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Research article

EFFECT OF PACLITAXEL ALONG WITH DI ALLYL SULFIDE ON IMMUNO COMPETENT CELLS, IMMUNE COMPLEXES AND IMMUNOGLOBULINS CHANGES IN 7,12 DI METHYL BENZ(A) ANTHRACENE INDUCED SKIN CANCER IN WISTAR RATS.

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ABSTRACT

Our recent studies have shown that naturally occurring dietary organo sulfure compounds such as di allyl sulfide and paclitaxel are capable of inhibiting polycyclic aromatic hydrocarbon (PAH) metabolism and subsequent PAH-DNA adduct formation in Wistar rats. In this study these plant phenols were tested for their effects against PAHs and 7,12 Di Methyl Benz (A) Anthracene -induced skin tumorigenesis in rats. Each compounds was evaluated as a possible anticarcinogen in an initiation and promotion and a complete skin tumorigenesis protocol. In the two-stage tumor protocol in Wistar rats using 7,12-dimethylbenz(a)anthracene as the initiating agent followed by twice weekly applications of acetone as tumor promoter each plant compounds afforded significant protection against skin tumorigenicity. The protective effects were verified both by prolongation of latency period and by subsequent tumor development. Our results suggest that these plants compounds have substantial though variable potential for modifying the risk of skin tumorigenicity induced by a wide variety of chemicals and of these combinations of Paclitaxel and Di allyl sulfide was shown to have maximal chemo protective effects.

Keywords: Paclitaxel, Di allyl sulfide, DMBA, Skin cancer.

INTRODUCTION

Skin cancer is the most common form of human cancer. It is estimated that over 1 million new cases occur annually.[1,2] The annual rates of all forms of skin cancer are increasing each year, representing a growing public concern. It has also been estimated that nearly half of all Americans who live to age 65 will develop skin cancer at least once[3]. The most common warning sign of skin cancer is a change in the appearance of the skin, such as a new growth or a sore that will not heal. In India, skin cancers constitute about 1-2% of all diagnosed cancers. Basal cell carcinoma is the commonest form of skin cancer worldwide, but various studies from India have consistently reported SCC as the most prevalent skin malignancy[4]. Although complete data of incidence is not available, various cancer registries in India reported cumulative incidence of skin cancer varying from 0.5 to 2 per 100 000 population[5]. Although, the incidence of skin cancers in India is lower as compared to the Western world, because of a large population, absolute number of cases is estimated to be significant. Skin cancer patients have stage IV receive chemotherapy and /or hormonal therapy to suppress cancer cells and control the disease. The goal of chemotherapy is to destroy, shrink primary tumors, slow the tumor growth, and to kill cancer cells that

may have spread (metastasized) to other parts of the body from the original tumor. Chemotherapeutic drugs elicit some toxicity towards normal cells also, that limits its usage.

Paclitaxel is a naturally occurring antineoplastic agent has shown great promise in the therapeutic treatment of certain human solid tumors particularly in metastatic breast cancer, skin cancer, lung cancer and refractory ovarian cancer[6]. Paclitaxel's antitumor activity was discovered in1960's during a large scale 35,000 plants-screening program sponsored by the National Cancer Institute (NCI), USA. Paclitaxel is a most effective drug in skin cancer, it has several important side affects particularly neutropenia, peripheral neuropathy and hypersensitivity reactions[7]. Myelo suppression or neutropenia is the principal dose limiting toxicity of paclitaxel on all administration schedules. It is undeniable that the need for new agents with both improved activity and acceptable safety profile is urgent. Nausea, vomiting, thrombocytopenia, mucositis, decreased appetite and diarrhea are the less common side effects of administration of paclitaxel. Ongoing clinical trials suggest that combining paclitaxel with other anticancer drugs may be an effective treatment for patients with skin cancer. Researchers are exploring ways to reduce the side effects of treatment improve the quality of patients' lives, and reduce pain.

