AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Effect of Taurine on Cytotoxic Markers *In* 7, 12- DimethylBenz (A) Anthracene Induced Mammary Carcinoma In Experimental Female Sprague-Dawley Rats

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ABSTRACT

Breast cancer comprises a diverse collection of diseases rather than a single homogeneous disease. Both preclinical and clinical research now commonly target specific subgroups of breast cancer with the aim of identifying biological markers or genetic phenotypes, and to reveal subgroup-specific therapeutic targets or indicators of prognosis. Since genetics is believed to account for only 10% of the reported cases, the environmental factors including diet are thought to play a significant role in predisposing breast cancer. The present study was aimed to evaluate the chemotherapeutic potential of taurine in 7, 12-dimethyl Benz (a) anthracene (DMBA) induced mammary carcinoma in rats. Oral treatment of taurine (100 mg/kg BW) to breast tumor bearing rats daily for ten to fifteen weeks was found to be effective against DMBA induced mammary gland carcinogenesis in female Sprague Dawley rats. The increased activities of 5′-ND, GGT and LDH in tissue of control and experimental breast cancer rats were significantly ($p < 0.05$) decreased to near normal levels. Thus, the results of the present study clearly indicate that taurine significantly suppresses DMBA induced breast cancer in rats.

Keywords: Breast cancer, 5'ND, GGT, LDH, Taurine.

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Received 09 February 2014, Accepted 24 February 2014

Please cite this article in press as: Vanitha MK *et al* Effect of Taurine on Cytotoxic Markers *In* 7, 12-DimethylBenz (A) Anthracene Induced Mammary Carcinoma In Experimental Female Sprague-Dawley Rats. American Journal of PharmTech Research 2014.

INTRODUCTION

Cancer is a group of diseases characterized by unregulated division and spread of cells.The cancerous cells may occur in liquids, as in leukemia. Most, however, occurs in solid tumor that originally appears in various tissues in various parts of the body. By their original locations they are classified into various types of cancer, such as lung, colon, breast, or prostate cancer. Localized tumors can be removed by surgery or irradiation with high survival rates. As cancer Progresses, however, it metastasizes – invading the surrounding tissues, entering the blood stream, spreading and establishing colonies in distant parts of the body. Only a third of patients with metastasized cancer survive more than five years. Invasive distensions spreading crab-like from a tumor in the breast were described by Hippocrates. From the crab, karkinos in Greek and cancer *in* Latin, came the name of the disease and the name of its inducing agents, carcinogen [1]. Breast cancer is a leading cause of cancer-related death and among one of the most aggressive metastasis disease worldwide. The growing mortality rate, with 410,000 deaths each year has yield more than 1.6% of all women deaths worldwide[2]. The major clinical problems of breast cancer are the recurrence of disseminated disease and metastatic behavior. In numerous Patients, miniature or clinically evident metastases have already occurred by the time the primary tumor is diagnosed.

For breast cancer diagnosis the traditional triple test are physical examinations, Mammography, and aspiration cytology. Unfortunately, all these methods are either not adequate or require an expert to identify breast cancer in early stages[3]. The etiology of breast Cancer is multifactorial. Significantly breast cancer risk factors 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 [4].

A major class of environmental carcinogens is a family of structurally related chemicals, the polycyclic aromatic hydrocarbons (PAH). These carcinogens and related halogenated Compounds have been implicated in mammary tumorigenesis by epidemiological and laboratory studies. DMBA (7, 12- dimethylbenz[*a*]anthracene) is a prototypical PAH that has been used to promote tumors in laboratory animals [5].

Oxidation is essential for many living organisms for the production of energy to fuel biological processes. However, oxidative stress resulting from increased free radical generation and decreased antioxidant status in the target cells and tissues play an important role in carcinogenesis [6]. Even though, all organisms possess antioxidant defense and repair systems that

have evolved to protect them against oxidative damage, these system are insufficient to prevent the damage entirely [7]. Considerable, natural products with antioxidant activity may be used to help the human body to reduce oxidative damage.

Taurine (2-aminoethanesulphonic acid) is a naturally- occurring sulphonic acid and an essential molecule in living creatures. It is known to maintain the level of cysteine, an important precursor of glutathione and also acts as an antioxidant [8.] It is synthesised in the pancreas via the cysteine sulfinic acid pathway and is present in tissues of many mammals in high concentrations. Recent investigations revealed that taurine protects several body organs[9,10] against toxicity and oxidative stress induced by various external agents like heavy metals[9,10,11] and drugs[12,13]. Although biochemical and physiological functions of taurine are still undefined, considerable evidence shows that it can act as a direct antioxidant by scavenging ROS (Reactive Oxygen Species) [14] or as an indirect antioxidant by preventing changes in membrane permeability due to oxidative impairment.

