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Efficacy of *Pleurotus ostreatus* (Jacq. Ex Fr.) P.kumm. on 7,12-dimethylbenz(a)anthracene induced mammary carcinogenesis in female Sprague-Dawley rats

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ABSTRACT

Neoplastic growth of the breast is the most common malignancy in women worldwide and its incidence has increased in most countries. In this study, we have evaluated the efficacy of *Pleurotus ostreatus* (Jacq. Ex Fr.) P.kumm. (*P. ostreatus*) an edible mushroom on modulating levels of xenobiotic metabolizing enzymes, hormonal status of estrogen and progesterone receptor (ER/PR), protein expressions and histopathological analysis in 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis using a rat model. DMBA was induced by single subcutaneous injection at a dosage of 25 mg in 1 mL vehicle. The ethanolic extract of *P. ostreatus* (POEet) was administered orally at a concentration of 600 mg/kg bwt as pre- and post-initiation stage of treatment throughout the experimental period which was also compared with standard tamoxifen (TAM) (10 mg/kg bwt). At the end of 16 weeks, our results showed the elevated phase I and depleted phase II metabolizing enzymes, over expression of (ER/PR) and the expression pattern of the proteins such as fas, fasL, caspase 3, caspase 8, caspase 9, Bax were found to be down regulated whereas p53, Bcl2, cox-2 and cyclin D1 were markedly upregulated in DMBA-induced Sprague-Dawley rats, which were significantly reversed on *P. ostreatus* administration. Moreover, pretreatment with *P. ostreatus* showed improved response when compared to that of posttreatment. Based on scientific appraisal, we conclude that the dietary consumption of *P. ostreatus* might offer maximum protection against DMBA-induced mammary carcinogenesis and improving human health if used as a regular basis.

Focal points:

- Benchside
  - The potential of *Pleurotus ostreatus* on DMBA induced rat mammary carcinogenesis were determined by the analysis of xenobiotic metabolizing enzymes, hormonal status of ER/PR, as well as the expression of protein using western blotting techniques and histological examination of liver and mammary tissues.
- Bedside
  - The pharmaceutical potential of *P. ostreatus* are analysed for is markedly available, edible one with high protein as well as fiber with low fat content and cost effective for patient convenience.
- Industry
  - Mushrooms are considered as a nutraceutical functional food which can contain enriched prolific produced of novel "mycochemicals" responsible for the human health potential on various diseases and malignancies.
- Community
  - Standardization and refinement of mycochemical from mushrooms were help to develop the new novel active compounds which contribute for better health and also help to reduce disease burden.

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- Regulatory agencies
  - The *P. ostreatus* contain several bioactive compounds which might be the key factor for the anticancer effect. This will need to be taken for labeling and pattering the compounds and tested in clinical trials.

1. Introduction

Breast cancer is the most frequent type of malignancy among women in global population, makes up about one-tenth of all new cancer diagnosis worldwide [1]. Although the etiology of breast cancer is multifactorial. Significantly breast cancer risk factor include age, early age at menarche, late age of menopause, and late age at first pregnancy, obesity, oral contraception, hormone replacement therapy, diet, family history, lactation and prior history of benign breast diseases [2]. Although, a vast number of cancer researches have been devoted in the development of antineoplastic drugs, the prognosis of the diseases is often challenging due to increased side effects. Thus the pursuit for anti-cancer drugs takes a compelling urgency for alternate preventive approaches through dietary means with more efficacy and low toxicity are considered to be the winning strategy in reducing the morbidity and mortality of breast cancer [3].