The chemotherapeutic and antitumor activity associated with garlic has been attributed to the presence of various organosulfide-based active compounds including Di Allyl sulfide[6]. A topical application of Di allyl sulfide is the most promising approach for treating skin tumors as it leads to a localized effect at the desired site with minimal side effects. Polycyclic aromatic hydrocarbons (PAHs) are commonly occurring environmental contaminants and are widely distributed in the environment as pollutants of air, water and soil[8]. Benzo (a) pyrene is the most toxic compound of PAHs.[9]

The purpose of the present study is to evaluate the combined effect of Paclitaxel and Di allyl sulfide against the DMBA induced skin carcinogenesis.

MATERIALS AND METHODS

Chemicals: 7,12 Dimethyl benz (a) anthracene and Di allyl sulfide were purchased from Sigma chemical company, USA. All the other chemicals used were of analytical grade.

Animal care and housing: Male Wistar rats, 6-8 weeks of age and weighing 150-200g, were used. The animals were procured from Central Animal House Block, Meenakshi Medical College and Research institute, Kanchipuran, Tamil Nadu, India and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, Ms.Hindustan Lever Ltd., Mumbai) and water *ad libitum*. This research work on Wistar male rats was sanctioned and approved by the Institutional Animal Ethical Committee

Experimental Design

The animals were divided in to six groups of 6 animals each.

Group I animals served as control,

Group II as animals treated with DMBA (5 µg/kg of body weight) per animal in acetone (100 µL), three times a week for 28 weeks to induce skin cancer. After tumor induction:

Group III animals were treated with Paclitaxel (33mg/kg b.wt) once in a week for 4 weeks in intra muscular.

Group IV animals were treated with garlic extract of Di allyl sulfide (250µg/animal) for 30 days daily.

Group V animals were treated with both Paclitaxel and Di allyl sulfide (as in group III and group IV) daily.

After the experimental period of 32 weeks, the animals were sacrificed by cervical dislocation. Blood sample was collected via cardiac puncture, and add 2-3drops of EDTA anticoagulant.

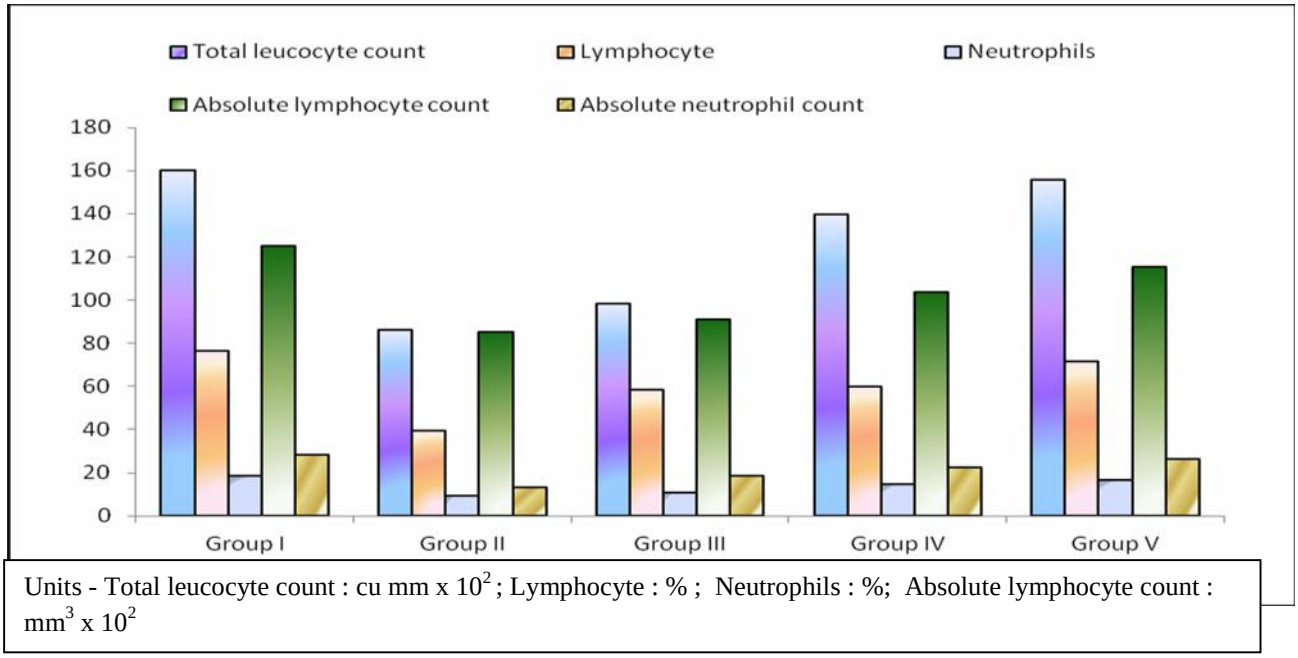
The following Biochemical analysis was done:

1. **Estimation of total white blood cells:** Enumerated by the method of John (1972)[10]
2. **Differential Leucocyte count:** By the method of John (1972) [10]
3. **Soluble immune complex** was estimated by the method of Seth and Srinivas (1981) [11]
4. **Nitro blue tetrazolium (NBT) reduction test**: was carried out by the method of gifford and malavista (1970) [12]
5. **Neutrophil function test: B**y the method of Wilkinson (1977) [13]
6. **Ig G was quantitatively measured**: by Tenant *et al* (1979) [14]

7. **Phagocytic index:** by the method of Wilkinson (1977) [13].
8. **Avidity index:** by the method of Wilkinson (1977) [13].

RESULTS

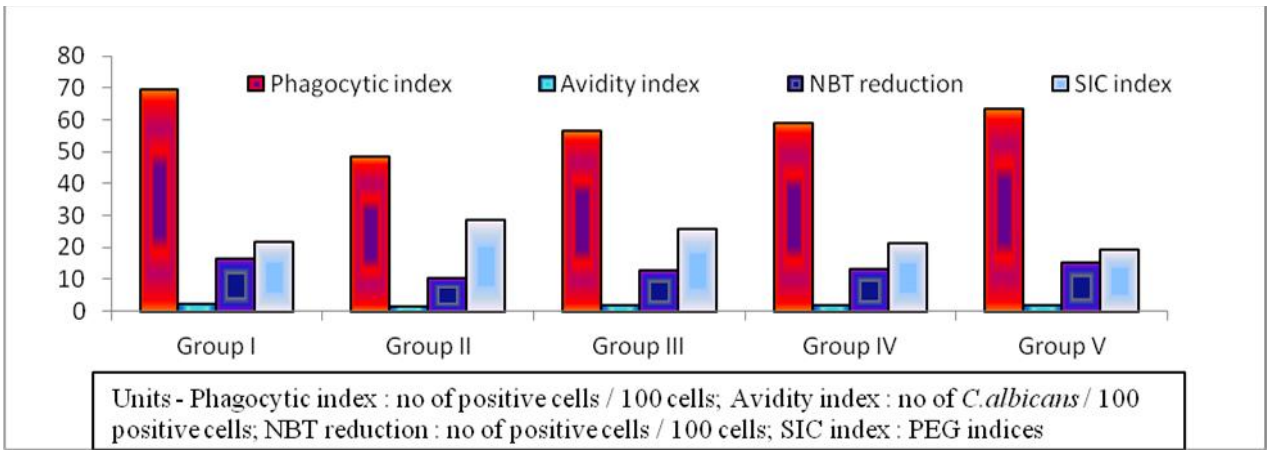
Immunocompetent cells : Fig. 1 represents the effect of paclitaxel and Di allyl sulfide on the status of immunocompetent cells in various experimental groups. Group II cancer bearing animals show a significant ($p<0.001$) decrease in the cell counts when compared with group I control animals. Paclitaxel and Di allyl sulfide treatment caused a significant decrease in leucocytes ($p<0.05$), lymphocyte ($p<0.01$), neutrophils ($p<0.01$), absolute lymphocyte count ($p<0.05$) and absolute neutrophil count ($p<0.05$). *Di Allyl Sulfide* along with paclitaxel treated group V animals caused a considerable changes ($p<0.001$; $p<0.01$) in cell count. However the effect was more pronounced in the group VI animals treated with both paclitaxel and *Di allyl sulfide* when compared with group I control animals.



