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AGARICUS BISPORUS LECTIN – A FORMIDABLE ANTICANCER AGENT AGAINST 7,12 DIMETHYLBENZ(A)ANTHRACENE INDUCED EXPERIMENTAL RAT MAMMARY CARCINOGENESIS VIA TRANSMOGRIFYING OXIDANT AND ANTIOXIDANT STATUS

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ABSTRACT

Introduction: *Agaricus bisporus,* a common edible mushroom is considered to be a valuable health food with high contents of polyphenols, vitamins, minerals, polysaccharides and importantly lectin. The lectin endowed called Agaricus bisporus lectin (ABL) is a persuasive fractional tetramer found to have health benefits and can be manoeuvred for treating profuse ailments.

Aim of the study: The main fanatical about the study was to investigate the effect of Agaricus bisporus lectin (ABL) on 7,12-Dimethylbenz(a)anthracene (DMBA) induced rat mammary carcinogenesis.

Methodology : The experimental design consisting of five groups each comprising 8 animals (Female Sprague Dawley rats) were used for the study. The mammary tumour was induced by DMBA after incubation period. After 7 to 8 weeks, ABL and Tamoxifen were administered to the tumour bearing rats and the experiment was ceased at the end of the 14th week and their plasma, tissue sections were processed for various biochemical and histological studies.

Results: In the study, we found that the levels of lipid peroxidation elevated and the levels of antioxidant status and the lipid profile was plummeted in the plasma and tissues of DMBA induced tumour bearing rats. As a result, with the administration of Agaricus bisporus lectin (ABL) the tumour growth was suppressed and restored the abnormal status with comparison to the standard Tamoxifen. Into the bargain, histopathological analysis also comparably confirmed that the ABL shields the cellular disruption caused by DMBA.

Conclusion: In conclusion, our study lay the foundation that Agaricus bisporus lectin (ABL) could be a proficient chemotherapeutic agent against the mammary carcinoma.

KEYWORDS: Agaricus bisporus lectin (ABL), 7,12-Dimethylbenz(a)anthracene (DMBA), Mammary carcinoma, Biochemical status.

1.INTRODUCTION

Breast cancer is one of the most common cancers in women worldwide, accounting for approximately 570,000 deaths in 2022. Over 1.5 million women (25% of all women with cancer) are diagnosed with breast cancer every year throughout the world. In America, it is estimated that 30% of all new cancer cases (252,710) among women are affected with breast cancer in 2022 (1). The etiology mainly comprises of Obesity, sedentary lifestyle, contraceptive medications, early menarche, late menopause, family history, prolonged hormone replacement treatment, tobacco and alcohol usage. This is a metastatic cancer and can commonly transfer to distant organs such as the bone, liver, lung and brain, which mainly accounts for its incurability (2). Early diagnosis of the disease can lead to a good prognosis and a high survival rate. However, because the treatment option has not proven effective, the researchers are turning to alternative medicine that substantially subsume natural products (3).

Mushrooms are fungi with large fruiting bodies which can be grouped into edible, medicinal and poisonous mushrooms (4). *Agaricus bisporus* is one of the most consumed edible mushrooms in the world, and its benefit to human health has been widely reported. The mushroom is a popular part of the human diet. It is commonly called white mushroom, button mushroom, or champignon. *A. bisporus* is rich in metabolites and other biologically active compounds (amino acids, simple sugar/saccharides, indole, phenolic compounds, fatty acids, sterols, statins, vitamins, trace elements, and minerals), complex carbohydrates, and proteins (5).

