

EFFECT OF PACLITAXEL ALONG WITH WITHNIA SOMINIFERIA ON LACTATE DEHYDROGENASE ENZYME ACTIVITY CHANGES IN 7, 12 DI METHYL BENZ (A) ANTHRACENE INDUCED BREAST CANCER WISTAR RATS

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ABSTRACT

Aim: The purpose of this study is to investigate the changes in the levels of lactate dehydrogenase enzyme (LDH) activity and efficacy of combination of paclitaxel along with Withania somnifera against breast cancer in experimental animals. Breast cancer is the commonest cancer among women in all developed countries (except Japan) as well as in North Africa, South America, and southeastern and western Asia. While the incidence of breast cancer appears to be increasing, mortality rates are now declining in at least some western countries. Breast cancer ranks third when both the sexes are considered together and is clearly a significant global public health problem. **Methods:** Breast cancer was induced in rats by 7, 12 Di methyl benz(a) anthracene (DMBA) at the dosage of 20mg dissolved in 0.5ml sunflower oil and administered into experimental animals for 28 weeks. In this study, we demonstrated that combination of paclitaxel and withania somnifera revert the changes in the rats from lethal dose of DMBA within 30 days. **Results:** All the isoenzymes LDH₁ – LDH₅ were observed in cancer bearing animals. Expression of these isoenzymes were found be reduced in paclitaxel and Withania somnifera treated animals. **Conclusions:** The treatment with combination of paclitaxel and withania somnifera effectively reduced LDH enzyme activity levels. So, from the obtained results it is concluded that paclitaxel and withania somnifera is capable of restoring the breast architecture.

Keywords: Withania somnifera , Di methyl benz(a) anthracene (DMBA), Paclitaxel, Lactate dehydrogenase enzyme (LDH) , Breast cancer.

INTRODUCTION

Breast cancer ranks third when both the sexes are considered together [1] and is clearly a significant global public health problem.

There are nearly 8,00,000 new cases of breast cancer worldwide each year .In approximately half of these patients, breast cancer will be the eventual cause of death. Incidence of breast cancer in Indian women is not as high as in western countries [2].It is the second most common cancer among women in south India. The age standardized rates vary from 22 to 28 per 1, 00,000 women [3]. Although the rates appear to be lower than those seen in developed countries, the burden of cancer in India is alarming.

Worldwide incidences of breast cancer continue to rise and geographical variations in breast cancer incidence indicate that environment factors contribute to overall risk [4]. Exposure to environmental carcinogens early in life is thought to be one of the first events in the development of breast cancer. Each year breast cancer is diagnosed in 910,000 women worldwide and 376,000 women die from the disease. Most of these cases are in industrial countries e.g, North America (180,000) and Europe (220,000).

Polycyclic Aromatic hydrocarbons an important class of chemical carcinogens that are widespread in the ambient environment due to fossil fuel combustion for energy production, transportation and industry. DMBA, a potent PAH recognized as an initiator of both skin and liver cancer [5]. The covalent binding of DMBA metabolites to DNA has been implicated as a critical step in the initiation phase of cancers. Paclitaxel (Taxol), a naturally occurring antineoplastic agent has shown great promise in the therapeutic management of certain human solid tumors particularly in metastatic breast cancer and malignancy involves skin, lung and refractory ovaries. It is the original member of the taxane group of anticancer drugs derived from the bark and needles of the pacific yew tree "Taxus brevifolia". Paclitaxel's antitumor activity was discovered in 1960's during a large scale 35,000 plants-screening program sponsored by the National Cancer Institute (NCI), USA. Withania is widely used in Ayurvedic medicine in India and in Unani and Middle Eastern traditional medicines, where it is highly regarded as a panacea, aphrodisiac, and rejuvenative. Withania somnifera (L). Dunal (Solanaceae) commonly called Ashwagandha (Sanskrit) is an

Ayurvedic Indian medicinal plant, which has been widely used as a home remedy for several ailments [6]. The use of ashwagandha in Ayurvedic medicine extends back more than 3000 to 4000 years (Upton, 2000) [7]. It has been widely extolled as a tonic, especially for emaciation in people of all ages, including babies, and enhances the reproductive function of both men and women. It has also been used for inflammation, especially in antitumor, arthritic and rheumatic conditions, for asthma, and as a major tonic to counteract aging and promote youthful longevity [8].

MATERIALS AND METHODS

Female 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, Kanchipuram, 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 female rats was sanctioned and approved by the Institutional Animal Ethical Committee (REG NO. 765/03/ca/CPCSEA). 7,12 Dimethyl benz (a) anthracene and Withania somnifera were purchased from Sigma chemical company, USA. All the other chemicals used were of analytical grade.

