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Research Article

IN-VIVO STUDIES ON ANTICANCER ACTIVITY OF TAXOL ISOLATED FROM AN ENDOPHYTIC FUNGUS PESTALOTIOPSIS PAUCISETA SACC. VM1

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ABSTRACT

Breast cancer is one of the leading malignant tumors and the second most prevalent cancer in the world among women. Taxol has been used for effective treatment of a variety of cancers including refractory ovarian cancer, breast cancer, non-small cell lung cancer, AIDS related Kaposi's sarcoma, head and neck carcinoma and other cancer types. The present study investigated for chemopreventive effect of fungal taxol derived from an endophytic fungus, *Pestalotiopsis pauciseta* Sacc. VM1 isolated from a medicinal plant *Tabebuia pentaphylla* Hemsl.. The enzymic and non-enzymic antioxidants i.e., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), Vitamin C, Vitamin E, reduced glutathione (GSH) were estimated in control and experimental groups. Lipid Peroxides (LPO), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were also evaluated. The results showed that the fungal taxol were found to be effective against 7, 12 dimethyl benz (a) anthracene induced mammary tumors in Sprague Dawley rats.

Keywords: Anticancer activity, DMBA, *Pestalotiopsis pauciseta*, Breast cancer, fungal taxol.

INTRODUCTION

Breast cancer is one of the most common types of cancers and cause of cancer death in women worldwide. Women with breast cancer have many treatment options. These include surgery, radiation therapy, chemotherapy, hormone therapy, and biological therapy. Many women receive more than one type of treatment. Surgery is the most common treatment for breast cancer. Radiation therapy uses high-energy rays to kill cancer cells. Chemotherapy uses anticancer drugs to kill cancer cells. Chemotherapy for breast cancer is usually a combination of drugs.

Taxol has been hailed by many in the cancer community as a major breakthrough in the treatment of cancer. The amount of taxol found in yew tree is relatively small, 0.01-0.03% dry weight and this has been a major factor contributing to its high price. The supply of taxol was limited due to several difficulties in obtaining the drug, the concentration of the compound in yew bark is low and the extraction procedure is complex and expensive. The yew tree is a limited resource and it grows very slow. As demand for paclitaxel increased, researchers have been exploring new ways to increase the availability of the drug. The projected increase in the use of taxol for basic research and cancer chemotherapy warrants effort to improve existing production processes for this important natural product. With the discovery that certain endophytic fungi are able to produce taxol has come the possibility that a cheaper and more widely available product may eventually available via industrial fermentation.[1,2] Very few studies are reported on anticancer activity of fungal taxol against DMBA induced mammary tumors in Sprague Dawley rats. Hence the present study was taken up to prove the chemopreventive effect of fungal taxol isolated from an endophytic fungus, *Pestalotiopsis pauciseta* Sacc. VM1 against 7, 12 dimethyl benz(a)anthracene induced mammary tumors in Sprague Dawley rats.

MATERIALS AND METHODS

Growth of the fungi in liquid media

The test fungus were grown in three litres Hopkins flasks containing 1500 ml of MID medium. The cultures were incubated for 18 days.

Extraction of taxol

The extraction procedure by Strobel[3]. After the incubation period, the cultures were filtered through four layers of cheese cloth to remove mycelia. To the culture filtrate 0.25 g Na_2CO_3 was added with frequent shaking in order to reduce the amount of fatty acids that may contaminate taxol in the culture. Then the culture filtrate was extracted with two equal volumes of solvent dichloromethane. The organic phase was collected and the solvent was then removed by evaporation under reduced pressure at 35ºC using rotary vacuum evaporator. The dry solid residue was re-dissolved in methanol for the subsequent separation and the fungal taxol was confirmed by spectral and analytical methods.

Ethical clearance

The Institutional Animal Ethical Committee approved experimental design performed in this study for the use of Sprague Dawley rats as an animal model for cancer activity (IAEC No. 03/014/08).