MATERIALS AND METHOD

The experimental animals were divided into four groups with six animals each.

Group I: Normal control animals Fed with standard diet and pure drinking water.

Group II: Animals treated with 25mg of 7, 12-dimethylbenz[*a*] anthracene (DMBA) in 1.0 ml Olive oil by gastric incubation to induce breast cancer.

Group III: Breast cancer bearing animals post treated with Taurine (100mg/Kg body weight) after the administration of 7, 12-dimethylbenz[*a*] anthracene (DMBA) from 10th week to 15th week.

Group IV: Control Animals treated with Taurine (as in Group III).

After the experimental period the animals were killed by cervical dislocation and blood, liver and breast tissues were used for the further analyses. Both the organs were excised immediately and was washed in ice cold saline to remove any extraneous matter, cleaned, blotted to dryness in filter paper. A 10% homogenate of breast and liver tissues were prepared in Tris-HCl buffer 0.1M (pH-7.4) using a Potter Elvejhem glass homogenizer as necessitated by the protocol. Dilutions were decided based on the protein concentration. The method of Lowry et al., (1951) was adopted for the estimation of protein content in the serum and tissue homogenates.

Animals

Healthy Female Sprague – Dawley Rats, 6-8 weeks of age and weighing 150-180g were used throughout the study. Rats were acclimated to laboratory condition with regular temperature

control ranging from 23±2˚C and were given ad libitum access to balanced diet (Gold Mohor rat feed, Ms. Hindustan Lever Ltd., Mumbai) and water. All the experiments were performed in compliance with the regulation of our institutional Animal Care and Use Committee. They were maintained in a controlled environment condition of alternative12h light/dark cycles. This research work on Female Sprague – Dawley Rats were sanctioned and approved by our Institutional animal ethical committee (IAEC/No-01/19/2012).

Drugs and chemicals

The 7, 12-dimethylbenz[*a*] anthracene [DMBA] and Taurine was purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratories, CDH division, Mumbai, India.

Biochemical Protocols

The activity of lactate dehydrogenase (LDH) was assayed using the method of King [15]. The activity of gamma glutamyl transpeptidase(GGT) was estimated according to the method of Orlowski and Meister[16] 5'-Nucleotidase (5'-ND) was assayed using the method of Luly *et al.* [17].

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to detect the significant changes between the groups. The student least significant difference (LSD) method was used to compare the means of different groups and the significance was denoted by 'P' value. A commercial software SPSS version 10.0 was employed to find out the statistical significance between the groups.

RESULTS AND DISCUSSION

The present study demonstrated that oral administration of taurine has been chemotherapeutic effects against 7, 12-dimethylbenz[*a*] anthracene (DMBA) in experimental Female Sprague – Dawley rats. Enzymes were one of the first protein molecules used as cancer biomarkers. Discovered in the early 1980's as a cancer biomarker for the early detection of prostate cancer, prostate specific antigen (PSA) is a serine protease [18].

Figure 1 shows the activities of the marker enzymes gamma-glutamyl transpeptidase, 5'-Nucleotidase and lactate dehydrogenase in breast tissue of control and experimental animals. In group II animals the activities of GGT, 5'- ND, LDH, were increased significantly when compare to control (group I) animals. The enzyme activities were reverted back to near normal in taurine treated group III breast cancer bearing animals, when compared to group II animals. In

Group IV, (Drug alone) where taurine was administrated animals; no significant changes were observed when compared to control (Figure 1).

Analysis of cancer marker enzymes serves as an indicator of cancer response to therapy [19]. The marker enzymes such as AHH, ADA, GGT, 5'-ND and LDH are specific indicators of lung damage [20, 21, 22]. The increase in the activities of these enzymes may be due to the increased tumor incidence [23].

GGT activity serves as a specific marker for the progress of carcinogenic events. The enzyme is membrane bound and its active site is oriented on the outer surface of cell membranes. GGT is a cell surface enzyme that cleaves extracellular glutathione there by providing the component for increased intracellular glutathione synthesis [24.] Elevated activities of GGT were observed in cancer conditions. Chemical carcinogens that enter the liver may initiate some systematic effects that induce GGT synthesis [25]. Increase levels of GGT were observed in cancerous cells [26]. This elevation may indicate the basic tumour burden.