The most studied proteins from *A. bisporus* are tyrosinase (also often called polyphenol oxidase, PPO) and lectin. Indeed, bioactive proteins from edible mushrooms mostly comprise of lectin, ribosome-inactivating protein, copper oxygenase/oxidase (laccase, tyrosinase), antifungal proteins/peptides, and immunomodulatory proteins. In this respect, it is important to note that ribosome-inactivating proteins are usually classified as lectin, which are known to display various activities. *A. bisporus* PPO and lectin are often employed in the search for human medicine, either as a bioactive molecule, as a molecular target, in the production of metabolites, or as a component in biochemical assays (6). Lectins are non-immune proteins that bind carbohydrates and are found in all living things, including plants.bacteria, fungi, viruses, and

animals. Their diverse biological activities, such as their anti-tumor, anti-insect, anti-bacterial, antiviral, and antifungal properties, have been demonstrated, indicating their potential applications in a multitude of therapeutic, research, and diagnostic domains.
Hence, we conducted the study to quantify the effect of ABL on altering biochemical and histopathological alterations in DMBA-induced rat mammary carcinoma.

2. MATERIALS AND METHODS

2.1. Chemicals

7,12-dimethylbenz(a)anthracene (DMBA), Agaricus bisporus lectin (ABL), Thiobarbituricacid (TBA), Phenazine methosulphate (PMS), Nitroblue tetrazolium (NBT), Reduced glutathione (GSH) and Dimethyl sulphoxide (DMSO) were purchased from Himedia and all other chemicals at analytical grade were purchased from the local commercial sources.

2.2. Experimental animals

Female Sprague-Dawley rats (weighed 130 – 150 g) were procured from Biogen Laboratory Animal Facility, Bangalore, India and the experiment protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) on 27.12.2021- IAEC PROPOSAL NUMBER – AU – IAEC / PR / 1317 / 12 / 21 and the experiment was carried out at the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, India. Before the trial, rats were given a week to become accustomed. The animals were housed in five large polypropylene cages lined with husk under conventional laboratory conditions [temperature (272 °C), humidity (55%), and a 12-hour light/dark cycle] Throughout the trial, the rats were fed conventional animal feed and given ample access to water.

2.3. Body weight

Periodically, the total body weight that the experimental and control animals produced over the course of the trials was noted. Using a microbalance, the absolute weight of the kidney and liver for each group of experimental animals was measured at the conclusion of the study.

2.4. Tumor Investiture

Female Sprague-Dawley rats were inculcated with DMBA (25 mg/kg body weight), a dose calculated to provoke enough tumour incidence requisite of the study. The DMBA was dissolved in a 1 mL emulsion of 0.75 mL sunflower oil and 0.25 mL physiological saline.

2.5. Experimental design

The experimental design was framed into five groups each consisting of eight animals. In the study, group I was served as control. The animals of the group II to IV were inculcated with a single dose of DMBA subcutaneously (25 mg/kg body weight), After seven weeks, the animals belonging to the group III were medicated with ABL intraperitoneally (5 mg/kg body weight), similarly group IV was medicated with TAM (50 µg/kg body weight) intraperitoneally and the group V were administered with ABL intraperitoneally (5 mg/kg body weight) thrice a week. After 14 weeks, the experiment was ceased and all rats were sacrificed by injecting ketamine at a dose of 60 mg/kg b.wt. The breast tissue was rapidly dissected and carefully cleaned with ice-cold saline. The tissues were then homogenised in 0.1M Tris hydrochloric acetic acid buffer (pH=7.4) was added before centrifugation at 3000g for 10 minutes at 40°C. The supernatant was collected and treated in order to evaluate particular biochemical characteristics. For histochemical investigations, the remaining tissues were stored in 10% formalin.

2.6. Quantification of Tumor incidence and Tumor volume

The number of tumours per rat was used to calculate tumour multiplicity. The tumour volume was calculated using the formula 4/3r3, where r is the tumor's diameter in millimetres. By multiplying the number of tumours in each group by the mean tumour volume in millimetres, the mean tumour load was calculated.

