

Original article

Exploring the anti-tumor efficacy of Paclitaxel – Diallyl Di sulfide mediated apoptosis in 7,12 Di Methyl Benz (a) Anthracene Induced skin cancer in Wistar rats

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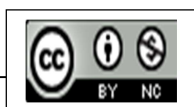
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Abstract

Background: Skin cancer is the most prevalent and common kind of cancer in Western countries. According to GLOBOCAN-2020 statistics, skin cancer is one among every three diagnosed malignancies worldwide. Apoptosis, a cellular self-destruction mechanism, is essential for maintaining tissue homeostasis, removing damaged cells, ensuring correct development. Apoptosis is characterized by morphological changes that occur on a regular basis, such as nuclear and inter-nucleosome DNA fragmentation, chromatin condensation, and plasma membrane blebbing. Both Paclitaxel and Di-allyl Di-Sulfide (DADS) exhibit anticancer properties by inducing cell-cycle arrest and activating apoptosis in various cancer cell lines. The aim of the study is to investigate the apoptotic changes induced by Paclitaxel and DADS in skin cancer-afflicted rats.

Methods: By evaluating the expression of Bcl2, p53 and caspase 3 apoptotic genes in skin cancer bearing male Wistar rats induced by Di-methyl Benz(a) Anthracene (DMBA), the efficacy of paclitaxel and Di-allyl Di-Sulfide combination were determined.

Results: The organo-Sulphur compound DADS and standard chemotherapeutic drug Paclitaxel revealed that they have the capacity to restore balance between aberrant and normal cellular circumstances.

Conclusion: This study suggests that natural bioactive compounds have regenerative and regulating capabilities when supplied to skin cancer animals.

Keywords: Skin Cancer, Di-methyl Benz(a) Anthracene, Di-allyl di sulfide, Paclitaxel, Apoptosis

Introduction

Cancer, an intimidating health concern, is on the rise globally, with projections indicating that it will continue to rise in the coming decades. Melanoma is a significant burden on millions of people globally, as indicated by GLOBOCAN-2020 figures indicating approximately 20 million new cases each year, with a 50% fatality rate, according to the World Health Organization (WHO) (1). The risk of getting skin cancer increases after fifty years, but exposure to harmful radiation from sun emphasizes the need to take preventative actions from a young age. One of the hot topics widely researched is about the anti-cancer property exhibited by various natural substances possessing medicinal properties. Adding these compounds to diet might protect the cells against genotoxins and carcinogenic substances. Several investigations on fruits and vegetables possessing anti-cancer properties revealed that people who intake fruits and vegetables experience multifaceted protection against cancer (2). Steinmetz and Potter's review emphasizes that a significant negative correlation exists between the risk of skin, liver, stomach, lung, and colon cancers and the consumption of fruits and vegetables. Scientific evidence suggests that good health promoting agents such as fruits, vegetables and spices contain numerous bioactive compounds and these have the ability to reduce cancer risk. So, natural compounds have caught attention among scientific community. Chemical compounds from natural sources are available in market and many of these substances are prescribed in traditional medicine. The active compounds present in garlic, turmeric, and citrus fruits contain protective phytochemicals which demonstrate potential protective efficacy

against cancer when administered in their natural form or as active constituents. These phytochemicals interfere with mutational mechanisms underlying cellular transformation, hyperproliferation, and the initiation of carcinogenesis. These inhibitory mechanisms prevent angiogenesis and metastasis processes.

