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Chemotherapeutic Efficacy of *Solanum trilobatum* along with Paclitaxel in Lung Cancer Bearing Swiss Albino Mice

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Abstract: The present study was conducted to ascertain the chemotherapeutic efficacy of *Solanum trilobatum* when administered along with paclitaxel against experimental lung carcinogenesis. Healthy male Swiss albino mice (6-8 weeks old) were treated with Benzo (a) pyrene (50 mg kg^{-1} body weight) to induce lung cancer. Paclitaxel at a dose of 33 mg kg^{-1} body weight intraperitoneally and *Solanum trilobatum* 200 mg kg^{-1} body weight orally was administrated for four weeks to lung cancer bearing animals. The level of lipid peroxides (LPO) was found to be markedly increased in carcinogen-administered animals, in contrast the activities/levels of the antioxidant status both in lung and liver were decreased in carcinogen administered animals. Upon *Solanum trilobatum* along with Paclitaxel administration the above pathological changes were bring back to near normal. From these findings we have concluded that *Solanum trilobatum* when administered along with paclitaxel prevents LPO and protects antioxidant system strongly aganist Benzo(a)pyrene induced lung cancer.

Key words: *Solanum trilobatum*, paclitaxel, LPO, antioxidants, benzo(a)pyrene, lung cancer

INTRODUCTION

Lung cancer is the leading cause of death in both men and women accounting for 29% of all cancer deaths. More people die each year due to lung cancer than that of other cancer like colon, breast, stomach and prostate cancer. The incidence of lung cancer still remains very high. Tobacco smoke contains over 60 established carcinogens. Among the constituents of smoke the Polycyclic Aromatic Hydrocarbons (PAHs) such as benzo (a)pyrene, play a major role in lung carcinogenesis (Stephen *et al.*, 2002). Benzo (a) pyrene is metabolized to (±)-B[*a*]P-r-7,t-8-dihydrodiol-t-9,10-epoxide (BPDE), the ultimate carcinogen. BPDE isomers then bind to the exocyclic nitrogen of deoxyguanosine in DNA via trans addition of the C-10 position in the epoxide molecule. This adduct may also cause activation of protooncogenes (Kristina *et al.*, 2000).

Paclitaxel (Taxol), a naturally occurring antineoplastic agent has shown great promise in the therapeutic treatment of certain human solid tumors particularly in metastatic breast cancer, refractory ovarian cancer (Yu, 1994). 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.

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Since paclitaxel remains one of the most effective antineoplastic agents available for the treatment of multiple tumors, strategies for modulating myelosupression have been actively investigated. The search for methods to suppress the toxic manifestation will be made to counteract the toxic side effects as well as to improve the therapeutic efficacy of paclitaxel by combining with the natural antioxidant.

Plants form an important source of novel chemical compounds with medicinal properties, may of which have been used for prevention and treatment of a variety of human ailments from time immemorial. Experimental and epidemiological studies over the past few decades have provided ample evidence in support of associations between plant food intake and reduce cancer risk. Many photochemicals are proven to have anticancer activities and many are in use for cancer therapeutics (Miranda *et al.*, 1999). People consuming diets rich in fruits and vegetables have lower incidences of diseases such as cancer (Ziegler, 1991).

Solanum trilobatum (Solanaceae) is a common shrub of Southren India, which has been used in the treatment of various diseases like respiratory disease, asthma, chronic febrile infections, tuberculosis, cardiac and liver diseases. Siddha physicians consider this drug as a specific and prepare ghee from this for use in tuberculosis and use as food for all kinds of lung diseases (Nadkarni, 1979). On exposure to 7, 12 Dimethylbenz (a) anthracene (DMBA), *Solanum trilobatum* extract significantly reduced the papilloma formation and thereby inhibited the skin carcinogenicity. This may be due to the significant free radical scavenging activity of the extract (Mohanan *et al.*, 1997). The extract potentiates the protective effect of cyclophosphamide induced toxicity. Apart from this, it has been reported that *Solanum trilobatum* extract did not produce any cytogenetic effects in the bone marrow cells of mice (Mohanan *et al.*, 1996).

The purpose of this study is to demonstrate the chemotherapeutic role of *Solanum trilobatum* along with paclitaxel by increasing the activities of antioxidant enzymes and decrease the level of LPO in lung cancer induced by benzo (a) pyrene. The objective of the present study was to emphasize the selective modulation of the enzymes by *Solanum trilobatum* along with paclitaxel in chemically induced lung cancer.

