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The traditional role of phytochemicals as the driving force behind the discovery of chemopreventive compounds and anticancer drugs has, in recent years, been reinforced by major advancements in our understanding to cancer biology and genetics. The emergence of relatively small yet highly diversified chemical libraries of phytochemicals is an important step towards enabling multiple pathways to be effectively probed. Furthermore, the success of novel chemopreventive approaches using screens for multiple diseases indicate that it might be useful in the selection of effective cancer chemotherapeutics and for the development of cytotoxic and oncogene-targeted agents.

Cancer is a disease in which disorder occurs in the normal processes of cell division, which are controlled by the genetic material of the cell. The process of carcinogenesis encompasses a slow journey of normal healthy cells to stages known as initiation, fixation of mutation, transformation, promotion, proliferation, and finally progression. These stages appear amenable to inhibition, reversal or retardation at various points. Here, cancer chemoprevention takes a crucial role, and can be defined as the prevention, inhibition or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. Many of these early precancerous lesions favor cell division over quiescence and protect cells against apoptosis.
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when signals are present. While it is generally accepted that a diet of large amounts of vegetables, fruits, and other plant products lowers cancer incidence, there is still a need to identify the most effective constituents of the diet as well as to elucidate their mechanisms of action. Especially for the skin cancer, chemoprevention could be an important armamentarium because skin is continuously exposed to various environmental carcinogens that include both chemical agents and solar ultraviolet radiations. A wide range of compounds, both synthetic and naturally occurring has been shown to possess cancer chemopreventive effects in in-vitro and in-vivo carcinogenesis models. Many of these phytochemicals have also shown chemopreventive effects against skin carcinogenesis. Studies from laboratories worldwide have shown that naturally occurring compounds present in human diet and beverages such as constituents of green/black tea, dialyl sulfide, resveratrol, curcumin, lupeol, indole-3-carbinol and [6]-gingerol affords protection against the development of skin cancer, both under in vitro as well as under in vivo situations. Various researches has addressed the chemotherapeutic potential of [6]-gingerol, the major phenolic ketones constituent of ginger rhizome.

In the view of these perspectives, the present study was designed to elucidate the cancer chemopreventive properties and the mechanism of [6]-gingerol in in vivo mouse skin carcinogenesis and in in vitro skin cancer cells with the following objectives:
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➢ To analyze the antimutagenic potential of [6]-Gingerol against carcinogen induced DNA damage.
➢ To investigate the influence of [6]-Gingerol on cell cycle regulation.
➢ To study apoptosis as a mechanism of cancer chemoprevention by [6]-Gingerol.
➢ To quantify the amount of [6]-gingerol in locally available ginger rhizomes.

With these aims and objectives, this thesis has been structured into four individual chapters:

In chapter I, we studied the role of [6]-gingerol in the induction of reactive oxygen species regulated mitochondrial cell death pathway in human epidermoid carcinoma A431 cells. Herein, [6]-gingerol was assessed for its anti-cancer effects in human epidermoid carcinoma A431 cells. Based on IC₅₀ value (300 µM) apparent from growth inhibition curve, we selected 250, 300 and 350 µM doses of [6]-gingerol for 48 h treatment for our further studies. Its treatment exhibited considerable cytotoxicity as indicated by growth inhibition of A431 cells mediated via generation of reactive oxygen species (ROS). This led to decrease in mitochondrial membrane potential (MMP) and subsequent induction of apoptosis shown by increase in sub G₁ cell population and DNA laddering pattern. Reverse transcriptase polymerase chain reaction (RT-PCR) results revealed that perturbations in mitochondrial membrane are associated with deregulation of Bax/Bcl-2 ratio at gene transcriptional level, where treatment with
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[6]-gingerol leads to upregulation of cytochrome c and Apaf-1 subsequently culminating in triggering of caspase cascade. This data asserted that it exhibits potent anti-cancer effects against in vitro skin cancer model.

In chapter II, we explored the role of [6]-gingerol against direct (ethyl) methanesulfonate (EMS), and indirect (7,12-dimethyl-1,2-benzanthracene (DMBA) and benzo[a]pyrene (B[a]P) carcinogen induced DNA strand breaks, using DNA alkaline unwinding assay. The chosen rodent model was Swiss albino mice to extrapolate the chemopreventive effect of [6]-gingerol on human beings. DMBA at a dose of 100µg/animal, B[a]P at a dose of 100µg/animal and EMS at a dose of 125µg/animal in separate sets of experiments, were utilized to assess the anti-mutagenic potential of [6]-gingerol. Increasing doses of [6]-gingerol were applied, prior / post to the single topical application of mutagen with the sampling time of 24, 48, 72 and 96 hours, respectively. [6]-Gingerol treatment showed a significant preventive effect against DNA strand breaks induced by the mutagens in dose and time dependent manner (p< 0.001). The pre-treatment of [6]-gingerol at the dose of 12 µg/animal showed up to 71.89%, 68.91% and 46.01% prevention, and post-treatment at the same dose showed upto 87.09%, 76.92% and 58.17% prevention, at 96 hours time interval, against DMBA, B[a]P and EMS, induced DNA damage, respectively. The results clearly demonstrates that topical application of [6]-gingerol prior to mutagen exposure led to a significant protection in time- and dose-dependent manner on the average number of DNA strand breaks. It may be due to its role as a blocking agent in the detoxification
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and inhibition of activation of promutagens and other xenobiotic compounds. While, post-treatment with [6]-gingerol, found to be better effective, could be because of its antioxidant ability which helps in reducing the frequency of occurrence of mutagens induced DNA strand breaks, predicting its role as a suppressing agent. Taken together the data imply that [6]-gingerol demonstrated a strong antimutagenic effects of against DNA alkylation damage induced by direct and indirect acting mutagens.