5'- nucleotidase enzyme hydrolyzes nucleotides with a phosphate group on carbon atom 5 of the ribose. It is found to be widely distributed in tumor tissues. A fast moving 5'- nucleotide phosphodiesterase is found to be elevated in metastases to liver from tumour of the lung and breast [25]. It is found to be widely distributed in tumour tissues and increased activity of this enzyme in leukaemia patients had already been reported [27].

LDH is recognized as a potential tumour marker enzyme in assessing the proliferation of malignant cells, and increased lung and serum LDH activity has been reported in lung cancer [28]. Proliferating malignant cells exhibit very high rates of glycolysis, which subsequently leads to elevate LDH activity.

In the present study, an increase in the levels of GGT, 5'-ND and LDH were found in breast cancer-bearing animals and these were significantly reduced after treatment with taurine. Many antioxidants are being identified as anticarcinogens that characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases [29,30] Taurine and its derivatives such as taurolidine and taurochloramin were found to display antineoplastic effect both in vitro and in vivo; through suppressing cell proliferation, enhancement of tumor cell apoptosis[31,32,33,34,35,36,37,38].and through an antiangiogenic effects [32,39] while enhancing the therapeutic index of some antitumor agent [33].

Administration of taurine caused the activities of these enzymes to return to normal levels. This may be due to anti neoplastic property of the drug indicating the protective role on tissue damage.

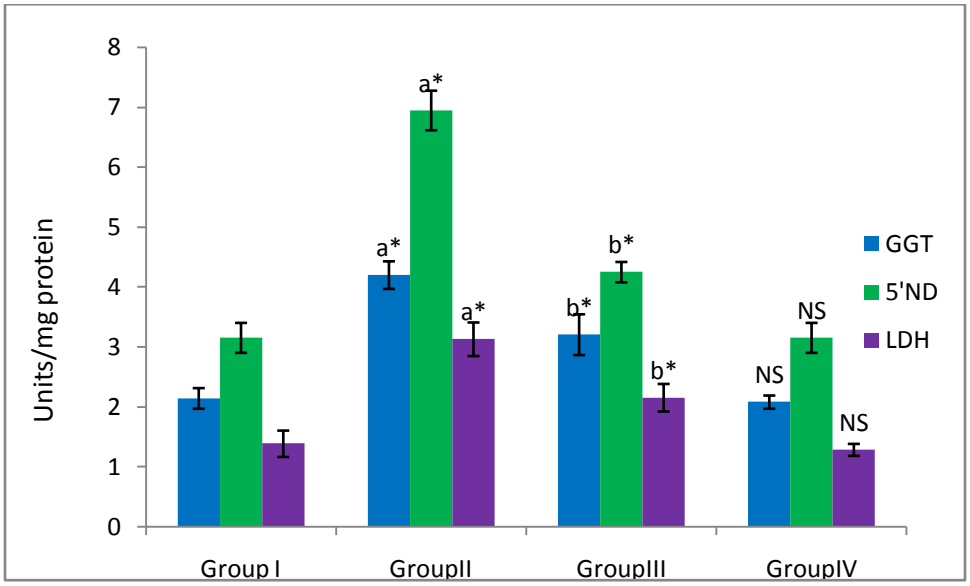

Figure 1: effect of taurine on cytotoxic marker enzymes in the breast tissues of control and experimental animals

Each value is expressed as mean ± S.D. for six rats in each group.

a: Group I compared with Group II

b: Group II compared with Group III

LDH – micro-moles of pyruvate liberated/min/mg protein;

GGT – nano-moles of p-nitroaniline formed/min/mg protein;

5'ND -n moles of p-inorganic phosphorus formed/min/mg protein.

Statistical significance: $^{**}p<0.001$, $^{*}p<0.05$

CONCLUSION

Hence our present study suggests that taurine inhibits proliferation of cancer cells and protects the cells from cytotoxic damage induced by free radicals by acting the antioxidant enzymes. From the review of literature it is clear that taurine has been reported to have potent anticancer activities in many types of cancer . Our results suggest that a taurine treatment offers a promising effect by acting as a potential chemotherapeutic chemopreventive drug against mammary carcinogenesis.

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