The process of cancer development can be initiated by environmental carcinogens such as 7, 12 Dimethyl Benz (a) anthracene(DMBA), cigarette smoke etc. and biological factors like Tumor Necrosis Factor (TNF) and H_2O_2 . These carcinogens modulate the transcription factors, anti-apoptotic proteins, proapoptotic proteins, protein kinases, cell cycle proteins, cell adhesion molecules, COX- 2, and growth factor signaling pathways. Paclitaxel (Taxol), a naturally occurring antineoplastic agent has shown great promise in the treatment metastatic breast cancer, skin cancer, lung cancer and refractory ovarian cancer. Paclitaxel is a potent inhibitor of eukaryotic cell replication, blocking cells in the late G2-M phase of the cell cycle(3). It is a unique antimicrotubular agent. Throughout recorded history, garlic (*Allium sativum*) possesses lipid-lowering, antimicrobial, chemo-preventative, and anticarcinogenic properties. The chemotherapeutic and anticancer attributes of garlic are attributed to its organo sulfide-based active compounds, notably Di-allyl Di sulfide (DADS) (4). A study asserts the efficacy of DADS across various cancers affecting diverse organs, including the skin, lungs, esophagus, colon, and liver (5). Adjuvants often increase the efficacy of standard chemotherapeutic agents. The combination effects of DADS along with standard chemotherapeutic drug paclitaxel is not studied extensively in vivo in skin cancer models. The present study centers around evaluating the efficacy of the combinatorial treatment of paclitaxel and DADS. The alterations in the molecular mechanisms are assessed when the combinatorial treatment is given for prevention and treatment of skin cancer.

Materials and Methods

Chemicals and Reagents

7,12 Di Methyl Benz(a) Anthracene, Di-allyl Di sulfide, Paclitaxel, Acetone purchased from Sigma Chemicals and other chemicals and Reagents purchased from SRL Chennai, India.

Animals

In the present study, the animals were taken care following CPCSEA guidelines and protocols. For this study ethical permission was obtained from the Institutional Animal Ethics Committee (IAEC no: 003/2019, dated 04.07.2019). The healthy adult male Wistar albino rats of the Wistar strain (*Rattus norvegicus*) were utilized for the study with the age between 150 to 180 days and weighing between 180 to 220gms. These rats were housed in clean polypropylene cages within the Animal House Facility at Meenakshi Medical College Hospital and Research Institute, where they were maintained under specific environmental conditions, including a humidity level of 65+ 5% and a temperature of 21 +2°C, with a consistent 12-hour light and 12-hour dark cycle. They were provided with a standard pellet diet (Lipton India, Mumbai, India) and had access to clean drinking water ad libitum.

Experimental Protocol

The rats were categorized into 6 groups. Each group comprised of 6 rats.

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|------|--------------------|--|
| i. | Group I : | Normal rats. |
| ii. | Group II : | Rats in which 7, 12 Di Methyl Benz (a) Anthracene (5µg/kg body weight) is applied topically three times a week for twenty-eight weeks. |
| | Group III : | Skin tumor induced rats treated with Paclitaxel (33mg/Kg. body weight) weekly once for four weeks. |
| iii. | Group IV : | Skin tumor induced rats treated with Di-allyl Di sulfide for Thirty days. (250µg/kg. body weight) |
| iv. | Group V : | Skin tumor induced rats treated with both paclitaxel and Di-allyl Di sulfide. (Concentration as Above) |

At the end of experimental period, 2ml of blood samples were collected from the retro orbital venous plexus and placed into collection tubes. The serum was then separated from these blood samples by centrifugation at 3500rpm for 15 minutes. After separation, the serum was stored in a freezer at -20°C. The skin and liver samples were meticulously dissected, cleaned with ice-cold saline and then stored at -70°C. After weighing the tissues right away, they were homogenized in 0.1 M Tris HCl buffer (pH 7.4). The serum and tissue analyses were performed for various biochemical parameters.

Immunoblotting for Bcl₂, p53 and Caspase 3

Protein blotting, also referred to as immuno- or western blot analysis, is a technique used to isolate particular proteins from a variety of samples. It entails employing antibodies to find particular proteins on PVDF or nitrocellulose membranes. The system consists of four basic methods: finding and imaging the target protein(s), electrophoresis of the target protein-containing sample, electrophoretic transfer (blotting) to a membrane, and labeling of the target protein(s) with certain primary and secondary antibodies. The protein concentration was measured by Lowry's method (1951). Bcl-2 (50 g of protein), p53 (40 g of protein) and Caspase 3 (40 g of protein) protein were fractionated by SDS- runner using 12 gel and transferred to nitrocellulose membrane as described by Towbin et al. (1979).