Animals

Healthy Female Sprague Dawley rats purchased from National Institute of Nutrition, Hyderabad, India were selected for the present investigation. The animal house was well ventilated and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with pellet feed supplied by M/s Hindustan Liver Ltd, (Bangalore, India) and water *ad libitum.*

Experimental Design

The experimental design of the present investigation was like the following five groups consisting of six animals in each group - **Group I :** Normal healthy animals served as controls. **Group II :** Rats was induced mammary carcinoma with 7,12, dimethylbenz(a) anthracene 25 mg/kg dissolved in 1 ml of olive oil, through gastric incubation (Welsch, 1985). **Group III:** Mammary carcinoma was induced in Group III animals also (as in Group II) and treated with fungal taxol (8 mg/Kg body weight in 1 ml saline) once in a week for four weeks, intraperitoneally. **Group IV**: Mammary carcinoma was induced in Group IV animals also (as in Group II) and treated with Paclitaxel (1 mg/Kg body weight in 1 ml saline) once in a week for four weeks intraperitoneally. **Group V:** Drug control animals received fungal Taxol alone in the same dosage as in Group III animals.

At the end of experimental period, the overnight fasted animals were sacrificed by cervical decapitation. Blood was collected from the rats. Serum was obtained after blood coagulation and centrifugation at 5000 rpm for 15 min to obtain a clear supernatant (serum) which was stored at -70ºC, until its use for further biochemical analysis. Breast tissues were immediately excised from the animals. A 10% homogenate was prepared in Tris-HCl buffer 0.1 M pH 7.4 using Homoginiser. The breast tissue homogenates were used for further biochemical analysis.

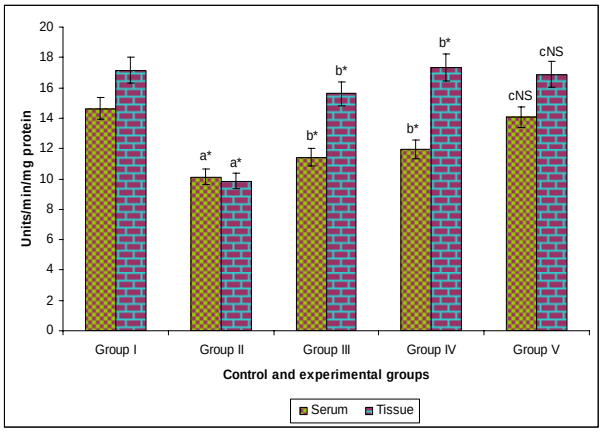

Fig. 1: Levels of SOD in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

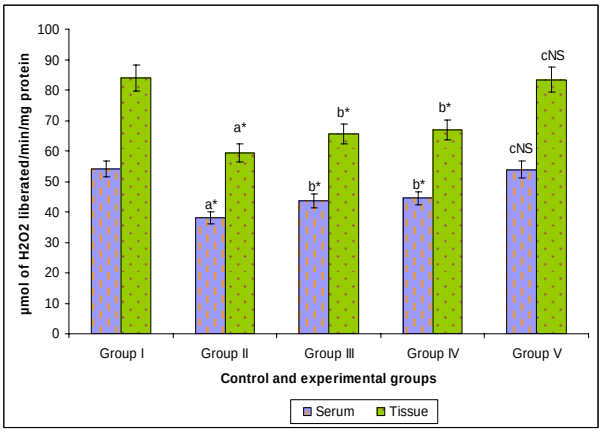

Fig. 2: Levels of Catalase in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

