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Chemopreventive and Chemotherapeutic Role of Taurine Against 7,12-Dimethylbenze(A)Anthracene Induced Mammary Carcinoma In Experimental Female Sprague-Dawley Rats

Vanitha Kalappan[1*], Dhanapal Sakthisekaran[1], Aruldass Ilakkia[1], Kuppusamy Periyasamy[1], Kuppusamy Basker[1], Sundaramoorthy Selvaraj[1],

1. Department of Medical Biochemistry, University of Madras, Chennai-600113

ABSTRACT

Breast cancer is one of the most serious problems in oncology. It is a leading cause of death among women in many countries. Environmental factors, of either biological or chemical origin, may act as initiators and promoters of the carcinogenesis. Chemical carcinogens such as 7,12-dimethylbenz[*a*] anthracene [DMBA],benz[a]pyrene [BP], and N-nitroso-N-methylurea are commonly employed to initiate and promote neoplastic transformation in experimental animals. DMBA is well established as a highly potent carcinogen. Cancer chemoprevention is recognized as the most promising and novel approach to prevent, inhibit or reverse the carcinogenic processes by intervention with natural products or synthetic chemical substances. Taurine is a sulfur containing beta amino acid with a wide range of vital, biological functions, ranging modulation, cell membrane stabilization to bearing an antioxidant and scavenging agent. In from neuro the last decade it has been widely used in the field of oncology as a chemo protective agent against hepatocarcinogenesis and colon carcinogenesis. The aim of this study was to get insight into the process of chemo resistance acquisition for a better understanding of the breast cancer therapy. Therefore, the current study has been undertaken to examine the effect of taurine on DMBA induced breast cancer in rats. At the end of the experimental period, the homogenized breast tissue was investigated and recorded the body weight, tumor weight and antitumor activity of Taurine in the experimental rats. Overall, these results suggested that the taurine treatment provided antioxidant defense with strong chemopreventive activity against the genesis of DMBA-induced mammary carcinoma.

*Corresponding Author Email: vanithakalappan@gmail.com,
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INTRODUCTION

Mammary carcinoma begins in breast tissue, which is made up of glands for milk production, called lobules, and the ducts that connect the lobules to the nipple. The remainder of the breast is made up of fatty, connective, and lymphatic tissue.[1] Mammary carcinoma is a complex multi-stage disease, involving the deregulation of a number of different signaling cascades .It is a most common type of cancer next to the lung cancer.[2]

The global burden of cancer continues to increase mostly because of the aging, factors like exposure to Ultra Violet radiation, pollution and a rise in the adoption of cancer-causing behaviors (obesity, poor diet, smoking habits etc.,). Mammary carcinoma is the most frequently diagnosed cancer and the second leading cause of cancer death among females.[3]

CARCINOGEN

Environmental factors, of either biological or chemical origin, may act as initiators, promoters, or both of carcinogenesis. Chemical carcinogens such as 7, 12-dimethylbenz[*a*] anthracene [DMBA], Benz [a] pyrene [BP], 4-nitroquinoline- 1-oxide, and N-nitroso-N-methylurea are commonly employed to initiate and promote neoplastic transformation in experimental animals. However, the most commonly experimental carcinogenesis is employed chemical carcinogen for inducing DMBA.[4] DMBA is well established as a highly potent carcinogen. It is used solely in carcinogenesis research. DMBA is absorbed through the skin and respiratory and intestinal tracts; and by intravenous and intraperitoneal injection, ingestion, and inhalation. It is carcinogenic and may irritate tissues and induce sensitivity.[5] There has been an increasing interest in carcinogenic chemicals such as DMBA to induce mammary carcinoma in rat models for the study of human mammary carcinomas. The role of polycyclic aromatic hydrocarbons (PAH) [6] is clearly implicated in the process of carcinogenesis especially DMBA, which is one of the most potent skin and breast carcinogens. [7]