Statistical Analysis

A One-way Analysis of Variance (ANOVA) and Tukey's Multiple Comparison Test were performed by using SPSS – 17 versions to calculate the significance of difference of means of treatment groups. All the data's were also subordinated to Student's 't' test (Paired) wherever needed, to calculate the significance of difference of means of the control and experimental groups using SPSS software tools. All the values were expressed as mean $\pm$ standard deviation. p value less than 0.05 was considered as significant.

Results

1) Bcl₂ protein expression

Figure .1 Antitumor efficacy of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on Bcl₂ protein expression in control and experimental groups of rats.

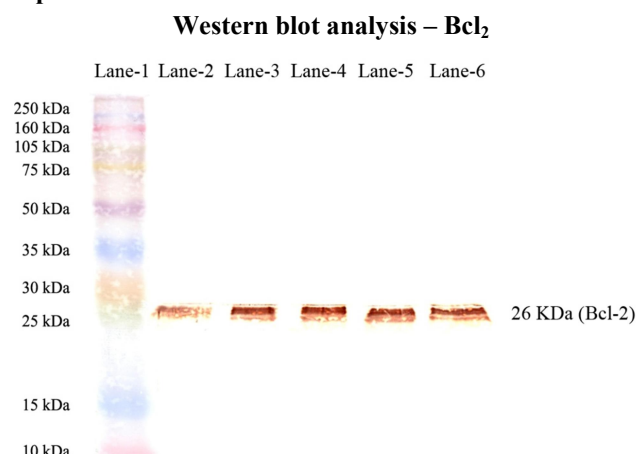


Fig 1: Western Blot Analysis of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on Bcl₂ protein expression in control and experimental groups of rats. Lane 1: Marker, Lane 2: Control, Lane 3: DMBA treated, Lane 4: Paclitaxel treated, Lane 5: Di allyl di sulfide treated, Lane 6: Paclitaxel and Di allyl di sulfide treated cancer bearing animal

Each value is expressed as Mean $\pm$ SD for the experiment. Comparisons are made between: a- as compared with Group I, b- as compared with Group II. Statistical significance - *P < 0.05.

Fig.1 depicts the Bcl₂ protein expression by western blot technique of control and experimental group of rats. The Bcl₂ protein, one of the main anti-apoptotic constituents of the Bcl-2 family, is necessary for transmitting the intracellular apoptotic signal. In group-II rats, it was observed that Bcl2 protein was much more expressed in comparison with group-I rats.

This protein expression was significantly low (P < 0.05) in group-V rats in contrast to group-II rats. Western blot study of malignant cells received the combined therapy of both paclitaxel and DADS revealed similar outcomes of Group-I rats. The outcome thus implies that paclitaxel and DADS help reduce tumor growth when combined.

2) p53 Protein Expression

Figure. 2 Antitumor efficacy of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on p53 protein expression in control and experimental animals

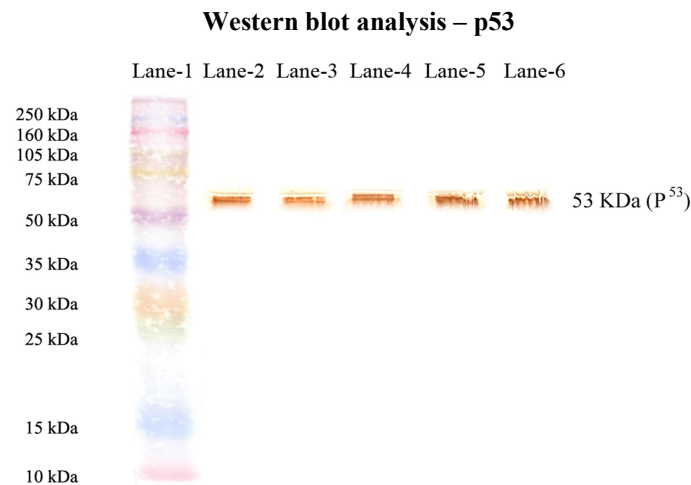


Fig 2: Western Blot Analysis of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on p53 protein expression in control and experimental animals. Lane 1: Marker, Lane 2: Control, Lane 3: DMBA treated, Lane 4: Paclitaxel treated, Lane 5: Di allyl di sulfide treated, Lane 6: Paclitaxel and Di allyl di sulfide treated cancer bearing animal.

