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# Role of Taurine on Glycolytic and Gluconeogenic Enzymes in 7, 12,-Dimethyl Benz (A) Anthracene–Induced Breast Cancer in Spraque–Dawley Rats

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**A B S T R A C T**

Breast cancer is a leading cause of cancer-related death and among one of the most aggressive metastasis disease worldwide. 7,12–Dimethyl Benz (a) anthracene (DMBA) is a well-known breast carcinogen. Taurine is sulfur containing β amino acid. Taurine interacts with RNA and DNA and exert its protective effects against alkylating agents, radiation and other carcinogens. In the present study, the effect of taurine on glycolytic & gluconeogenic enzymes in DMBA induced breast cancer was studied. DMBA (25 mg/kg body weight) induced breast cancer bearing animals showed abnormal alterations in the activities of key glycolytic and gluconeogenic enzymes in the liver and breast tissues. The effect of taurine (100 mg/kg body weight) post-treatment was studied on DMBA induced breast cancer bearing rats. Post-treatment with taurine significantly decreased the activities of hexokinase, phosphogluco isomerase & aldolase; and increased the activities of glucose-6-phosphatase & fructose-6-phosphatase. This clearly shows that taurine may interrupt the energy requirement of neoplastic tissues leading to the suppression of cancer progression. In conclusion, the results suggest that taurine has a definite positive influence on energy metabolism that proves its chemotherapeutic efficacy against DMBA- induced breast cancer.

**Keywords:** Taurine, DMBA, breast cancer, glycolytic enzymes, gluconeogenic enzymes

**A R T I C L E I N F O**

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## 1. Introduction

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death among females.[1] Environmental factors, of either biological or chemical origin, may act as initiators, promoters, or both of carcinogenesis.

Polycyclic aromatic hydrocarbons (PAH) especially DMBA, is one of the most potent skin and breast carcinogen[2] DMBA is well established as a highly potent carcinogen. It is absorbed through the skin and respiratory and intestinal tracts; and by intravenous and intraperitoneal injection, ingestion, and inhalation.[3] DMBA undergoes 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.[4] 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.[5] In healthy humans, dietary foodstuffs are the main source of taurine high concentrations is found in animal source whilst undetectable in vegetables. Methionine and cysteine are precursors of taurine, however synthesis ability varies widely amongst species. In the last decade it has been widely used in the field of oncology as a chemo protective agent against different types of cancer.[6-8]

The aim of the present study is to assess the activities of key glycolytic and gluconeogenic enzymes during breast cancer and to determine the effect of taurine upon treatment.

## 2. Experimental

**Materials**

7,12-dimethyl benz[a]anthracene (DMBA) and taurine (both with 99% purity) were purchased from M/s.Sigma chemicals, St. Louis, USA. All other chemicals were of analytical grade procured from M/s. SRL Chemicals Pvt. Ltd., Mumbai, India.

**Animals**

Healthy Female Sprague – Dawley rats, 6-8 weeks of age and weighing about 150-180 g were used. The animals were procured from Central Animal House Facility, Dr. ALM PG IBMS, University of Madras, Taramani, Chennai -600 113, India. Rats were acclimatized to laboratory condition with 12 hrs light/dark cycle under constant temperature and humidity and were given ad libitum access to balanced diet (Gold Mohor rat feed, M/s. Hindustan Lever Ltd., Mumbai. The Institutional Animal Ethical Committee (IAEC NO. 01 /19 /2012) approved this Research work.

**Experimental design**

Experimental animals were divided into four groups of six rats each as follows.

**Group I:** Normal control animals fed with standard diet and pure drinking water

**Group II:** Animals treated with a single dose of 7,12-dimethylbenz[a] anthracene (DMBA) (25mg /kg b.wt) in 1.0 ml olive oil by gastric intubation to induce breast cancer

**Group III:** Breast cancer bearing animals were post treated with Taurine (100mg/ kg b.wt in pure drinking water by gastric intubation) after the 10th week of DMBA administration and continued upto 15th week9

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

At the end of the experimental period, animals were sacrificed by cervical decapitation under ether anesthesia and breast tissues were excised immediately and washed with ice-cold saline. A 10% homogenate of the washed tissue (breast) was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 12,000 x g for 30 min in a refrigerated high-speed centrifuge at 4oC. Blood was also collected and the serum was separated for other estimations. The following biochemical estimations were carried out in the supernatant and in the serum.

**Biochemical analysis**

Estimation of protein and phosphorus

The protein content was estimated by the method of Lowry et al., 10 inorganic phosphate was estimated by the method of Fiske and Subbarow.11

**Assay of glycolytic enzymes**

Hexokinase was assayed by the method of Branstrup et al.,12 phosphoglucoisomerase by the method of Horrocks et al. 13 and aldolase by the method of King et al.14

**Assay of gluconeogenic enzymes**

The activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase was determined. 15

**Data analysis**

All data were expressed as mean ± S.D for six rats. The results were computed statistically (SPSS Software Package) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. ($P < 0.05$) was considered significant.

## 3. Results and Discussion

Results

Table 1 shows the activities of breast and liver glycolytic enzymes in control and experimental group of animals. In breast cancer animals (group II), there was markedly ($P < 0.05$) increased activities of key glycolytic enzymes hexokinase, phosphoglucoisomerase and aldolase when compared to group I control animals. Taurine treatment significantly ($P < 0.05$) restored the activities of above glycolytic enzymes to near normalcy in Group III animals when compared with cancer bearing animals of group II.