Experimental Design: The animals were divided into six groups of 6 animals each. Group I animals served as control, Group II, III, IV, V as animals treated with DMBA (20mg) per animal in sunflower oil (0.5ml), three times a week for 28 weeks to induce breast cancer. After tumor induction Group III animals were treated with Paclitaxel (33mg/kg b.wt) once in a week for 4 weeks. Group IV animals were treated with Withania somnifera (250µg/animal) for 30 days. Group V animals were treated with both Paclitaxel and Withania somnifera (as in group III and group IV). These were Group VI Control animals treated with paclitaxel and Withania somnifera for 28 weeks plus 30 days. After the experimental period of 32 weeks, the animals were sacrificed.

Biochemical analysis: The isoenzymes pattern of lactate dehydrogenase was separated by the method of Dietz and Lubrano (1967).

Statistical analysis: For statistical analysis, one way analysis of variance (ANOVA) was used, followed by the Newman-Keuls Multiple Comparison test.

RESULTS

Plate 1 depicts the Isoenzyme pattern of lactate dehydrogenase in serum of control and experimental animals. All the isoenzymes LDH₁ – LDH₅ were observed in cancer bearing (group II) animals. Expression of these isoenzymes were found to be reduced in paclitaxel (group III) and Withania somnifera (group IV) treated animals. However a much significant reduction in the LDH isoenzyme expression pattern was observed in-group V animals treated with both paclitaxel and Withania somnifera.

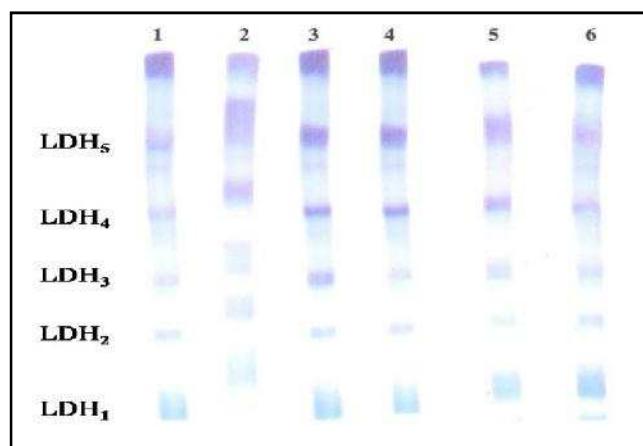


Fig 1: Lactate dehydrogenase (LDH) isoenzyme pattern in serum of control and experimental animals

Lane 1: Control

Lane 2: DMBA treated

Lane 3: Paclitaxel treated

Lane 4: Withania somnifera treated

Lane 5: Paclitaxel and Withania somnifera treated cancer bearing animal

Lane 6: Paclitaxel and Withania somnifera treated control animal

DISCUSSION

Lactate dehydrogenase (LDH) is the most common clinical enzyme used in the cancer patients for prognostic purpose [9]. It has an important role in germ cell functions and can predict responses to chemotherapy and the prospects of remission. Human cancer tissues typically exhibit 2-3 fold increases in glycolytic enzymes and LDH activity. Sandhya Mishra et al. (2004) have also reported the increased level of LDH in breast cancer patients [10]. reported greater LDH activity in breast cancer conditions. There was a significant increase in LDH level in serum of cancer bearing Group II animals [11]. This might be due to the membrane disruptions that caused the release of these enzymes from the cancer cells or the overproduction by the tumor cells [12]. The elevated LDH activity may also have resulted from differences in the rate of synthesis, degradation or the excretion of the enzymes in the mammary cancer bearing animals [13] has reported that among the isoenzymes LDH₅ was six times greater than LDH₁ in mammary tumor cells.

Flavonoids have proved to possess antitumor effect on various animal models [14]. The biological and pharmacological activity of Withania somnifera was associated with phenolic compounds mainly to flavonoids, aromatic acids and esters [15,16]. Antioxidant activity of flavonoids may also be due to their structural features and its action on membrane [17,18,19]. Paclitaxel being rich in flavanoid content possess antitumor and antiproliferative activities that stabilizes the membrane permeability and reduces the release of LDH [20, 21].

Conclusion

From the present study, the effect of Paclitaxel- Withania somnifera combination proved to be effective chemotherapeutic agent against DMBA induced Breast cancer

in wistar rats compared to that of paclitaxel or Withania somnifera confirmed analyzing the LDH isoenzymes levels in serum.

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Conflict Of Interest: Nil

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