MATERIALS AND METHODS

Chemicals

Benzo (a) pyrene was purchased from Sigma Chemical Company, St. Louis, MO, USA. Paclitaxel was purchased from Dabur India Ltd., India. *Solanum trilobatum* was collected from Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd., Chennai, India. All other chemicals used for the experiments were of analytical grade.

Preparation of Plant Extract

Dried leaves of *Solanum trilobatum* was extracted in ethanol (95% v/v) in a hermetically closed glass vessel for 4 days at 37°C under occasional shaking. The ethanol extract was then filtered and evaporated in a rotary evaporator under reduced pressure at 60°C. The extract was stored in a vacuum desiccator.

Animal Experiments

Healthy male Swiss albino mice (*Mus muscullus*) (6-8-weeks old) were used throughout the study. They were maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed with standard pellet diet (Gold Mohor rat feed, Ms. Hindustan Lever Ltd., Mumbai) and water *ad libitum*. Animal experiments were conducted according to the guidelines of institutional animal ethical committee.

Experimental Setup

The experimental animals will be divided into four groups of six animals each. Group I - Control animals treated with corn oil (vehicle) orally. Group II - Benzo (a) pyrene (50 mg kg^{-1} b.wt, orally) treated animals. Goup III - Lung cancer bearing animals treated with Paclitaxel (33 mg kg^{-1} b.wt,ip) alone. Group IV - Lung cancer bearing animals treated Paclitaxel along with *Solanum trilobatum* extract (33 mg kg^{-1} b.wt, i.p. + 200 mg kg^{-1} b.wt, orally).

Experimental Procedure

At the end of the experimental period (18 weeks), the animals were sacrificed by cervical decapitation. Lung and liver tissues were collected and homogenized in Tris-HCl buffer 0.01 M (pH 7.4). The tissue homogenates were used for biochemical assays.

Biochemical Assays

The protein content was determined according to Lowry *et al.* (1951). Lipid peroxidation (LPO) was determination by the method of Ohkawa *et al.* (1979). The activity of Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund (1974). Catalase (CAT) was assayed by the method of Sinha (1972). Glutathione Peroxidase (GPx) was assayed by the method of Rotruck *et al.* (1973). Glutathione Reductase (GR) was assayed by the method of Staal *et al.* (1969). Total reduced glutathione (GSH) was determined by the method of Moron *et al.* (1979). Vitamin C was assayed by the procedure of Omaye *et al.* (1979).Vitamin E was estimated by the method of Desai (1984).

Histopathological analyses were carried out to confirm the tumor incidence and the activity of the drug. Lung samples were subjected for routine histopathological examination. Small pieces were collected in 10% formal saline (10 parts of formaldehyde and 90 parts of normal saline) for proper fixation. These tissues were processed and embedded in paraffin wax. Sections were cut and stained with haemotoxylin and eosin (Luna, 1966).

Statistical Analysis

The results were expressed as mean±Standard Deviation (SD) for six animals in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using the SPSS software package for Windows. Post hoc testing was performed for inter-group comparisons using the Least Significance Difference (LSD) test.

RESULTS

The chemotherapeutic efficacy of *Solanum trilobatum* along with paclitaxel was measured against Benzo (a) pyrene induced lung carcinogenesis. Table 1 shows the level of LPO in lung and liver of control and experimental animals. The level of LPO was significantly (p<0.001) increased in cancer bearing animals (Group II) when compared with (group I) control animals. Paclitaxel (group III) treatment caused a significant (p<0.01) decrease in their levels when compared with cancer bearing

Table 1: Effect of *Solanum trilobatum* along with paclitaxel on the levels of lipid peroxidation in lung and liver of control and experimental animals

Particulars	Group I	Group II	Group III	Group IV
Lung	0.58±0.04	1.02±0.09a*	0.85±0.06b@	0.65±0.04b#
Liver	0.62±0.05	1.08±0.07a*	0.91± 0.08b@	0.72±0.05b#

Results are given as mean±SD for 6 mice. Units: nmoles of MDA formed/min/mg protein. Comparisons are made between a: Group II compared with Group I. b: Group II compared with Group III and IV. Statistical significance at *p<0.001, @p<0.01 and #p<0.05, respectively