Units - Total leucocyte count : cu mm x 10^2 ; Lymphocyte : % ; Neutrophils : %; Absolute lymphocyte count : mm^3 x 10^2

Fig 1: Effect of paclitaxel and Di allyl sulfide on the status of immunocompetent cells

Immune complexes: Fig. 2 depicts the effect of paclitaxel and *Di Allyl Sulfide* on immune complexes like phagocytic index, avidity complex, NBT reduction and SIC in various experimental groups. Group II cancer bearing animals showed a significant ($p<0.001$) decrease in the immune complexes when compared with group I control animals. Paclitaxel treatment caused a significant ($p<0.01$; $p<0.05$) decrease in the levels of immune complexes. Upon paclitaxel and *Di allyl sulfide* treatment there found to be a significant ($p<0.001$; $p<0.01$) increase in the levels of immune complexes.



Units - Phagocytic index : no of positive cells / 100 cells; Avidity index : no of *C.albicans* / 100 positive cells; NBT reduction : no of positive cells / 100 cells; SIC index : PEG indices

Fig 2: Effect of paclitaxel and *Di Allyl Sulfide* on immune complexes

Immunoglobulins

Fig 3 display the levels of immunoglobulins like IgG, IgA, and Ig M in various experimental groups. IgG and IgM levels were decreased considerably ($p<0.001$) in cancer bearing group II animals with an increase ($p<0.001$) in IgA level when compared with group I control animals. Upon paclitaxel treatment the levels of IgG, IgM were significantly ($p<0.05$) decreased where as IgA level was increased ($p<0.01$) in group III animals. *Di allyl sulfide* along with paclitaxel treated group V animals showed considerable alterations in the levels of Immunoglobulins ($p<0.001$) when compared with group II cancer bearing animals. In group VI animals treated with both paclitaxel and *Di allyl sulfide* show no significant changes when compared with group I control animals.

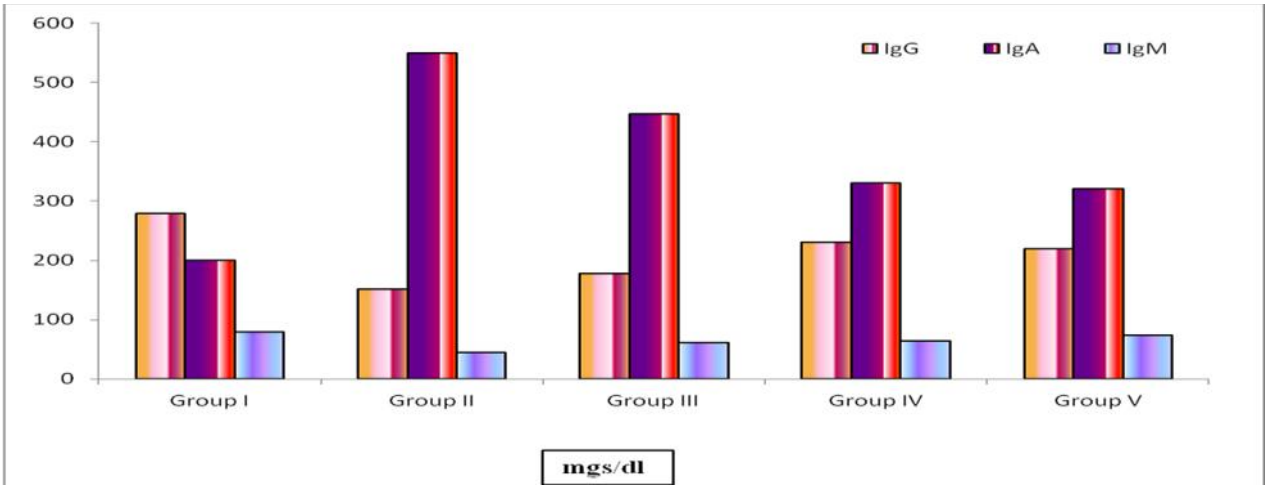


Fig 3: Levels of immunoglobulins like IgG, IgA, and Ig M in various experimental groups.