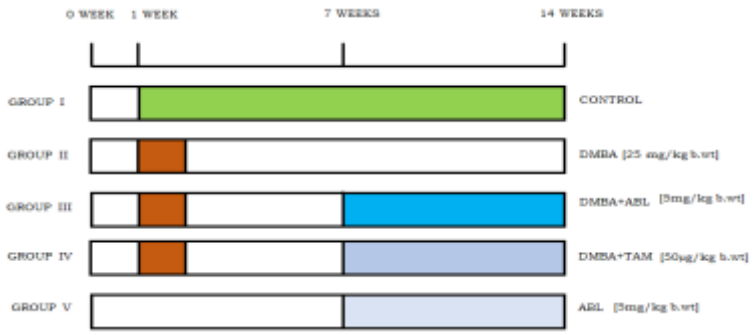


Fig 1. Experimental design

2.6. Biochemical assessments

2.6.1. Lipid peroxidative assessment

The engrossment of thiobarbituric acid reactive substances (TBARS) in breast tissue were deployed to measure lipid peroxidation. Tissue samples for TBARS were determined using the Ohkawa et al. technique.

2.6.2. Antioxidative enzymes assessment

The concentrations of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assessed using Kakkar et al., Sinha et al. and Rotruck et al. techniques, whereas non-enzymatic antioxidants like reduced glutathione (GSH), vitamin C (Vit-C), and vitamin E (Vit-E) levels in mammary tissue were evaluated using Ellman et al, Omaye et al.and Desai et al. techniques.

2.7. Histological assessment

The mammary tissues of control and experimental rats was removed, preserved in neutral buffered formalin (10% formaldehyde), dried with ethanol solutions, and then embedded in paraffin wax. Tissues were stained with Mayer's haematoxylin and eosin (H&E) after being sliced to a thickness of 3-5 m for the microanatomical observation and periodic acid schiff's staining was utilized for the presence of polysaccharides and it was further examined through the light electric microscope (40x) to perform additional investigations.

2.8. Statistical analysis

A statistical analysis was performed using SPSS V26.0. The data were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used to correlate the difference between the variables. A value of $P < 0.05$ was considered statistically significant

3. RESULTS

3.1. Effect of ABL on Initial and final body weight changes in control and experimental rats

GROUPS	INITIAL(g)	FINAL(g)
GROUP I	**130.25±8.50**	**165.25±10.20**
GROUP II	**132.20±10.00**	**100.50±7.30**$^{###}$
GROUP III	**142.50±12.20**	**120.40±9.25***
GROUP IV	**135.25±10.10**	**158.30±12.20*****
GROUP V	**145.25±11.50**	**160.10±8.10**

Table 1 - Effect of ABL on Initial and final body weight changes in control and experimental rats

Values are expressed as mean ± SD for six rats in each group. Significant levels are $^{###}P < 0.001$ when compared with control group and $^{*}P < 0.05$, $^{***}P < 0.001$ when compared with DMBA group

The table 1 portrays the initial and final body weight changes of the normal and the experimental rats where, Initially, there have been no significant differences in body weight amongst control and experimental rats. Terminally it was noticed that the body weight of DMBA-induced tumor carrying rats was dramatically reduced as contrasted to control rats at the terminational point of the experiment. In addition, tumor bearing rats medicated with ABL and Tamoxifen showed increase in body weight when compared to DMBA induced rats and the ABL alone treated showed results similar to the control group rats.

3.2. Effect of ABL on tumor incidence, total number of tumors and tumor volume in control and experimental rats

Groups	Tumor incidence (%)	Number of tumors (N)	Tumor volume (mm^3/rat)
I	-	-	-
II	100**%**	8/8	28.14±1.45$^{###}$
III	50**%**	4/8	14.20±3.50*
IV	37.5%	3/8	9.50±0.50***
V	-	-	-

Table 2 - Effect of ABL on tumor incidence, total number of tumors and tumor volume in control and experimental rats

Values are expressed as mean ± SD for six rats in each group. Significant levels are $^{###}P < 0.001$ when compared with control group and $^{*}P < 0.05$, $^{***}P < 0.001$ when compared with DMBA group

The table 2 depicts the effect of ABL on the carcinogenic parameters like tumor incidence, cumulative numbers of tumors, and tumor volume of both the control and experimental animals. The results revealed the DMBA group (II) showed 100% tumor incidence when compared with ABL (group III) and tamoxifen (group IV) treated rats with 50% and 37% tumor

incidence respectively. Hence the administration of ABL suppressed the tumor growth more or less equal to the amount of suppression by tamoxifen.