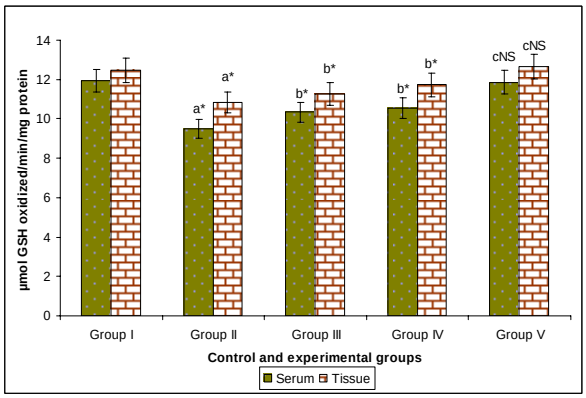

Fig. 3: Levels of GPx in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

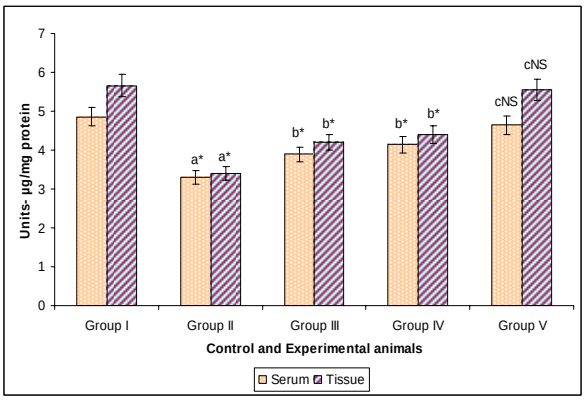

Fig. 4: Levels of Vitamin C in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

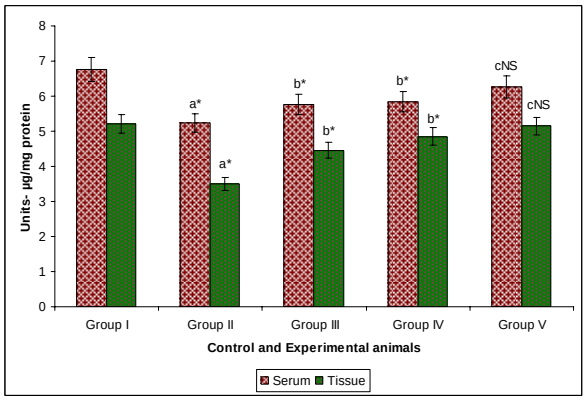

Fig. 5: Levels of Vitamin E in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

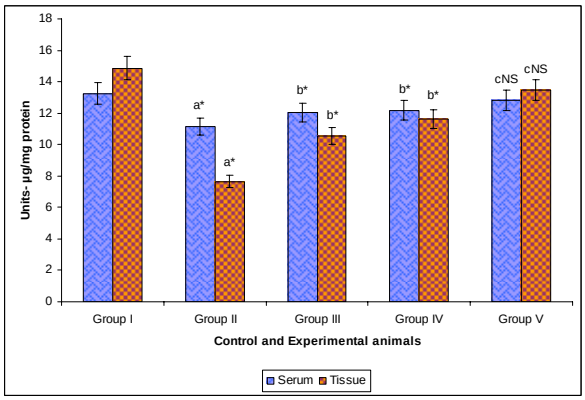

Fig. 6: Levels of Reduced Glutathione (GSH) in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS- Non Significant.

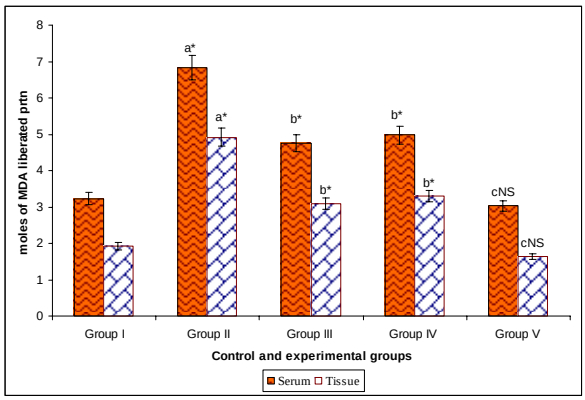

Fig. 7: Levels of LPO in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS-Non Significant.