In mammary epithelial cells, DMBA undergo metabolic activation to form its active metabolite, dihydrodiolepoxides, which can damage DNA and form DMBA-DNA adduct, contributing to carcinogenesis. Over production of Reactive Oxygen Species (ROS) occur during metabolic activation of DMBA to diolepoxide, can also cause oxidative damage to structure and functions of DNA, proteins and Lipids, contributing to neoplastic transformation.[8]

Chemoprevention

An alternative approach to cancer avoidance could be to increase the intake of chemopreventive compounds, which might be reasonably expected to interfere with the initiation, promotion or

progression of carcinogenesis. [9] A larger numbers of chemopreventive agents have been elucidated in epidemiological and experimental studies, preclinical and clinical observations.[10] However the toxic side effects produced by some of these agents have limited their extensive use. Therefore, there is a need to identify synthetic or natural compounds that have significant chemopreventive potential without undesirable toxic effects. The chemopreventive mechanisms are thought to involve multiple biochemical and biological mechanisms including enzyme induction and anti-oxidation.[11]

Taurine is sulfur containing β amino acid with a wide range of vital biological functions, ranging from neuromodulation, cell membrane stabilization to being an antioxidant and scavenging agent. [12, 13,14,15,16] In healthy humans, dietary foodstuffs are the main source of taurine high concentrations is found in animal source whilst undetectable in vegetables. [17, 18] Methionine and cysteine are precursors of taurine, however synthesis ability varies widely amongst species (1); the maximal human synthesis rate is unknown. The average daily synthesis in adults ranges between 0.4 – 1.1mmol (50 – 125mg).[19,17,20]

In the last decade it has been widely used in the field of oncology as a chemo protective agent against hepatocarcinogenesis [16,21,22,23,24] and colon carcinogenesis. [25,26,27]

Experimental Design

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 mammary carcinoma.

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

MATERIALS AND METHOD

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 glutathione reductase (GR) was assayed by the method of Stahl et al., (1969)[28] and expressed as nmoles of NADPH oxidized/min/mg protein. Reduced Glutathione (GSH) was determined by the method of Moron et al., (1979)[29] which is based on the reaction of GSH with 2,2'-dithio-bis- nitrobenzoic acid (DTNB) to give a colored compound that absorbs at 412nm. The level of ascorbic acid was estimated by the method of Omaye et al., (1979)[30] and expressed as µg/ mg protein. The level of vitamin E was estimated by the method of Desai (1984) [31] and expressed as µg/ mg tissue. Superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund (1974).[32] The enzyme activity is defined as Units /mg protein. The activity of catalase (CAT) was estimated by the method of Sinha (1972)[33] and expressed as nmoles of H_2O_2 consumed/min/ mg protein. Activity of glutathione peroxidase (GPX) was assayed by the method of Rotruck et al. (1973),[34] expressed as µmoles of GSH oxidised/min/mg protein. Lactate dehydrogenase, Aspartate amino transferese (AST) andAlanine aminotransferese(ALT) activity was estimated by the method of King (1965).[35]

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:

In the last decade, a new trend to investigate the possible correlation between antioxidants and the pathogenesis of cancer developed.[36,37] Taurine was used successfully as a chemotherapeutic agent against the powerful hepatocarcinogenic agent, diethy1 nitrosamine (DENA)-treated rats.[38] Also, successful results were recorded when used to treat colon cancer induced by azoxymenthan and hydrophobic bile acid. [39,40,41]

Effect of taurine on anti-tumour activity in control and experimental animals.