Edible mushrooms are often used in the traditional system of medicine for the treatment of human ailments. In worldwide, mushrooms have been attracted the attention of the public due to presence of chemical composition particularly for antitumor property. *Pleurotus ostreatus* (Jacq.ex.fr) P.kumm. is a traditional Chinese medicinal and edible fungus which is famous for its delicious taste and high quantities of proteins, carbohydrates, minerals and vitamins as well as low fat [4]. Chemical investigations from different region of the world confirmed that the lectins, polysaccharides, polysaccharide-peptides, polysaccharide-proteins have been identified in *Pleurotus* mushrooms and many of these compounds have been found to have promising biological effects which protect the body against free radicals that damage body cells to induce cancer. These compounds are regarded as biological response modifier (BRM) they cause no harm and place no additional stress on the body, but help the body to adopt to environmental and biological stress [5]. We earlier reported that supplementation of *P. ostreatus* exhibits the modulatory effect on oxidant/antioxidant status in DMBA induced mammary carcinoma in experimental rats [6].

The present study was designed to ascertain the inhibitory effect of *P. ostreatus* on 7,12-dimethylbenz(a)antheracene induced rat mammary carcinoma by assessing the changes in phase I and phase II enzymes levels, hormonal status with respect to histological grading as well as the expression pattern of proteins.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin, cytochrome C, 1-chloro-2,4-dinitrobenzene (CDNB), 2,6-dichlorophenolindophenol (DCPIP), methylene blue, reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dinucleotide (NADPH), sodium dithionite, 7,12-dimethylbenz(a)anthracene (DMBA) and tamoxifen were purchased from Sigma Chemical Pvt. Ltd., Bangalore, India. Antibody's used for western blotting were purchased from Santa Cruz Biotechnology, CA, USA and Neo Markers USA. All other chemical and solvents used were of analytical grade.

2.2. Material

P. ostreatus mushrooms were collected in and around areas of Udhagamandalam, Nilagiri district, Tamil Nadu. The mushroom was taxonomically identified and a voucher specimen (No: 233) was deposited in the herbarium of Botany, Department of Botany, Annamalai University.

2.3. Preparation of mushroom ethanolic extract

The fresh fruiting bodies of *P. ostreatus* were dried in shade conditions and the dried materials were pulverized in a blender to get coarse powder. For *P. ostreatus* fruiting bodies ethanolic extraction, five grams of the powder was extracted with 100 mL of 95% ethanol using a Soxhlet apparatus. The solvent was evaporated on a rotary evaporator (Buchi Rotarvapour, Switzerland) under reduced pressure and controlled temperature (40–50 °C) [7]. A dark semisolid material (6% yield) thus obtained was stored at 4 °C until use. A known amount of the residual extracts were suspended in distilled water and was orally administrated to the animals by gastric intubation.

2.4. Animals

The whole experiment was carried out according to the guideline of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the animal ethical committee of Annamalai University (Reg. No:160/1999, Proposal number: 947 CPCSEA). The study was conducted on six week old adult female Sprague-Dawley rats, weighing approximately 130–150 g were obtained from National Institute of Nutrition, Hyderabad and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University. The rats were housed in polypropylene cages at room temperature (27 ± 2 °C) with relative humidity 55 ± 5%, in an experimental room. In Annamalainagar, the LD (light:dark) cycle is almost 12:12 h. The rats were maintained as per the principle and guidelines of the ethical committee for animal care of Annamalai University in accordance with the Indian National Law on animal care and use. The rats had free access to standard pellet diet (Amrut Laboratory Animal Feed, Mysore Feed Limited, Bangalore, India) and water *ad libitum* were available to the animals throughout the experimental period and replenished daily. The standard pellet diet comprised of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrate) and it provides metabolizable energy of 3600 kcal/kg.

2.5. Experimental design

Animals were assorted into six groups of six animals each according to the following experimental regimen. Animals in Groups 1 were induced with DMBA 25 mg in 1 mL of vehicle (0.5 mL of sunflower oil in 0.5 mL of saline) [8]. In Group 2 and 3 rats was received (600 mg/kg bwt) POEet extract as pre-initiation and post-initiation phase alone with DMBA. Group 4 rats were treated with tamoxifen (TAM) (10 mg/kg bwt) along with DMBA. Group 5 rats received (600 mg/kg bwt) POEet alone and group 6 rats was treated as control. The experiment was first terminated at the end