3.3. Effect of ABL on Lipid peroxidative marker

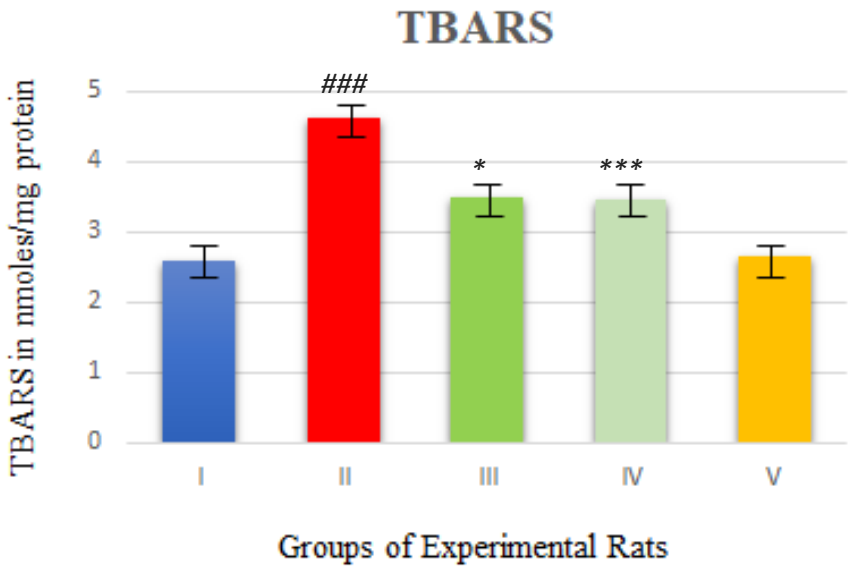
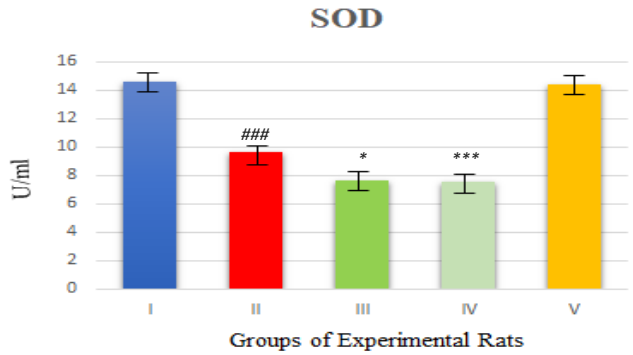


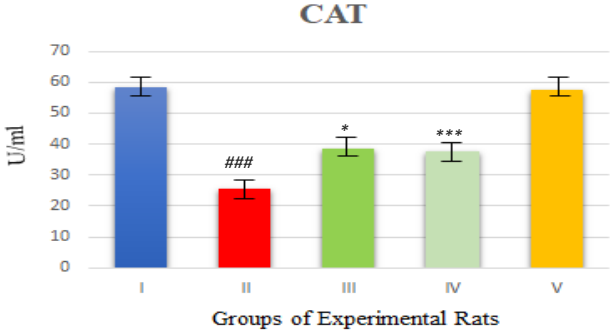
Fig 1. Effect of ABL on lipid peroxidative marker

TBARS: µmol of MDA released per microgram of protein per minute; Values are expressed as mean ± SD for six rats in each group; Significant levels are $^{###}P < 0.001$ when compared with control group and $^{*}P < 0.01$, $^{***}P < 0.001$ when compared with DMBA group.