The present study demonstrated that oral administration of taurine has chemopreventive effects against 7, 12-dimethylbenz[*a*] anthracene (DMBA) in experimental Female Sprague – Dawley rats. Figure 1 and 2 represents the body and organ weights of the control and experimental rats recorded periodically once in a week from the day of tumor induction, till the completion of the experimental period. Initially, there wasn't any significant change in the body and organ weight of the control and experimental animals, but for the later period an explicit decrease in the body weight and increase in breast weight was observed with the induction group (group II) indicating the severity in the assault of the carcinogen. On treatment with taurine, a gradual improvement was noticed in post treated groups (group III). The control and drug control animals in group I and IV did not show any change in the body and organ weight throughout the experimental period. The considerable weight loss observed in cancer-bearing animals could be because of cancer cachexia, anorexia or mal-absorption which reportedly contributes to progressive wasting notably in skeletal muscle and adipose tissue.[42] The significant increase in tumourigenesis observed in the breast of cancer-induced experimental animals could be because of the uncontrolled proliferation of cancerous cells but the increase in body weight upon administration of taurine could be because of the protective efficacy of this drug. This indicates the antineoplastic property of the drug. Nutritional therapy is a key component for the treatment of cancer cachexia and to actually help in controlling malignant disease in some situations.[43]

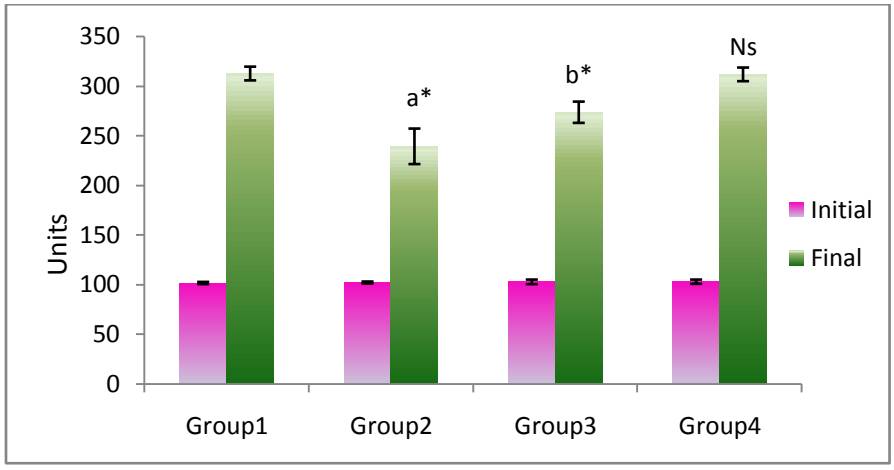

Figure.1 Effect of taurine on anti-tumour activity in control and experimental animals.

Values are expressed as mean ± SD for Six animals.

a is compared with group I (Control);

b is compared with Group II (DMBA);

significant levels were ** p<0.01, * p<0.05, and a ^{NS} p>0.05.

Units: Body Weight (gm)

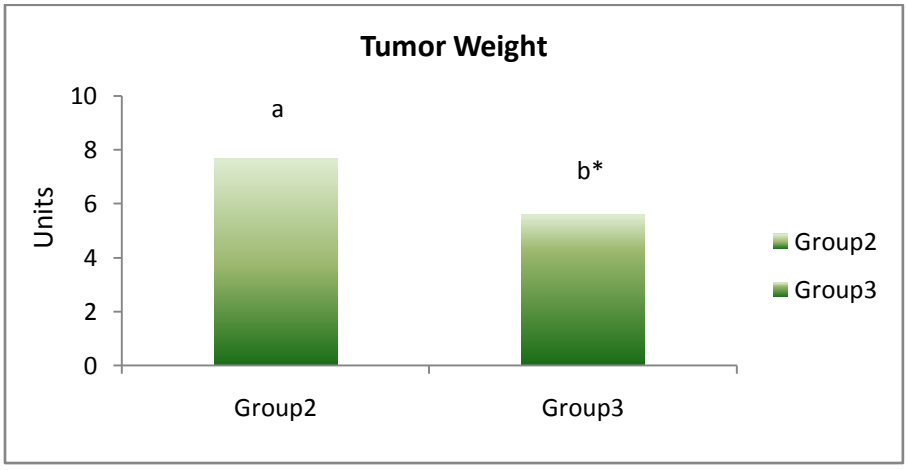

Figure.2 Effect of taurine on anti-tumour activity in control and experimental animals.