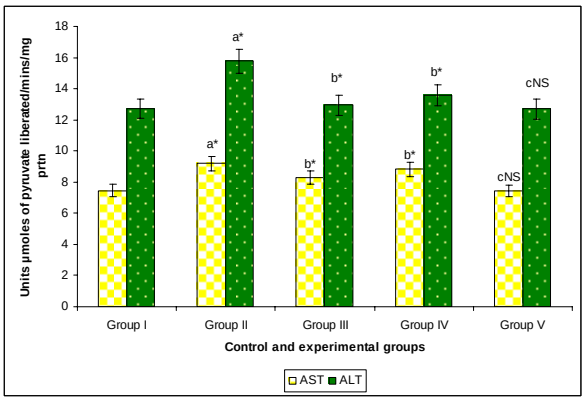

Fig. 8: Levels of AST and ALT in control and experimental groups. Values are given as mean ± SD for groups of six rats each. a – Group II Vs Group I, b- Group III and IV Vs Group II, c- Group V Vs Group I. The symbol * represents the statistical significance at P<0.05, NS-Non Significant.

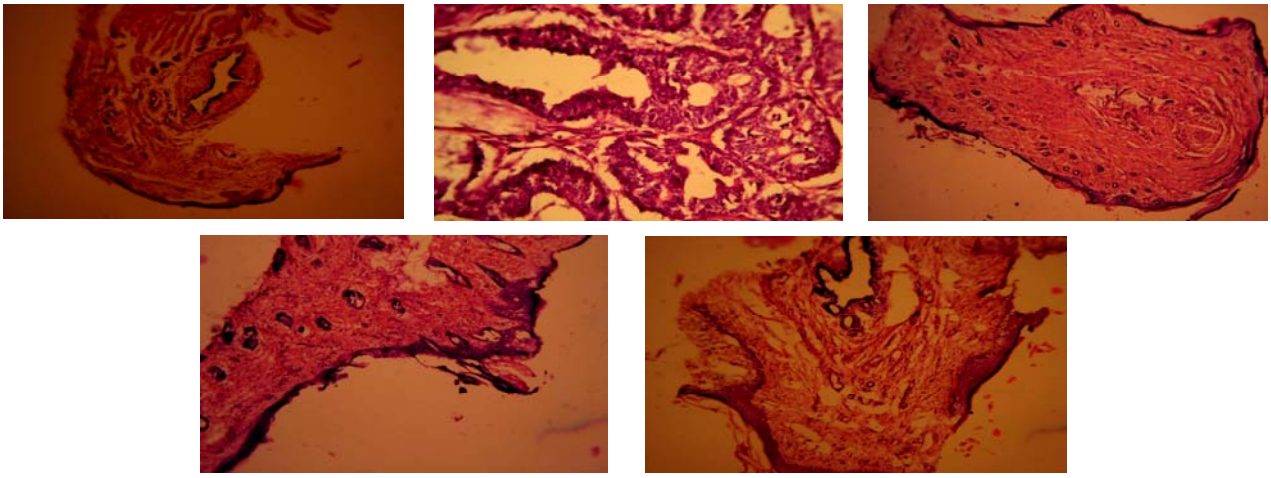

Fig. 9: Histopathological examination of breast tissue in control and experimental groups of rat. A. Tissue of normal rat, B. Cancer-induced breast tissue of rat, C. Cancer-induced rat treated with fungal taxol, D. Cancer-induced rat treated with authentic taxol, E. Rat treated fungal taxol alone.

Biochemical and Histopathalogical Studies

The Glutathione Peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) activity were estimated by the method of Rotruck[4], Sinha[5] and Misra & Fridovich[6]. The activity of Vitamin-C, Vitamin-E and Reduced glutathione (GSH) was estimated by the method of Omaye[7], Quaite[8] and Moron[9] Lipid peroxidation was measured by the method of Ohkawa[10]. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activity was estimated by the method of King[11].Mammary tissue was fixed in 10% buffered formalin, embedded in paraffin using a conventional automated system. The blocks were cut to obtain 5-µm-thick sections and stained with haemotoxylin-eosin. Serial paraffin sections of each tissue image were captured by light microscopy[12].