Figure 1 shows the concentrations of lipid peroxidative marker (TBARS) in mammary tissues of both the control and experimental rats. DMBA administered rats (Group II) showed significantly elevated levels of TBARS than control rats (Group I) and the administration of ABL (Group III) and Tamoxifen (Group IV) significantly diminished TBARS levels when compared with DMBA induced rats (Group II). In addition to this, ABL alone administered (Group V) rats expressed similar levels of Control (Group I) rats. In terms of limiting TBARS levels, ABL was proven to be effective with similar effects with Tamoxifen.

3.4. Effect of ABL on Enzymatic and Non- Enzymatic Anti-Oxidative Markers





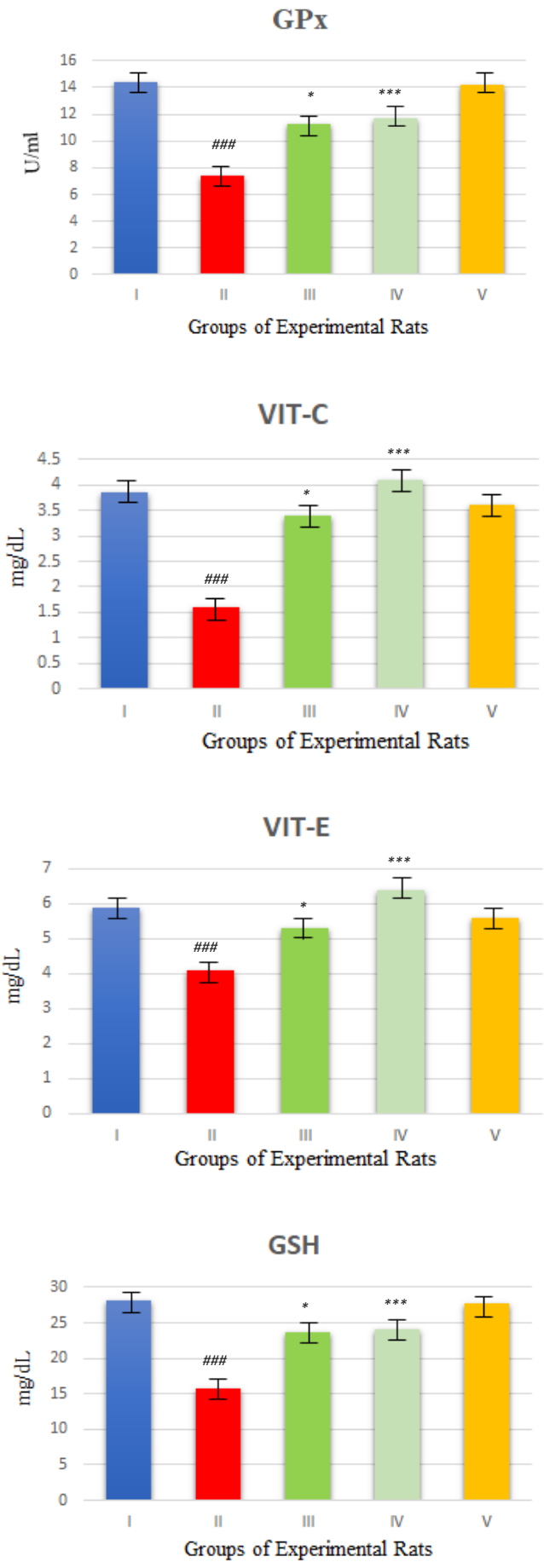


Fig 2 - Effect of ABL on Enzymatic and Non- Enzymatic Anti-Oxidative Markers

Values are expressed as mean ± SD for six rats in each group. Significant levels are $^{###}P < 0.001$ when compared with control group and $^{*}P < 0.05$, $^{***}P < 0.001$ when compared with DMBA group.