Values are expressed as mean ± SD for Six animals.

a is compared with group I (Control);

b is compared with Group II (DMBA);

significant levels were ** p<0.01, * p<0.05, and a ^{NS} p>0.05.

Units: Tumor Weight (gm)

Effect of taurine on superoxide dismutase(SOD), catalase (CAT), and glutathione peroxidase (GPx) in breast tissue of control and experimental animals.

Figure 3 shows the activities of the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in breast tissue of control and experimental animals. In group II animals the activities of SOD, CAT, and GPx, were decreased significantly when compare to control (group I) animals. The enzyme activities were reverted back to near normal in taurine treated group III mammary carcinoma 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 3).

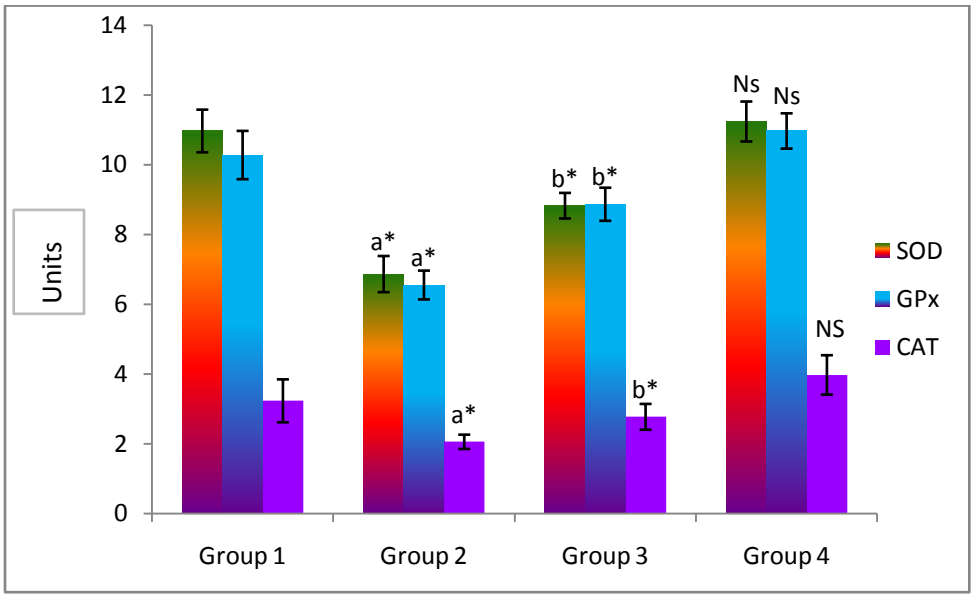

Figure.3 Effect of taurine on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in breast tissue of control and experimental animals.

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

a: Group I compared with Group II

b: Group II compared with Group III

Units: Superoxide Dismutase – one enzyme unit = amount of enzyme required to prevent 50% auto oxidation/min/mg protein; Catalase – nmoles of H2O2 consumed/min/mg protein; Glutathione Peroxidase- µmoles of GSH oxidized/min/mg protein. Statistical significance: #$p<0.001$,*$p<0.05$

Reactive Oxygen Species (ROS) are generated during normal aerobic metabolism and increased levels are present during oxidative stress. It has been proposed that ROS is necessary for life and essential for the regulation of essential physiologic functions. However, at high concentrations, ROS are cytotoxic. ROS are important in cell differentiation, apoptosis, and cell proliferation. These functions are regulated by redox-sensitive signal transduction pathways. The amount of antioxidants in the cells is high and so cells prevent or repair the damages caused by ROS.

ROS-induced damage can result in cell death, mutations, chromosomal aberrations and also carcinogenesis.[44] There are three major types of antioxidant enzymes in mammalian cells: superoxide dismutase, catalase, and peroxidase, of which glutathione peroxidase is the most important component of these.[45] Both endogenous and exogenous antioxidants play an important and interdependent role in preventing cancer. Superoxide radicals may be reduced by the enzyme superoxide dismutase to from 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.[46] The present results corroborate these findings. Oral administration of taurine brought back the status of these enzymes to near normal level, which indicates their free radical scavenging properties.