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD). Data was analysed by the method of One way analysis of variance (ANOVA) followed by Tukey multiple comparison was carried out to compare the mean value of different groups by using SPSS 7.5 student version.

RESULTS

Effect of fungal taxol on Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-peroxidase (GPx) in serum and breast tissue of control and experimental groups

The activities of enzymic antioxidants in serum and breast tissue of control and experimental rats are shown in Fig. 1, 2, & 3. The SOD, CAT and GPx were significantly reduced in cancer bearing animals (Group II). In the drug treated animals (Group-III and IV) the activities of SOD, CAT and GPx were significantly increased when compared to cancer bearing animals (Group II). The drug alone group where only fungal taxol was administrated exhibited no difference when compared to control group.

Effect of fungal taxol on Vitamin-C, Vitamin-E and Reduced glutathione (GSH) in serum and breast tissue of control and experimental groups

The levels of non-enzymic antioxidants such as vitamin C, vitamin E and GSH in breast tissue are shown in Fig. 4, 5, & 6. The levels of vitamin C, vitamin E and GSH were reduced in group II cancer bearing animals when compared to control animals. The drug treated groups (III & IV) showed increased levels of non enzymic

antioxidant, which were significantly increased when compared to cancer induced animals. Whereas the drug alone group, where only fungal taxol administrated rats, no significant difference was observed when compared to control group.

Effect of fungal taxol on Lipid peroxides (LPO) in serum and breast tissue of control and experimental groups

The effect of fungal taxol on LPO levels in serum and breast tissue of control and experimental groups was analyzed. Lipid peroxides levels were significantly higher in cancer bearing rats (Group II) when compared to control rats (Group I). The drug (fungal taxol) treated (Group III) and commercial drug treated (Group IV) rats showed decreased LPO levels when compared to DMBA administrated rats (Group II). In Group V, (drug alone) where only fungal taxol was administrated rats, no significant changes were observed when compared to control (Figure 7).

Effect of fungal taxol on Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activity in control and experimental groups

The AST and ALT levels are higher in DMBA administrated cancer induced (Group II) animals when compared to normal control rats. Whereas, the fungal taxol (Group III) and authentic taxol (Group IV) treated rats significantly reduced the AST and ALT when compared to cancer bearing rats. Drug alone (Group V) rats did not show any significant changes when compared to control rats (Figure 8).

Histopathological changes in Breast tissue of control and experimental groups

The normal control (Group I) rats showed normal alveolar structure of mammary tissue with dilated duct in fibrocollagenous stroma (Figure 9a). In DMBA induced (Group II) rats, the section shows malignant neoplasm compost of neoplastic ducts closely packed (Figure 9b). The fungal taxol (Figure 9c) and commercial taxol (Figure 9d) treated (Group III & IV) rats showed mild ductular proliferation with focal epithelial hyperplasia. The drug alone group, where only fungal taxol was given, (Group V) the section showed no observable distinct change from the normal control (Figure 9e).

DISCUSSION

The present study was carried to evaluate the anticancer activity of fungal taxol isolated from *Pestalotiopsis pauciseta* Sacc. VM1 on mammary tumor bearing Sprague Dawley rats. The SOD, CAT and GPx were significantly reduced in cancer bearing animals (Group II). In the drug treated animals (Group-III and IV) the activities of SOD, CAT and GPx were significantly increased when compared to cancer bearing animals (Group II). The drug alone group where only fungal taxol was administrated exhibited no difference when compared to control group. Superoxide radicals may be reduced by the enzyme superoxide dismutase to form H_2O_2 and oxygen. Catalase is an enzyme which converts H_2O_2 to neutral products O_2 and H_2O. Glutathione peroxidase (GPx), catalyses destruction of H_2O_2 and other lipid hydrogen peroxides using glutathione as electron donor. Several studies have reported the decreased activities of GPx in various cancerous conditions. There was a decline in the activities of GPx in the present study, which may be due to the altered antioxidant defence system caused by enormous production of free radicals in DMBA induced carcinogens[13]