Fig. 2. shows the levels of mammary tissue enzymatic antioxidants (SOD, CAT, and GPx) and non-enzymatic antioxidants (GSH, Vit C, and Vit E) in both control and experimental group animals. SOD, CAT, GPx, GSH, Vit C, and Vit E levels significantly lower in DMBA-induced rats (Group II) when compared with control rats (Group I). With the administration of ABL (Group III) shows significantly escalated levels of SOD, CAT, GPx, GSH, Vit C, and Vit E on compared with DMBA rats (Group II). Along with this, no differences were recorded in (Group V) alone treated rats when compared to control rats (Group I).

3.6. Histopathological alterations in the Mammary tissues [Haematoxylin and Eosin] staining

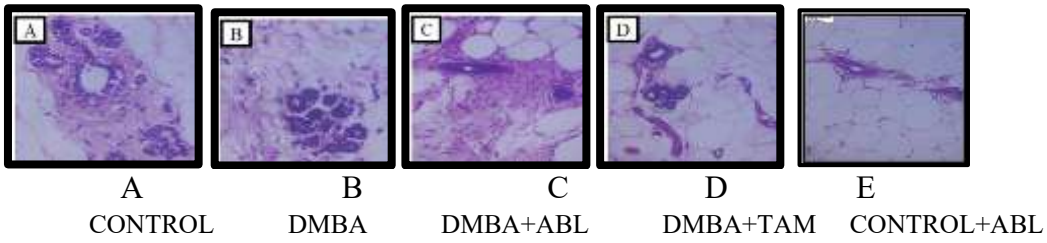


Fig 3 - Histopathological alterations in the Mammary tissues [Haematoxylin and Eosin] staining

Histopathological alterations in mammary tissue sections of control and experimental rats were depicted in Fig.3. The mammary tissue morphology of control rats (Group I) and ABL alone treated Group V treated rats appeared normal. In contrast, rats administered with DMBA (Group II) exhibited invasive ductal carcinoma with aberrant cellular proliferation. Substantial ductal hyperplasia and modest tumor infiltration have been detected in (Group III) (C) treated rats. It was found that ABL was potent in comparison with Tamoxifen.

4. DISCUSSION

Breast cancer constitutes a deadly human health challenge that is accompanied by the biochemical and histopathological alterations. These cellular indications warn the occurrence of breast cancer. In this era, the current medications that undergo rejection in therapy may be related to underlying characteristics of mistargeting that underpins all of the biochemical anomalies reported in the malignant evolution (7). Presently the natural derived compounds deliver multiple enhancement in the treatment of cancer. Taking into consideration of the statement, *Agaricus bisporus* mushrooms derived lectin called *Agaricus bisporus* lectin was fabricated to restore the biochemical alterations in the DMBA induced rat mammary carcinogenesis (8). DMBA, a polyaromatic hydrocarbon which is the chemical carcinogen induced the formation of free radicals by increasing the oxidative stress inside the cellular environment (9). This oxidative stress triggers an enhancement in the reduction of cells and hence the abnormal growth of cancerous cells resulting in the elevated levels of TBARS. These changes may affect the need or functional lipids in the membrane composition as well as the lipid formation and degradation which might result in an imbalanced energy homeostasis thus increased cholesterol and fatty acid synthesis leads to elevated lipid grades that have altered signaling characteristics in several aspects of carcinogenesis thus it appears to be potential molecular mechanism for preventing the development of breast cancers. Our study highlighted the transmogrification of lipid peroxidative marker and the enzymatic and non-enzymatic antioxidants with administration of *Agaricus bisporus* lectin, where the administration of *Agaricus bisporus* lectin in the tumor bearing rats suppressed the tumor growth and restored the abnormal antioxidant status in comparison with the standard tamoxifen.

5. CONCLUSION

In recent years, research into natural medicinal products has progressed exponentially but much remains to be done. In conclusion, the DMBA induced upregulation of lipid peroxidation and ROS production were altered by Agaricus bisporus lectin. This our study lay the foundation that ABL could be a proficient chemotherapeutic agent in comparable with Tamoxifen against rat mammary carcinogenesis without any toxic effects.

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