Each value is expressed as Mean $\pm$ SD for the experiment. Comparisons are made between: a- as compared with Group I, b- as compared with Group II. Statistical significance - *P < 0.05.

Figure.2 displays the Antitumor efficacy of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on p53 protein expression in control and experimental rats after Western blot analysis. When group-II rats were treated with Paclitaxel – Di-allyl Di sulfide, it was discovered that the amount of p53 protein was slightly higher by $p < 0.05$, showing cell cycle arrest. Paclitaxel – Di-allyl Di sulfide was observed to significantly ($p < 0.05$) elevate p53 protein expression when combined, as opposed to either medication alone.

3) Caspase-3 Protein Expression

Figure.3 Antitumor efficacy of Paclitaxel and Paclitaxel – Di-allyl Di sulfide onCaspase–3 protein expression in control and experimental animals

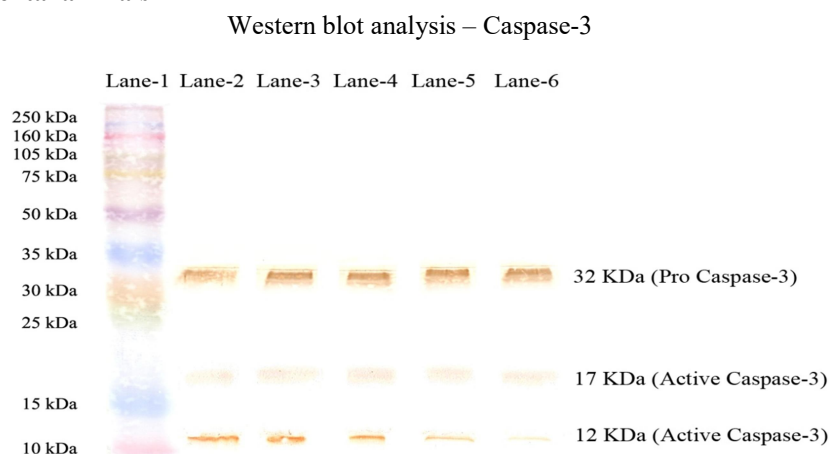


Fig.3: Western blot analysis of Paclitaxel and Paclitaxel – Di-allyl Di sulfide on Caspase–3 protein expression in control and experimental animals. Lane 1: Marker, Lane 2: Control, Lane 3: DMBA treated, Lane 4: Paclitaxel treated, Lane 5: Di allyl di sulfide treated, Lane 6: Paclitaxel and Di allyl di sulfide treated cancer bearing animal.

Each value is expressed as Mean $\pm$ SD for the experiment. Comparisons are made between: a- as compared with Group I, b- as compared with Group II. Statistical significance - *P < 0.05.

Figure 3 shows the caspase 3 protein expression level in control and experimental groups of rats by western blot analysis. Compared with the rats of group-I, the caspase-3 was observed to be reduced in the rats of group-II. Caspase-3 proteins were significantly ($P < 0.05$) elevated in the rats of group V treated with a combination of Paclitaxel – Di allyl di sulfide than in groups III & group IV (different drug treatments).

Discussion

Apoptosis occurs in all organisms which is pivotal for elimination of damaged cells from the body. Apoptosis, referred as programmed cell death, represents a genetically controlled physiological, cellular, and biochemical suicide procedure which is indispensable for maintaining homeostasis and promoting growth. Current understanding shows that alterations in the regulation of cell death can lead to the formation of tumors. Cancer development is often associated with heightened cell proliferation and diminished apoptosis. The induction of apoptosis has the potential to impede or decelerate cancer growth (6).