Effect of taurine on vitamin C, vitamin E and reduced glutathione (GSH) in breast tissue of control and experimental animals.

The levels of non enzymic antioxidants such as vitamin C, vitamin E and GSH in breast tissues are shown in Figure 4. The level of vitamin C, vitamin E and GSH were reduced in group II mammary carcinoma bearing animals when compared to control animals. The drug treated group III animals showed increased levels of non enzymic antioxidant, which were significantly increased when compared to mammary carcinoma induced animals. Drug alone (group IV) animals did not show any significant changes when compare to control animals. Vitamins C and E and reduced glutathione comprise the non- enzymic antioxidant system that protects the cells against free radicals and ROS. Antioxidant vitamins have a number of biological activities such as immune stimulation, scavenging the free radicals and alteration in metabolic activation of carcinogens.[47] They can utilize reactive oxygen metabolites, protecting biopolymers and reduce oxidative DNA damage.[48] The major antioxidant viz., Vitamin E protects the cells. It is a potent oxygen radical scavenger that protects cells from carcinogenic chemicals by inhibiting LPO- and free radical-mediated consequences. In addition to its antioxidant properties Vitamin E also functions as a biologic response modifier influencing the production of second messengers and products of arachidonic acid cascade which have profound effect on cell proliferation.[49] Our data suggests that administration of taurine significantly decreases the toxic complications of carcinogenesis and increases the level of free radical scavenging enzymes.

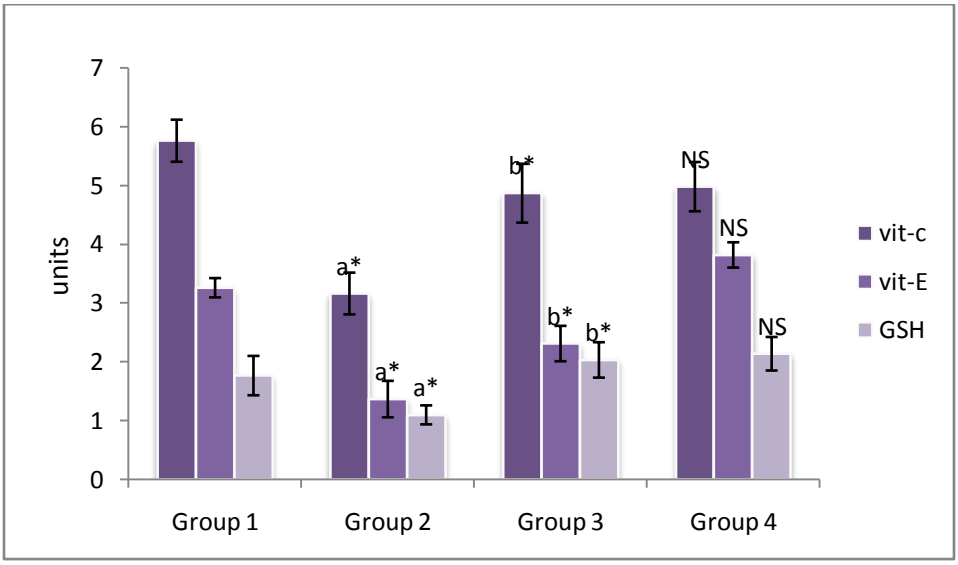

Figure 4 Effect of taurine on vitamin C, vitamin E and reduced glutathione(GSH) in breast tissue of control and experimental animals.

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

a: Group I compared with Group II

b: Group II compared with Group III

Statistical significance: #p<0.001, *p<0.05

Units: GSH - µmol/g tissue; Vitamin-E - µg/g tissue; Vitamin-C -µg/g tissue.

Effect of taurine on lipid peroxides (LPO) in breast tissue of control and experimental animals.