Chapter III was aimed at unraveling the molecular mechanisms underlying antitumorigenic potential of [6]-gingerol against benzo[a]pyrene (B[a]P) induced mouse skin tumorigenesis. Topical treatment of [6]-gingerol (2.5μM/animal) was given to the animals prior and post to B[a]P (5μg/animal) administration up to 32 weeks. Chemopreventive properties of [6]-gingerol were reflected by delay in onset of tumorigenesis, reduced cumulative number of tumors and reduction in tumor volume. Histopathological examination and p53 immunohistochemical staining were performed to visualize the influence of [6]-gingerol supplementation on the aggressiveness of tumors induced by B[a]P. The appearance of sub-G1 peak, indicative of apoptosis, was found to be significant on [6]-gingerol supplementation in cell cycle studies. Results revealed that perturbations in cell cycle were associated with the induced expression of the p53 and pro-apoptotic Bax, with concomitant decrease in anti-apoptotic protein Bcl-2. Alteration in Bax/Bcl2 ratio by [6]-gingerol treatment resulted in apoptosis, which was associated with the release of cytochrome c and induction of apoptotic protease-activating factor-1 (APAF-1). Further, this effect was found to result in cleaved
fragments of caspase-9,-3, and poly (ADP-ribose) polymerase (PARP). Also, throughout the experiment, the post treatment of [6]-gingerol demonstrated elevated apoptotic propensity against B[a]P induced tumorigenesis. These findings demonstrate for the first time that [6]-gingerol induces apoptosis through commencement of p53 activity in mouse skin tumors, thereupon suggesting its chemopreventive activity, through the modulation of proteins involved in mitochondrial pathway of apoptosis.

Next, the reason that [6]-gingerol is a major constituent of ginger impelled us to determine its concentration in the rhizomes of Indian ginger. Chapter IV includes the exercise of quantification of [6]-gingerol in different ginger rhizomes purchased randomly from the local market by high-performance liquid-chromatography (HPLC). The HPLC method was validated by defining the linearity of different standards employed (R² value). Therefore, the amount of [6]-gingerol was calculated individually for 3 locally available rhizomes of ginger which was found to vary widely from 104.57±8.78 to 965.25±9.56 μg/g of the rhizome. So, the chromatographic analysis of [6]-gingerol may be useful to standardize the components of ginger for use as dietary supplements along with their doses. Such information would more precisely aid in identifying the safest and most effective dose range.

Conclusively, all these observations confirm that [6]-gingerol acts as a potent chemopreventive agent against skin carcinogenesis. We can conclude through this study that [6]-gingerol can regulate intrinsic apoptotic pathways by directly
triggering apoptosis-promoting signaling cascades as mechanism of cancer chemoprevention (Fig. 1). It is hopeful that further characterization of pathways regulating cell cycle progression and apoptosis will facilitate novel drug discovery programs to exploit [6]-gingerol for the prevention and treatment of several human cancers. Our hypothesis is that the topical treatments with [6]-gingerol acting as a natural source inhibitor of different stages of skin carcinogenesis result in an efficient prevention of skin cancer.
Fig.1. Concept diagram to show apoptosis as a mechanism of cancer chemoprevention by [6]-gingerol using *in vitro* and *in vivo* cancer models.
List of Publications
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RESEARCH PUBLICATIONS


BOOK CHAPTER/ REVIEW

1. Nigam N, George J, Shukla Y. Molecular Targets and Therapeutic uses of Ginger (6-gerinol). In: Molecular Targets and Therapeutic Uses of Spices: Modern Uses for Ancient Medicine. Eds : Bharat B. Aggarwal, Ajai Kumar B. Kunnumakkara. Cytokine Research Laboratory, Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, USA (In press).


ABSTRACTS IN CONFERENCES/ SEMINARS


2. Nigam N. and Shukla Y. Preventive Effects of Lupeol on DMB \ Induced DNA Alkylation Damage. International Conference on Biomarkers in the


4. Roy P., Nigam N. and Shukla Y. Induction of apoptosis by tea polyphenols through Bax translocation, cytochrome c release and caspases activation in mouse skin tumors. XXVII Annual Conference of Society of Toxicology and International Workshop on Toxicology held at Bangalore. October 6-12, 2007.