Table 2: Effect of *Solanum trilobatum* along with paclitaxel on the levels of enzymic and non-enzymic antioxidants in lung of control and experimental animals

Particulars	Group I	Group II	Group III	Group IV
SOD	4.80±0.37	2.93±0.18a*	4.36±0.28b@	4.63±0.38b*
CAT	255.00±23.6	123.67±12.1a*	162.50±12.6b#	247.33±23.5b*
GPx	42.39±4.12	22.57±1.9a*	33.19±2.9b@	45.79±3.13b*
GR	2.51±0.19	1.72±0.14a*	2.42±0.18b#	2.61±0.19b*
GSH	1.47±0.08	0.84±0.07a*	1.17±0.13b#	1.56±0.14b*
Vitamin C	0.47±0.04	0.26±0.02a*	0.33±0.02b@	0.46±0.04b*
Vitamin E	0.58±0.04	0.33±0.021a*	0.42±0.03b@	0.56±0.05b*

Results are given as mean±SD for 6 mice. Units of enzyme activity: SOD: Units/mg protein, One unit is equal to the amount of enzyme that inhibits the autooxidation reaction by 50%; Catalase: μmoles of H_2O_2 consumed/min/mg protein; GPx: μg of GSH consumed/min/mg protein; GR: nmoles of NADPH oxidized/min/mg protein; GSH, Vitamin C and Vitamin E: μg/mg protein. Comparisons are made between a : Group II compared with Group I. b: Group II compared with Group III and IV. Statistical significance at *p<0.001, @p<0.01 and #p<0.05, respectively

Table 3: Effect of *Solanum trilobatum* along with paclitaxel on the levels of enzymic and non-enzymic antioxidants in liver of control and experimental animals

Particulars	Group I	Group II	Group III	Group IV
SOD	5.58±0.52	2.78±0.017a*	4.29±0.03b#	5.57±0.25b*
CAT	338.33±32.2	233.50±20.01a*	291.17±25.1b@	335.50±29.2b*
GPx	43.82±3.90	27.63±2.5a*	31.87±2.7b@	43.71±3.6b*
GR	3.82±0.21	2.48±0.18a*	3.20±0.24b#	3.69±0.28b*
GSH	1.84±0.14	1.04±0.09a*	1.54±0.08b@	1.94±0.16b*
Vitamin C	0.55±0.04	0.26±0.01a*	0.35±0.02b#	0.55±0.04b*
Vitamin E	0.72±0.06	0.43±0.03a*	0.60±0.04b@	0.75±0.06b*

Results are given as mean±SD for 6 mice. Units of enzyme activity: SOD: Units/mg protein, One unit is equal to the amount of enzyme that inhibits the autooxidation reaction by 50%; Catalase: μmoles of H_2O_2 consumed/min/mg protein; GPx: μg of GSH consumed/min/mg protein; GR: nmoles of NADPH oxidized/min/mg protein; GSH, Vitamin C and Vitamin E: μg/mg protein. Comparisons are made between a: Group II compared with Group I. b: Group II compared with Group III and IV. Statistical significance at *p<0.001, @p<0.01 and #p<0.05, respectively