Effect of taurine on Lipid peroxides (LPO) in breast tissue of control and experimental groups were analysed. Lipid peroxides levels were significantly higher in cancer bearing animals (group II) when compare to control animals (Group I). The drug treated (group III) animals showed decreased LPO levels when compare to DMBA induced animals (Group II). In Group IV, (Drug alone) where taurine was administrated animals, no significant changes were observed when compared to control (Figure 5)**.** Oxidative stress, especially lipid peroxidation is known to be involved in carcinogenesis[50]. Increased levels of lipid peroxidation products play a role in the early phases of tumour growth (Rice-Evans and Burdon, 1993).[51] Faber et al. (1995)[52] and Huang et al. (1999)[53] have shown that the patients with mammary carcinoma have higher Melondialdehyde (MDA) levels, which are regarded as indicators of lipid peroxidation when compared to controls. In the present study, an increase in the levels of lipid peroxidation was found in mammary carcinoma-bearing animals and these were significantly reduced after treatment with taurine.

On the other hand, taurine has been reported to protect cells against oxidative injury. [54,55,56,57,58,59,60] Several mechanisms may play a role in taurine-mediated reduction in oxidative stress. Taurine was reported to protect cells by scavenging oxygen free radicals by up-regulating the anti-oxidant defenses, by forming chloramines with Hypochlorous acid (HOCl), or by binding free metal ions such as Fe2+ by its sulphonic acid group. [54,55,56] Because cysteine is a precursor of taurine and GSH, taurine supplementation may cause enhancement in GSH levels by directing cysteine into the GSH synthesis pathway. [55,61] Therefore, increased GSH levels after taurine treatment may play an additional role in decreasing oxidative stress. In the present study, an increase in the levels of lipid peroxidation was found in mammary carcinoma-bearing animals and these were significantly reduced after treatment with taurine and a significantly increase in GSH levels were detected in experimental rats with taurine treatment.

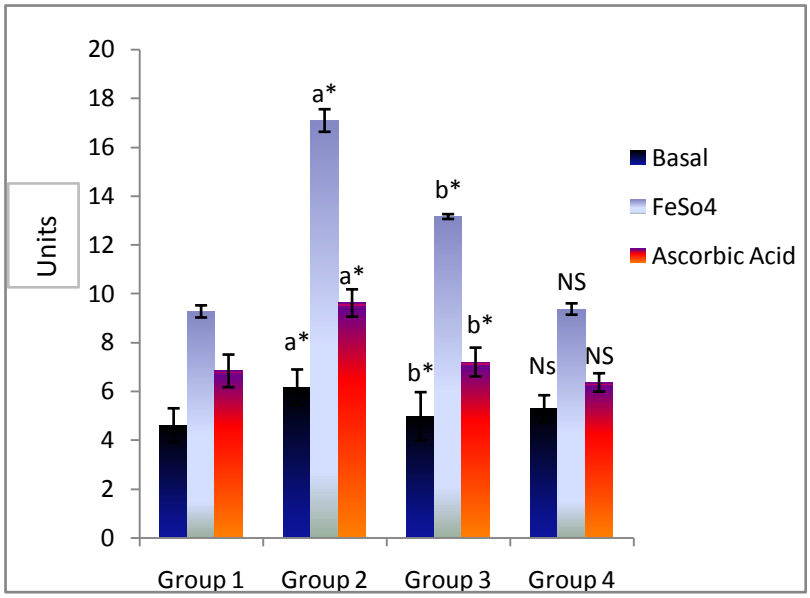

Figure.5 Effect of taurine on lipid peroxides (LPO) in breast tissue of control and experimental animals.

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

a: Group I compared with Group II

b: Group II compared with Group III

Units: n moles of MDA formed/min/mg protein.

Statistical significance: #p<0.001, *p<0.05

Effect of taurine on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenate (LDH) in breast tissue of control and experimental animals.

Effect of taurine on Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT) and Lactate dehydrogenate (LDH) in breast tissue of control and experimental groups were analyzed.