Fig. 1 represents the activities of liver gluconeogenic enzymes in control and experimental group of animals. Breast cancer challenged animals (group II) showed significantly decreased ($P < 0.05$) activities of key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase. The activities of all the above gluconeogenic enzymes were significantly ($P < 0.05$)

increased to near normalcy in taurine post treated Group III animals when compared to group II breast cancer animals.

**Discussion**

Studies on experimental cancers have shown that metabolic alterations occurring in the tumors are often accompanied by the changes in the activities of various enzymes, including key enzymes of glucose metabolism. [16,17] Ability to perform a high rate of glycolysis, is among the most characteristic biochemical phenotype for animal and human cancers. Rapidly growing, highly malignant tumor cells can obtain upto 60% of their total ATP production from glycolysis. [18] The degree of elevation of these glycolytic enzymes is directly related to the extent of morphological differentiation and growth rate of cancers.[19] Hexokinase, the first enzyme of the glycolytic pathway plays a pivotal role in glucose metabolism in transformed cells and its activity, mRNA levels and transcription rate are strikingly increased in tumor cells relative to normal cells.[20] Hence the glycolytic capacity of cancer cells depends mostly on hexokinase activity for its metabolic fuel. Phosphoglucoisomerase serves as a good index of tumor growth and is significantly elevated in cancerous conditions.[21] It is reported that phosphor glucoisomerase is an indicator of metastatic growth and was elevated in patients with neoplasms especially after metastasis.[22] Aldolase, the other key enzyme of glycolysis was also found to be elevated in cancer bearing animals and the elevated activity of aldolase may be due to cell impairment and necrosis in cancer bearing animals.[23] Levels of enzymes in the gluconeogenesis pathway were drastically reduced, along with transcription factors involved in the expression of the genes encoding those enzymes in breast cancer.[24] Since glucose-6-phosphatase is also essential for liver cells to convert glycogen (the storage form of glucose) to glucose, suppression of this enzyme can block all pathways leading to glucose production by the liver.[24] These findings are in accordance with our present study, as we noticed elevations in the activities of key glycolytic enzymes hexokinase, phosphoglucoisomerase, aldolase and decline in the activities of gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase in breast cancer bearing animals. The gradual decreases in the activity of glycolytic and increase in the activity of gluconeogenic enzymes' levels during taurine treatment correspond to a return of the tumor towards near normal state. This could be attributed by the potent chemotherapeutic activity of taurine. Thus, taurine has a potent beneficial influence on modulating the energy metabolism during breast cancer that supports its chemotherapeutic property against DMBA induced breast carcinoma.

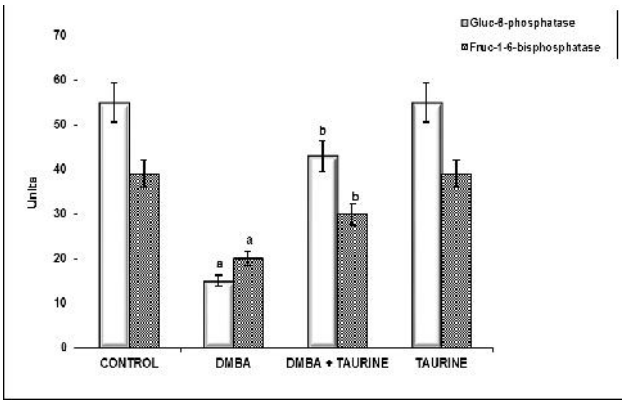


**Figure 1:** Shows the activities of liver gluconeogenic enzymes in control and experimental group of animals.

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P<0.05, as compared with [a]Group I, [b]Group II.

**Units:** Glucose-6-phosphatase, fructose-1,6-bisphosphatase -nmoles of inorganic phosphate liberated/ min/mg protein.

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**Table 1:** Activities of key glycolytic enzymes in the breast and liver of control and experimental group of animals

| Parameters | Group I (Control) | Group II (DMBA) | Group III (DMBA+Taurine) | Group IV (Taurine) |
|---|---|---|---|---|
| **Breast:** Hexokinase | 27.6 ± 2.8 | 76.3 ± 7.1 [a] | 39.5 ± 3.2[b] | 26.6 ± 2.2 |
| Phosphoglucoisomerase | 14.3 ± 1.42 | 58.7 ± 5.0 [a] | 21.0 ± 2.1 [b] | 14.8 ± 1.38 |
| Aldolase | 13.5 ± 1.31 | 66.1 ± 6.5 [a] | 19.4 ± 1.81 [b] | 14.5 ± 1.5 |
| **Liver:** Hexokinase | 44.2 ± 4.3 | 93.2 ± 9.2 [a] | 56.5 ± 5.1 [b] | 43.2 ± 4.2 |
| Phosphoglucoisomerase | 37.3 ± 3.6 | 87.5 ± 8.6 [a] | 43.3 ± 4.5 [b] | 37.9 ± 3.6 |
| Aldolase | 40.7 ± 4.1 | 89.0 ± 8.2 [a] | 59.7 ± 5.8 [b] | 40.9 ± 4.0 |

Results are expressed as mean ± S.D for six rats in each group. Statistical significance at P<0.05, as compared with aGroup I, bGroup II. **Units:** Hexokinase – nmoles of glucose-6-phosphatase liberated/mg protein; phosphoglucoisomerase – nmoles of fructose liberated/mg protein; aldolase – nmoles of glyceraldehyde liberated/mg protein.

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