DISCUSSION

Immunomodulatory activities: Chemotherapy remains the major hope for the treatment of cancer and is always associated with some degree of haemopoietic tissue toxicity and immune suppression. Although cancer itself is immunosuppressive, cytotoxic antineoplatic therapy is the primary contributor to the clinical immunodeficiency observed in cancer patients. Severe leucopenia, thrombocytopenia alterations in circulating platelets, white and red blood cells are the main side effects of chemotherapy leading to the decrease of chemotherapy dose or discontinuation of treatment.[15] The most common complication associated with cytotoxic antineoplatic therapy occurs with the onset of neutropenia.[16] Though paclitaxel is a potent anticancer agent the major limiting side effect is myelosuppression. It induces troublesome neutropenia of grade 3-4 with decrease in WBC count in more than 50% of the patients.

Abnormal content of immunoglobulin indicate the concised humoral immunity and reduction in immune response. Thompson *et a.l*[17] have reported decreased levels of IgG and IgM in skin cancer conditions. The levels of IgG and IgM were also decreased in various other cancerous conditions.[18-20] IgA content alone was found to be increased in the skin cancer bearing rats. Chandy *et al*[21], have reported that the elevated serum IgA levels may be due to the failure of clearance mechanism by the damaged liver. This indicates the severity of liver damage which directly correlates with the progression of the disease.

A significant alteration in the neutrophil functions has been observed in all our study. The killing ability of the neutroplil as indicated by the NBT reduction and phagocytic ability of the neutrophils as indicated by the phagocytic index and the avidity index has been significantly decreased in the cancer bearing animals which was further decreased upon treatment with paclitaxel.

Soluble serum immune complexes serve as an indicator of immune responses either due to presence of excess antigens or antibodies. This may be due to decreased antibody production during cancer. Immunomodulation through natural or synthetic substances may be considered as an alternative for the prevention and cure of neoplastic diseases.[22, 23] Flavanoids are polyphenol substances of plant origin, having biological and antioxidative properties.[24] Several reports have demonstrated the beneficial effect

of flavanoids in preventing toxicity of different agents.[25-27]

Flavanoids display a remarkable array of biochemical and pharmacologiocal actions some of which suggest that certain members of this group of compounds significantly affect the function of the immune system. They also affect the function of enzyme system critically involved in the immune system. Di allyl sulfide contains organo sulfur compounds that play a very important role in scavenging free radicals. In our study also Di allyl sulfide exhibited positive effect on the immune system which can be attributed to its flavanoid content. Ali *et al.* (2000) have reported that Di allyl sulfide stimulates immune response in rats. Mesbah Lahouel[28] have reported the effect of Di allyl sulfide on haemotoxicity of chemotherapeutic drugs.

Considering the possible mode of antitumor action of Di allyl sulfide it is likely that it could be mediated by immunomodulatory activity of Di allyl sulfide. The present study has given the hope that Di allyl sulfide can confer in the reduction of side effects due to chemotherapeutic agents and may be used in humans in future.

CONCLUSION

From the present study, the effect of Paclitaxel- DAS combination proved to be effective chemotherapeutic agent against DMBA induced skin cancer in wistar rats compared to that of paclitaxel or Di allyl sulfide confirmed analyzing the total white blood cells, Differential leukocyte count, Soluble immune complex, neutophil function tests, IgG, IgM, IgA levels and Phagocytic , Avidity indexs in blood samples

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