The enzymes levels were significantly higher in cancer bearing animals (group II) when compare to control animals (Group I). The drug treated (group III) animals showed decreased Enzymes levels when compare to DMBA induced animals (Group I). In Group IV, (Drug alone) where taurine was administrated animals, no significant changes were observed when compared to control group I animals (Figure 6).

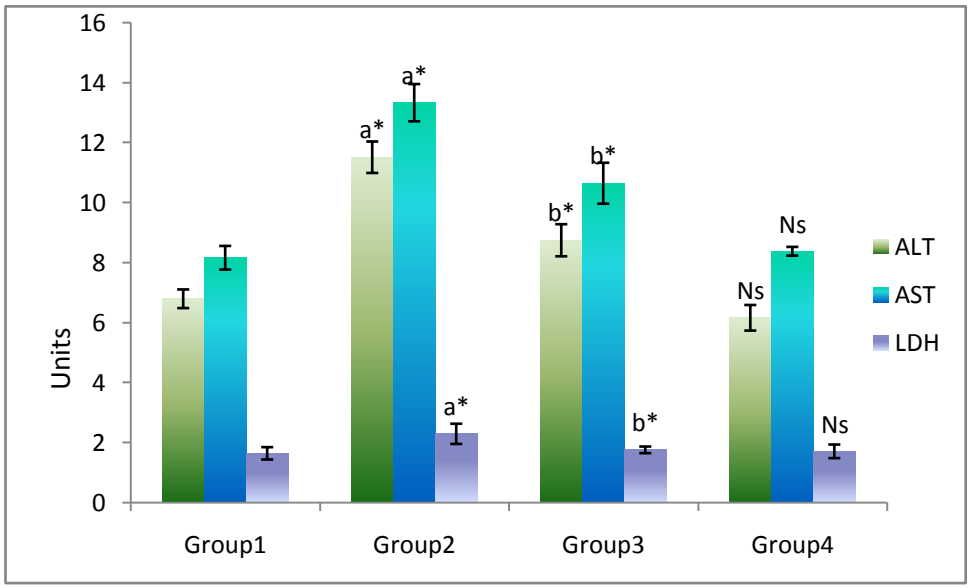

Figure.6 Effect of taurine on aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenate (LDH) in breast tissue of control and experimental animals.

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

a: Group I compared with Group II

b: Group II compared with Group III

ALT, AST, LDH – micro-moles of pyruvate liberated/min/mg protein

Statistical significance: #$p<0.001$, *$p<0.05$

Alanine aminotransferase and aspartate aminotransferase are diagnostic tumor markers in liver and heart diseases. The decreased activities of these enzymes in liver indirectly indicate the progression of tumor growth as tumor markers are directly associated with the malignancy in the cancerous conditions and is a potential molecular biomarker for assessing exposure to any toxic agents.[62] 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 mammary carcinoma condition may be due to the progression of tumor growth.[63] Hayes (1994)[64] and Palani et al. (1998)[65] have also reported increased activities of these aminotransferase in plasma and serum

of cancer bearing rats. 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.

Lactate dehydrogenase (LDH) enzyme is a tetramer recognized as a marker with potential use in assessing the progression of the proliferating malignant cells. In the present study, increase in the activities of LDH in carcinoma bearing animals, could be attributed to over production of enzymes by proliferated cells and further release of their isoenzyme from destructed cells and it is a fairly sensitive marker for solid neoplasm.[66,67] Numerous other reports also revealed the elevated levels of LDH in various types of cancers.[68] The rise in LDH may also be due to the higher glycolysis in the cancerous condition, which is the only energy-producing pathway for the uncontrolled proliferating malignant cells.

CONCLUSION:

The present study thus demonstrated the chemopreventive and chemotherapeutic potential of taurine in DMBA induced mammary carcinogenesis. Our results thus suggest that taurine significantly protected the antioxidant status and protected the structural integrity of cell surface during DMBA induced carcinogenesis.

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