India, Burma (myanmar), indo-china and china and Japan. Besides it's cultivated through Southeast Asia and in many other parts^[7]. The species has also been naturalized in Brazil and is also found in some areas of central and South Florida.

In terms of chemical constituents, Kava pyrone (DDK) is a medicinal compound has been detected in leaves, rhizomes, flowers and seeds of *Alpinia Zerumbet* ^[8]. It possesses several biological properties such as plant growth inhibition ^[18], insecticidal activity and antifungal activity. In addition it has antiplatelet, antiulcerogenic and antithrombotic effects. Some phenolic compounds have also been identified in *Alpinia Zerumbet*'s leaves ^[9]. In view of the above described properties the objective of the presented work is to evaluate the radioprotective properties of *Alpinia Zerumbet* as a herbal radioprotector.

3. METHODOLOGY ADOPTED

After processing of the plant leaves, extract was prepared using maceration/ percolation technique. The solubility of the extract was evaluated with multiple solvents such as DDW, 50% methanol, multiple DMSO concentrations: 10%, 20%,30%, 40% and 50%. 50% methanol was found to be the most suitable solvent.

Next, Phytochemical analysis of the extract was done wherein, tests were done to evaluate the presence of reducing sugars, proteins, steroid and triterpenoid, cardiac glycosides, Anthraquinone Glycosides, Cynogenic Glycosides, Coumarin Glycoside, flavonoids, alkaloids, gums, tannins and phenolic compounds. Further Thin layer chromatography analysis was done to determine the chemical constituents of the extract.

Cytogenetic analysis was undertaken to finally evaluate the radioprotective properties of the extract against various levels of radiation (UV) exposure. Cytogenetic analyses are essential to the diagnosis and treatment of different forms of cancer, especially leukemia, cancers of the blood cell-forming system. The results of cytogenetic tests can help to confirm the diagnosis of a form of leukemia, and all the chromosomal abnormalities ^[10].

The cytogenetic analysis was done using cell culture. Below were the key aspects which were considered during the analysis.

1. Pre-treating cells in a hypotonic solution, which swells them and spreads the chromosomes
2. Arresting mitosis in metaphase by a solution of colchicine
3. Squashing the preparation on the slide forcing the chromosomes into a single plane
4. Cutting up a photomicrograph and arranging the result into an indisputable karyogram.

Peripheral Blood Lymphocyte culture was done with heparinized blood and using RPMI-1640 media in aseptic condition. The test samples were exposed to different duration of radiation exposure along with 50% methanolic extract of *Alpinia Zerumbet* at different dosage were introduced. Drug control evaluation was set where the cultures were exposed only to various concentration of 50% methanolic extract of *Alpinia Zerumbet*, and only to radiation exposure for 0.5 and 1 hr. The cultured vials were incubated for 72 hours.

The cultures were then harvested for micronucleus and apoptosis assay.

The above described methodology may be utilized for any cytoprotective evaluation process.

4. RESULTS AND DISCUSSION

Prior to performing in vitro cytoprotective assay, phytochemicals analysis of the extract was performed and presence of different phytochemicals such as sugars, protein, steroid tri-terpinoid, cynogenic glycosides, phenolic compound, anthraquinone, tannins flavonoids, and alkaloids was detected.

radioprotective potentiality of the extract was studied on the basis of number of micronucleus containing cells (MN cells) and apoptotic cells (AP cells) found in the peripheral blood lymphocytes culture.

To evaluate cytoprotective effect of the *Alpinia Zerumbet*, 5 major groups for radiooprotection analysis were taken as part of the experiment these included

To evaluate cytoprotective effect of the *Alpinia zerumbet*, 4 major groups for radioprotection analysis were taken as part of the experiment these included

1. NC- Normal control group (only Blood).
2. RC- Radiotherapy control group treated with UV radiation with different exposure times.
3. DC- Drug (*Alpinia zerumbet* extract) control group treated with 5 different concentrations of extract of *Alpinia zerumbet* to assess the genotoxicity of the drug.
4. R&D- Drug and radiation therapy control group treated with different UV exposure times and drug administered either at the time of exposure or 24hrs after radiation exposure.

The number of micronucleus (MN) and apoptotic (AP) cells were observed per 100 cells and scored per group. Summary of the results for each group is presented below.