Group I

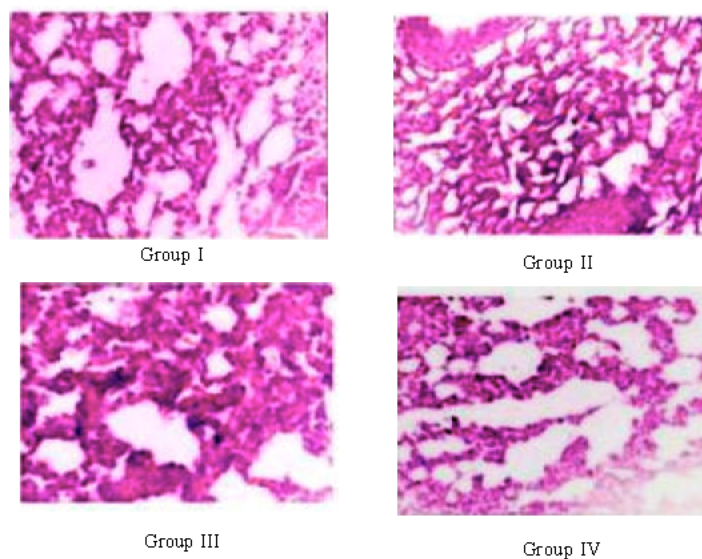Group II

Group III

Group IV

Fig. 1: Histopathological investigation of the lungs of control and experimental animals. Group I, Control animals showing normal architecture (H and E X 10); Group II, B (a) P treated animals showing alveolar damage as seen from increased number of hyper chromatic, irregular nuclei in the cells of alveolar wall (H and E×10); Group III, Paclitaxel treated animal showing significantly reduced hyperplasia or cell proliferation (H and E X 10); Group IV, Paclitaxel and propolis treated animal showing no signs of hyperplasia or neoplasia (H and E X 10)

animals. On combination of *Solanum trilobatum* and paclitaxel (Group IV) showed a much more significant (p<0.001) decrease in the levels of lipid peroxidation when compared with the cancer induced group.

Table 2 and 3 show the activity of enzymic and nonenzymic antioxidant enzymes (SOD, CAT, GPx, GR, GSH, Vitamin E and Vitamin C) in lung and liver of control and experimental animals. The level of enzymic and non enzymic antioxidant significantly (p<0.001) decreased in cancer bearing animals (Group II) when compared with (group I) control animals. Treatment with Paclitaxel (Group III) caused a significant (p<0.01, p<0.05) increase in these enzyme activities when compared with cancer bearing animals. However combination treatment with *Solanum trilobatum* and paclitaxel (Group IV) showed a much more significant (p<0.001) increase in the activities of the enzymic and non enzymic antioxidants. The combination therapy showed more effect than that of paclitaxel treated alone.

Figure 1 portrays the histological analysis of control and experimental groups. The lung from control animals revealed a normal architecture (Group I). In lung cancer bearing animals (Group II) the lung showed alveolar damages and irregular hyperchromatic cells. Paclitaxel treated (G-III) animals showed slightly reduced alveolar damage and in paclitaxel and *Solanum trilobatum* treated (G-IV) animals a well reduced alveolar damage was seen.

DISCUSSION

Benzo (a) pyrene is a very effective carcinogen interacting with membrane lipids and consequently inducing free radical formation (Sikkim *et al.*, 2000). Benzo (a) pyrene activates oxidative stress especially lipid peroxidation is known to be involved in carcinogenesis(Trush and Kensler, 1991). Lipid peroxidation can cause a cascade of effect a lipid derived radicals there by causing additional membrane damage. Increased levels of lipid peroxidation products play a role in the early phases of tumor growth (Rice-Evans and Burdon, 1993). Byproducts of lipid peroxidation have been shown to cause profound alterations in the structural organization and functions of the cell membrane including decreased membrane fluidity, increased membrane permeability, inactivation of membrane bound enzymes and loss of essential fatty acids (Thirunavukkarasu *et al.*, 2001; Van Ginkel and Sevanian, 1994).

Free radicals react with lipids causing peroxidation, resulting in release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. MDA has also been reported to cause mutagenesis in various tissues by forming DNA adducts. Plasma MDA level has been regarded to be an indicator of lipid peroxidation. Faber *et al.* (1995) have shown that the patients with lung cancer have higher MDA levels when compared to controls. In the present study an increase in the levels of lipid peroxidation was found in lung cancer bearing animals and these were significantly reduced after treatment with *Solanum trilobatum* along with paclitaxel.

Herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases, by combating the Reactive Oxygen Species (ROS) induced oxidative damage (Li Shijun *et al.*, 2000). Reference to antioxidant enzyme status in lung and liver, all the antioxidant enzymes examined in this study viz., superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase were found decreased in lung cancer bearing animals. Superoxide dismutase (SOD), converts superoxide radical into hydrogen peroxide whereas GPxs and CATs convert H_2O_2 into water (Le *et al.*, 1995). SOD, Catalase and GPx activity have been augmented significantly by the *Solanum trilobatum* along with paclitaxel administration. Indeed SOD elevation along with catalase and GPx may combine decreased lipid peroxidation, which is an indicator of oxidative cell damage that persists in the cell. Unlike SOD, catalase and GPx require several secondary enzymes like

Glutathione Reductase (GR) and cofactors like GSH, NADPH, to function at high efficacy. Glutathione Reductase (GR) another major antioxidant enzyme that catalyzes the NADPH dependent reduction of glutathione disulfide to glutathione thus maintaining the GSH levels in cell (Katiyar *et al.*, 1993). A significant increase of GR following administration of *Solanum trilobatum* along with paclitaxel treatment. Thus helping the cell to maintain the basal level of GSH, which is important for many other GSH dependent detoxification reactions.