The majority of chemotherapeutic drugs function by either hindering cell division or actively engaging in the genetically controlled process of programmed cell death. The key to the success of this therapeutic strategy lies in the capability to encourage tumor cells to actively participate in the programmed cell death program (7).

The apoptosis mechanism involves DNA fragmentation, cell shrinkage and disassembly into membrane-enclosed vesicles, chromatic condensation, and membrane blebbing (8). These modifications happen in a duplicable sequence, are predictable and can be accomplished within minutes.

Bcl2 and Apoptosis

Among the pivotal regulators of apoptosis are the expanding families of Bcl oncogenes. Bcl-2, Bcl-XL, and BFL-1 are examples of components that exert inhibitory effects on apoptosis, whereas Bim, Bax, and Bak serve as stimulators of apoptosis (9). The B cell lymphoma/leukemia gene 2 (Bcl2) is notably associated with various cancers, initially believed to result solely from specific translocations. These translocations position the Bcl2 gene in proximity to a potent enhancer promoter sequence of the immunoglobulin H gene.

A plethora of genes have been found and placed under the Bcl2 family name based on their sequence similarity with Bcl2. These genes include both negative and positive apoptosis regulators. Cells are protected against apoptosis by the Bcl2 proto-oncogene. Bcl2 has two unique intracellular localizations, according to morphological and biochemical studies: the mitochondria and the endoplasmic reticulum (ER).

While mitochondria are well-known as apoptosis-related organelles, Bcl2 also has an anti-apoptotic effect in response to cytochrome C. This is accomplished by inhibiting mitochondrial permeability transition and finally releasing cyt-C. Bcl2 obstructs cell death pathways downstream of cytochrome C, presumably at the ER level, according to the findings. Another study found that ER-targeted Bcl2 might prevent myc-induced apoptosis in a mouse fibroblast cell line, lending evidence to this theory (10).

In various cell types, Bcl2 proteins play a crucial role as pivotal regulators of the apoptotic process, as indicated by in-vitro research. The discovery of Bcl2 was facilitated by translocation studies, particularly prevalent in follicular and non-Hodgkin lymphomas (11). Di-allyl Di sulfide has demonstrated efficacy in treating ovarian, skin, lung, and breast carcinomas, significantly suppressing Bcl2 protein expression in treated mice. While the exact mechanism of cell death remains unclear, tumor cells treated with Di-allyl Di sulfide exhibited DNA fragmentation patterns indicative of apoptosis. The ability of Di-allyl Di sulfide to induce apoptosis has been associated with the phosphorylation of the anti-apoptotic Bcl2 protein. It's worth noting that negative regulation of the Bcl2 protein can also lead to the initiation of apoptosis (12). In the current study, Bcl2 was similarly downregulated in rats with skin cancer which received a combination of paclitaxel and Di-allyl Di sulfide. Based on the current investigation's findings, it is hypothesized that Di-allyl Di sulfide's anticancer action involves induction of apoptosis.

p53 and Apoptosis

The TP53 gene encodes p53, a transcription factor also known as protein 53 or tumor protein 53. Within multicellular organisms, p53 assumes a crucial role in regulating the cell cycle and functions as a tumor suppressor, contributing to the prevention of tumorigenesis. The genomic location of p53 has been identified on chromosome 17.

Under the influence of the p53 protein, a distinct gene within the cell is activated to synthesize the p21 protein. This p21 protein then interacts with a protein that promotes cell proliferation, namely cyclin-dependent kinase 2 (cdk2).

When the p21 and cdk2 form a complex, the cell is hindered from progressing to the subsequent stage of cell division. In the mitosis process, the p21 protein acts as a signal to halt cell division. Unbridled cell division can lead to the formation of tumors. The p53 tumor suppressor, recognized for its role in sequence-specific transcription, is capable of inducing growth arrest in cells or triggering apoptosis in response to cellular stress. The selection of these cellular

responses is influenced by various factors, including cell type, stress levels, the activity of p53 co-activators, and the specific nature of the cell (13).