The levels of vitamin C, vitamin E and GSH (non enzymic antioxidant) were reduced in group II cancer bearing animals when compared to control animals. The drug treated groups (III & IV) showed increased levels of non-enzymic antioxidant, which were significantly increased when compared to cancer induced animals. No significant difference was observed in fungal taxol administrated rats (drug alone group) when compared to control group. The water soluble vitamin C, plays several important roles *in vivo*[14].It is a good scavenger of most reactive oxygen species[15] and protects lipid and plasma membranes[16] thereby preventing degenerative disease including cancer [17]. It demonstrates a synergistic interaction with tocopheroxyl radical, resulting in the regeneration of α -tocopherol. Ascorbate imparts its protection by undergoing oxidation ultimately forming dehydroascorbate [18]. GSH is required for the reduction of dehydroascorbate back to ascorbate. The paclitaxel induced depletion of water soluble and lipid soluble antioxidants leads to increased susceptibility of the tissues to free radical damage. Vitamin E acts as a biological antioxidant. As a free radical quencher, vitamin E accounts for much of the lipid soluble chain breaking antioxidant capacity of the human blood plasma and erythrocyte membrane[19]. Administration of fungal taxol reveals its effectiveness in affording protection of cell membranes by maintaining the antioxidants (GSH, Vitamin C and Vitamin E).

The formation of Melondialdehyde is considered as an index of lipid peroxidation that causes cell injury. Elevation of Lipid Peroxides, as indicated by increased MDA was observed in breast cancer bearing animals. Significant increase in LPO in carcinogenic process may be due to abnormal levels of reactive oxygen species (ROS). ROS production in excess of cellular antioxidant capacity may result in damage to lipid, protein, RNA and DNA or other effects[20]. In the present study, Lipid peroxides levels were significantly higher in cancer bearing rats (Group II) when compared to control rats (Group I). The drug (fungal taxol) treated (Group III) and commercial drug treated (Group IV) rats showed decreased LPO levels when compared to DMBA administrated rats (Group II). In Group V, (drug alone) where only fungal taxol was administrated rats, no significant changes were observed when compared to control. The naturally occurring free radical scavenger fungal taxol lowered the MDA level suggesting reduced LPO. Due to the free radical scavenging property, the fungal taxol helps to improve the antioxidants defence system and prevent the damage induced by free radicals.

Aspartate transaminase (AST) and Alanine transaminase (ALT) are familiar markers of liver function and to ascertain the non-involvement of systemic toxicity, the activities of marker enzymes like Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed. In our study, the AST and ALT levels are higher in DMBA administrated cancer induced (Group II) animals when compared to normal control rats. Whereas, the fungal taxol (Group III) and authentic taxol (Group IV) treated rats significantly reduce the AST and ALT when compared to cancer bearing rats. Drug alone (Group V) rats did not show the significant changes when compared to control rats. As a marker for liver metastases in breast cancer patients and also as a marker for hepatotoxicity, aspartate transaminase and alanine transaminase were found to be increased. It was concluded that the elevations in the activities could be due to the decreased synthesis of degradative products which could have resulted in the elevation in the circulation. Elevations in liver transaminases following tamoxifen administration have been observed [21]. Tissue damage is the sensitive feature in the cancerous conditions so any deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues. Hence elevation of these liver specific enzymes observed in breast cancer condition may be due to the progression of tumor growth[22]. Padmavathi [23] are reported the reduction of the activities of AST and ALT treatment with propolis, taxol and propolis combination with taxol in cancer bearing rats.

The results of the present study are encouraging as fungal taxol has shown improvement in the biochemical parameters of the hosts and decreased the lipid peroxidation. The above parameters are responsible for the anticancer activity of fungal taxol isolated from *Pestalotiopsis pauciseta* Sacc. VM1.

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