Apart from the enzymic antioxidants, non-enzymic antioxidants like reduced glutathione, ascorbic acid and α - tocopherol play an excellent role in preventing the cells from oxidative threats. Reduced glutathione plays an important role in a variety of detoxification process, including nullification of peroxidative damage. Kosower and Kosower (1983) have shown a direct link between the thiol status of the membrane and cellular glutathione. The function of glutathione is to serve as an agent for reducing membrane protein disulphides and to arrest membrane oxidation.

Glutathione is one of the most abundant compounds in the body and its biological functions depend mainly on the thiol group of its cysteinyl residue. Apart from the numerous functions like transport of acids, ions and/or sugar, synthesis of protein and DNA, maintenance of membrane integrity etc. reduced glutathione (GSH) participates in spontaneous scavenging of electrophiles or free radicals and in reaction catalyzed by enzymes like GST and GPx. Recent study hypothesize that GST catalyzed GSH conjugation is an important mechanism for the detoxification of anti-BPDE. In the present study, Glutathione activity was decreased level in the lung and liver tissues investigated in the lung cancer bearing animals. Since Benzo (a) pyrene is proved to be a potent human carcinogen which produce lung and forestomach neoplasia it becomes very important to identify compounds that can interact and inhibit the process. There are reports that fruits, vegetables as well as several herbs and plants have the diversified pharmacological properties that can inhibit this process(Badary *et al.*, 1999; Prochaska and Fernandes, 1993; Van Lieshout *et al.*, 1998). In this present study, treatment with *Solanum trilobatum* along with paclitaxel has significantly augmented the glutathione level.

Enzymic antioxidants are inactivated by hydroxyl radicals and hence the presence of non-enzymic antioxidant is presumably essential for the removal of these radicals. Vit-C is a water soluble antioxidant that removes free radicals from cytosol by reacting directly with them (Allen, 1991). Thus, the decreased level of Vit-C found in Group II lung cancer-bearing animals maybe due to the utilisation of antioxidant to scavenge the free radicals. The availability of Vit-C is a determined factor in controlling and potentiating many aspects of host resistance against cancer. The ascorbate molecule must be involved in the feedback inhibition of lysosomal glycosides responsible for malignant invasiveness (Cameron *et al.*, 1979).

The Vit-C can protect cell membrane and lipoprotein particles from oxidative damage by regenerating the antioxidant from Vit-E (Buettner, 1993; Beyer, 1994). Thus, Vit-C and Vit-E act synergistically in scavenging wide variety of ROS. Vit-E is the major lipid soluble radical scavenger that prevents the LPO by terminating the chain reactions initiated in the membrane lipids (Wiseman, 1996). Vit-E is a chain breaking antioxidant by donating its labile hydrogen atom from phenolic hydroxyl groups to propagating lipid peroxyl and alkoxyl radical intermediates of LPO (Daoud and Griffin, 1985). Decreased Vit-E content in Group II lung cancer-bearing animals might be due to the excessive utilisation of this antioxidant for quenching enormous free radicals produced in these conditions. Besides, Vit-E has been found to have potent antioxidant activity due to its ability to penetrate to a precise site into the membrane, which maybe the important feature of protection against highly reactive radicals (Packer and Slater, 1979). But these conditions were found to be increased in the treatment with *Solanum trilobatum* along with paclitaxel. The combination chemotherapy has enhanced various cellular antioxidants and thiol content in tissues, which in turn reduces free radical mediated cellular damage.

Our results obtained in the present study with reference to Lipid peroxidation, antioxidant enzymes and histopathological analysis suggest that *Solanum trilobatum* and paclitaxel possesses significant chemotherapeutic activity in experimental lung cancer. The antioxidant and chemotherapeutic properties of *Solanum trilobatum* and paclitaxel may be responsible for the observed chemotherapeutic action. Hence these results suggested that combined chemotherapy of *Solanum trilobatum* along with paclitaxel could be more advantageous in cancer.

ACKNOWLEDGMENTS

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