In the present study, it was observed that skin cancer cells exhibited markedly reduced levels of p53 expression. The diminished expression of p53 may contribute to the promotion of cell cycle progression, potentially fostering the growth of skin cancer cells. However, immunoblot analysis revealed a significant upregulation of p53 levels in skin cancer cells treated separately with paclitaxel and di-allyl di sulfide, as well as in those treated with a combination of both substances. This observed increase in p53 levels could be attributed to the anti-tumor capabilities of di-allyl di sulfide, coupled with the mitotic arrest induced by di-allyl disulfide, thereby enhancing the cytotoxic activity against skin cancer cells.

Furthermore, studies have shown that di-allyl disulfide-induced apoptosis at low dosages (0.01 M) is mediated via the p53 signaling system. It has also been revealed that di allyl disulfide-induced apoptosis is contingent on both concentration and the cell cycle dynamics (15). In apoptosis-resistant SCC-VII tumors, di allyl disulfide upregulated the p53 gene (14).

While the current study observed an increase in p53 expression with di-allyl disulfide therapy at 0.5 M, its precise impact on mediating apoptosis remains unclear. Previous research utilizing di allyl disulfide has indicated that nuclear p53 protein levels rise following di allyl disulfide treatment, aligning with the occurrence of DNA fragmentation. This suggests the crucial involvement of the p53-associated signaling pathway in Di-allyl di sulfide-mediated apoptotic cell death (15). Consequently, the utilization of di-allyl disulfide and paclitaxel emerges as a highly effective strategy to enhance the p53 pathway, thereby reducing the viability of skin cancer cells.

Caspases and apoptosis

The proteolytic system, which contains caspase family proteases as main components, is fundamental to the apoptotic system. Upon receiving a pro-apoptotic signal, these enzymes become involved in triggering a cascading effect. Caspases play a pivotal role in inactivating proteins that otherwise inhibit apoptosis in living cells. The mechanism through which caspases induce apoptosis involves the direct destruction of cellular structures, exemplified by the disruption of the nuclear lamina (16). Apoptosis induction was observed in skin cancer animals following treatment with di allyl di sulfide and paclitaxel, evident through the augmentation of caspase-3 activity. Di-allyl disulfide specifically targets mitochondria, leading to the early upregulation of caspase activation during apoptosis (17). In the current study, we found a significant increase in caspase-3 activity in the serum and skin tissues of rat treated with di-allyl disulfide, both alone and in combination with paclitaxel. This increased caspase-3 activity was also seen in skin cancer-bearing rat given paclitaxel alone. Paclitaxel, alone or in conjunction with di-allyl disulfide, may activate caspase-3 by a variety of methods, including activation of the TNF-receptor family, which is recognized for conveying signals for cell death and is hence referred to as a death receptor (18). The increased caspase-3 activity promotes cancer cell death, which is consistent with the found apoptosis-inducing activity in skin cancer-bearing rats treated with Paclitaxel and di allyl disulfide.

DNA Damage

Main objective of measuring DNA damage assessed DNA single strand breaks, DNA protein cross links and the levels of 8-OH deoxyguanosine in the tumor tissue. The single cell gel electrophoresis or comet assay is a novel method for the assessment of DNA strand breakage in a single cell. Narendra P Singh (2016) 934) have reported elevated levels of DNA damage in cancer cases. In the present study also, we have observed significant increase in the comet tail length in cancer bearing animals which is in agreement with the reports by Narendra P Singh (2016).

Conclusion

This study underscores the global prevalence of skin cancer, particularly melanoma, and its widespread impact on millions of individuals. The research delves into the fundamental mechanism of apoptosis, highlighting its pivotal role in maintaining cellular homeostasis and thwarting cancer proliferation. The investigation centers on exploring the potential anticancer effects of Paclitaxel and Di-allyl Di Sulfide in rats afflicted with skin cancer, revealing their capacity to induce apoptosis and modulate crucial proteins such as Bcl2, p53 and Caspase-3. As per the findings, the synergistic application of these naturally occurring bioactive substances may serve to normalize cellular conditions, presenting a promising therapeutic avenue for skin cancer. The outcomes underscore the potential of natural compounds in regulating and restoring skin tissue, contributing to the ongoing battle against skin cancer.