1. In the case of normal control (NC) group, in which no treatment was given to the culture, no MN cells were found. 1% of the cells were seen to undergo apoptosis.
2. The radiation group R1C, in which cells were exposed to UV radiation for half an hour, showed 12% of MN cells and 14% apoptotic cells. In the radiation group R2C, in which cells were exposed to UV radiation for one hr, we found 18% of MN cells and 20% of AP cells.
3. In case of DC group, to see the genotoxic effect of extract, the culture was treated with various drug conc. i.e. 20%, 40%, 60%, 80%, 100%, minimal damage to cells was observed as 3-6% of the cells were found to contain MN cells, 60% drug conc. had maximum toxicity with 6% of MN cells, apoptosis was observed in 9%, in case of 60% and 80% drug conc., 11% in 20% and 100% drug conc. and 10% in 40% drug conc.
4. In case of R&D group, MN cells and AP cells were scored in the case of groups that were irradiated at the time of culture, with simultaneous treatment with various conc. of the drug. The groups exposed to half an hr UV radiation showed decrease in MN cells with respect to increase in drug conc. MN cells decreased to 8%, 6%, 6%, and 5% in culture treated with 20%, 40%, 60%, and 80% respectively. At 100% drug conc., no MN cells were found. However, not much decrease in number of AP cells was observed 14%, 12%, and 15%

AP cells were scored 20%, 40%, and 80% drug conc. respectively. It decreased up to 5% and 7% AP cells was seen in 60% and 100% drug conc. group.

5. The cultures exposed to UV radiation for one hr, after drug treatment were observed for % of MN and AP cells. The drug concentrations were found to exhibit considerable protective effect as the % MN cells scored for conc. 20%, 40%, 60%, 80% and 100%, the frequency of MN cells was reduced to 8%, 6%, 8%, 3% and 5%, respectively when compared to the 18% found in radiation control group (R2C). However, not much decrease in frequency of AP cells was observed. 13% cells were found to have undergone apoptosis in 20% and 60% drug conc. treated groups, and 10%, 11%, and 8% were found in 40%, 80%, and 100% drug conc. treated groups, respectively.

MN and AP cells were also scored in the culture treated with drug at the time of culture and exposed to UV radiation after 24 hrs for duration of half an hr and one hr. A large decrease in the number of MN cells was observed in case of drug pretreated culture irradiated after 24hrs with UV radiation for one hr duration. Except 20% drug conc., which had 4% MN cells, all other groups (40%, 60%, 80% and 100%) were found to possess 1-2% MN cells. However, decreased in AP cells were observed with respect to increasing drug conc. 9% AP cells were found in 20% drug conc. treated groups, 5% AP cells in case of 40% and 60% drug conc. groups, and 4% AP cells were found in respect to 80%, and 100% drug conc.

Summary of the results are presented in Table 2 and statistical analysis figure 2,3,4,5:

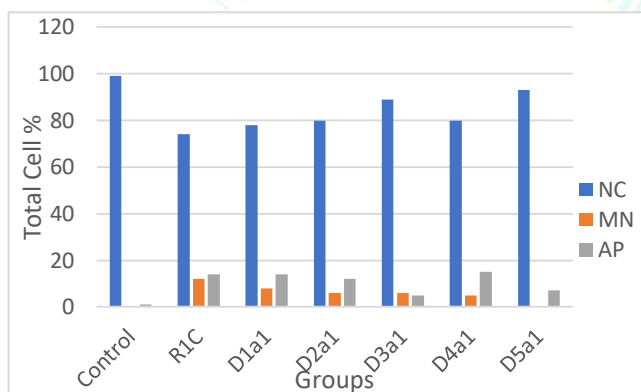


Figure 2

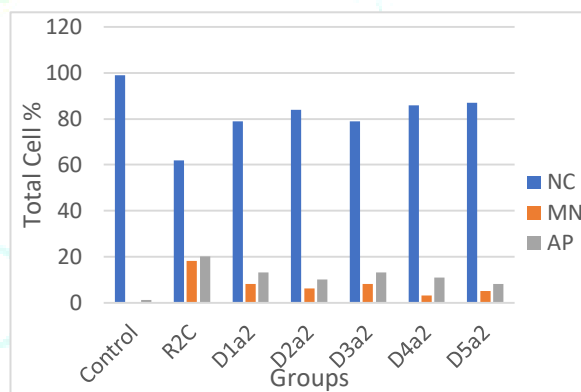


Figure 3

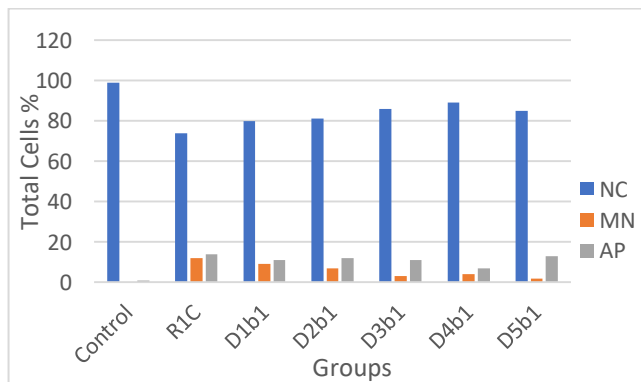


Figure 4

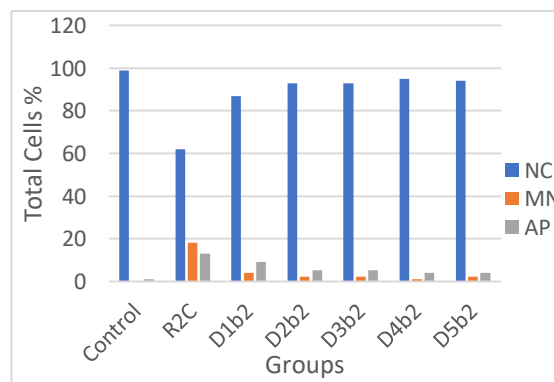


Figure 5

Figure 2: Comparison of NC, MN and AP cells in control, radiation control (exposure 1/2 hr) and drug treated groups when drug is given as an adjuvant.. Figure 3: Comparison of NC, MN and AP cells in control, radiation control and drug treated group (1 hrs radiation) Figure 4 : Comparison of NC, MN and AP cells in control, radiation control (1/2 hr exposure after 24hrs of culture) and drug groups. Figure 5: Comparison of NC, MN and AP cells in radiation control and radiation drug control group (1 hrs radiation after 24 hrs of culture).

Table 2: Represents the Micronucleus, Apoptotic cells And Total cells in In-Vitro Lymphocyte culture treated with Radiation therapy and Aqueous Extracts of *Alpinia Zerumbet* leaf.

S.No.	Group	Sub Group	Total cell	% of Normal cell	% of Micronucleus cells	% of Apoptotic cells
1	NC	NC	100	99	0	1
2	RC	R1C	100	74	12	14
3		R2C	100	62	18	20
4	DC	D1	100	84	3	11
5		D2	100	86	4	10
6		D3	100	85	6	9
7		D4	100	87	5	9
8		D5	100	85	4	11
9	RD	D1a1	100	78	8	14
10		D2a1	100	80	6	12
11		D3a1	100	89	6	5
12		D4a1	100	80	5	15
13		D5a1	100	93	0	7
14		D1a2	100	79	8	13
15		D2a2	100	84	6	10
16		D3a2	100	79	8	13
17		D4a2	100	86	3	11
18		D5a2	100	87	5	8
19		D1b1	100	80	9	11
20		D2b1	100	81	7	12
21		D3b1	100	86	3	11
22		D4b1	100	89	4	7
23		D5b1	100	85	2	13
24		D1b2	100	87	4	9
25		D2b2	100	93	2	5
26		D3b2	100	93	2	5
27		D4b2	100	95	1	4
28		D5b2	100	94	2	4

Abbreviation:

NC	No Control
RC	Radiation Control
DC	Drug Control
RD	Radiation and Drug control
R1	0.5hr radiation exposure
R2	1 Hr Radiation exposure
D1,D2,D3,D4,D5	10%,20%,30%,40%,50% drug conc.
a1	Drug treatment at time of exposure
a2	Drug treatment 24hr after exposure

5 CONCLUSIONS

Radioprotective potential of alcoholic extract of *Alpinia Zerumbet* against various exposure levels of radiation was conducted.

The effect of UV radiation on lymphocytes was studied. Also, radio protective effect of drug was studied to determine whether it has better protective activity as an adjuvant to radiation exposure or when it is administered prior to the exposure.

The study shows that with increase in drug concentration, that radio protective effect exerted against UV radiation increases. When the drug was administered as an adjuvant, considerable decrease in the number of micronucleus containing cells was observed, for both the radiation

exposure levels of half hour and one hour. This shows that the drug was effective in preventing chromosomal damage caused by radiation. However, it was not effective enough in prevention of the radiation induced apoptosis of cells.

In the study, when the lymphocytes were pretreated with the drug 24 hrs before the radiation exposure, the drug was found to be effective in preventing chromosomal damage as well as induction of apoptosis. However, the protective effect was observed to be more in case of long exposure time, rather than the short half an hour exposure to UV radiation.

This protective effect of the *Alpinia Zerumbet* leaf extract can be attributed to the presence of compound like flavonoids and phenolics, which are known to possess free radical scavenging activity.

6 CONFLICT OF INTEREST

None

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