Références

1. Radiation: Ultraviolet (UV) radiation and skin cancer.[Internet]. WorldHealthOrganization. 2017. [Retrieved 2024 10 15]. Available at:<https://www.who.int/uv/faq/skincancer/en/index1.html>.
2. Willett, W.C. Diet and health: what should we eat?.*Science* (New York, N.Y.), 1994;264(5158), 532–537.
3. Schiff,P.B., Fant, J., & Horwitz, S.B. Promotion of microtubule assembly in vitro by taxol. *Nature*. (1979);277(5698), 665-667.
4. Reddy, L.A.L.I.N.I., Odhav, B. and Bhoola, K.D. Natural products for cancer prevention: a global perspective.*Pharmacology & therapeutics*,2003; 99(1),1-13.
5. Arora, A., Kalra, N. and Shukla, Y. Regulation of p21/ras protein expression by diallyl sulfide in DMBA induced neoplastic changes in mouse skin.*Cancer letters*,2006;242(1), 28-36.
6. Pistrutto G, Trisciuglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. *Aging* (albany NY). 2016 Mar 27;8(4):603.
7. Mollaei M, Hassan ZM, Khorshidi F, Langroudi L. Chemotherapeutic drugs: Cell death-and resistance-related signaling pathways. Are they really as smart as the tumor cells?. *Translational Oncology*. 2021 May 1;14(5):101056.
8. Elmore S. Apoptosis: a review of programmed cell death. *Toxicologic pathology*. 2007 Jun;35(4):495-516.
9. Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. *Frontiers in oncology*. 2022 Oct 12;12:985363.
10. Lee ST, Hoeflich KP, Wasfy GW, Woodgett JR, Leber B, Andrews DW, Hedley DW, Penn LZ. Bcl-2 targeted to the endoplasmic reticulum can inhibit apoptosis induced by Myc but not etoposide in Rat-1 fibroblasts. *Oncogene*. 1999 Jun;18(23):3520-8.
11. Klanova M, Klener P. BCL-2 proteins in pathogenesis and therapy of B-cell non-Hodgkin lymphomas. *Cancers*. 2020 Apr 10;12(4):938.
12. Mitra S, Das R, Emran TB, Labib RK, Islam F, Sharma R, Ahmad I, Nainu F, Chidambaram K, Alhumaydhi FA, Chandran D. Diallyl disulfide: a bioactive garlic compound with anticancer potential. *Frontiers in pharmacology*. 2022 Aug 22;13:943967.
13. Borrero LJ, El-Deiry WS. Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2021 Aug 1;1876(1):188556.
14. Saito M, Korsmeyer SJ, Schlesinger PH. BAX-dependent transport of cytochrome c reconstituted in pure liposomes. *Nature cell biology*. 2000 Aug;2(8):553-5.
15. Chiu TH, Lan KY, Yang MD, Lin JJ, Hsia TC, Wu CT, Yang JS, Chueh FS, Chung JG. Diallyl sulfide promotes cell-cycle arrest through the p53 expression and triggers induction of apoptosis via caspase-and mitochondria-dependent signaling pathways in human cervical cancer Ca Ski cells. *Nutrition and cancer*. 2013 Apr 1;65(3):505-14.
16. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harbor perspectives in biology*. 2013 Apr 1;5(4):a008656.
17. Nagaraj NS, Anilakumar KR, Singh OV. Diallyl disulfide causes caspase-dependent apoptosis in human cancer cells through a Bax-triggered mitochondrial pathway. *The Journal of nutritional biochemistry*. 2010 May 1;21(5):405-12.
18. Khing TM, Choi WS, Kim DM, Po WW, Thein W, Shin CY, Sohn UD. The effect of paclitaxel on apoptosis, autophagy and mitotic catastrophe in AGS cells. *Scientific Reports*. 2021 Dec 6;11(1):23490.