

RESEARCH ARTICLE

Therapeutic effect of *Ananus comosus* peel on breast cancer induced by 7, 12- dimethylbenz(α)anthracene

M. Kalaiselvi, D. Gomathi, G. Ravikumar, K. Devaki & C. Uma

Abstract

Ananus comosus (L.) Merr. (pineapple), a tropical species with distinctive juicy fruits, has demonstrated a wide variety of biological activities which makes it a good plant source for the treatment of many oxidative stress mediated diseases. The present study was aimed to evaluate therapeutic potential of *A. comosus* by assaying the activities of liver marker and lysosomal enzymes in 7, 12 dimethylbenz(α)anthracene induced breast cancer bearing rats. Animals were divided into five groups of six numbers each. Group 1 served as control, Group 2 had rats induced with mammary carcinogenesis by 7, 12 dimethylbenz(α)anthracene, Group 3 and 4 were treated with plant extract and tamoxifen and Group 5 had animals treated with plant extract alone. All the animals were sacrificed after 120 days of experimental period, serum and mammary tissue were used for the analysis of liver marker and lysosomal marker enzymes using standard protocols. The rats induced with breast cancer by DMBA showed altered levels of liver markers and lysosomal marker enzymes in serum and mammary tissue. On the other hand, oral administration of ethanolic extract of *A. comosus* peel and standard drug tamoxifen to breast cancer bearing rats for 30 days, helped to bring back those marker levels to near normal whereas upon treatment with plant extract alone did not

produce any toxic effect. The histology of mammary tissues inevitably supports the biochemical alterations and this was due to the interaction of *A. comosus* peel through the induction or inhibition of metabolism and also the modulating property in the marker and lysosomal enzymes.

Key words: *Ananus comosus*; breast cancer; liver marker and lysosomal marker enzymes; modulating property.

Introduction

Breast cancer is the second most prevalent cancer worldwide and their incidence increases gradually (Vinothini *et al.*, 2009). Annually, 910,000 new patients are diagnosed with breast cancer and 376,000 women die from the disease. The etiology of breast cancer is multifactor and the risk factors include early menarche, late menopause, nuliparity, and late age at first birth, postmenopausal obesity, extended use of oral contraceptives, hormone replacement therapy, family history and previous benign breast disease (McPherson *et al.*, 2000).

Exposure to environmental pollutants such as polycyclic aromatic hydrocarbon (PAH) is associated with the development of numerous cancers in human (Nebert *et al.*, 2004). PAHs are products formed by incomplete combustion of organic matter. They are the most important class of carcinogens implicated in the development of mammary carcinogenesis in humans (Tsuchiya *et al.*, 2005). Polycyclic aromatic hydrocarbons are universally present in the atmosphere, industrial and domestic oil furnaces, gasoline, char-broiled meat, tobacco smoke and diesel exhaust. The resulting PAH may be released to the environment in airborne particulates, or in solid or liquid by-products of the pyrolytic process (Al-Attar, 2004). Once these chemicals are consumed, our body will metabolize and transform these compounds into DNA-attacking mutagens (Chan *et al.*, 2002).

Enzymatic activation of PAHs leads to the generation of active oxygen species like peroxides and superoxide anion

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radicals, which induce oxidative stress in the form of lipid peroxidation. Uncontrolled increase of these highly reactive molecules lead to free radical mediated chain reactions of which indiscriminately damage proteins, lipids and DNA resulting at last resort in cell death (Lenaz, 2001). Higher amounts of PAH-DNA adducts have been found in human breast tumors than in normal breast tissues (Nandakumar *et al.*, 2011). 7, 12-dimethylbenz(α)anthracene (DMBA) is one of the polycyclic aromatic hydrocarbon chemical groups. DMBA is a well known cytotoxic, carcinogenic, mutagenic and immunosuppressive agent (Buters *et al.*, 2003). Experimental studies showed that DMBA-induced skin, oral, mammary and ovarian tumors (Han *et al.*, 2002; Li *et al.*, 2003; Suzuki *et al.*, 2003).

Many plant extracts and plant products have been identified as good protectors against the free radicals by triggering antioxidant gene expression. For that account, natural antioxidants from plant sources have been viewed as promising therapeutic drugs (Mittal *et al.*, 2001). For example, the peel of *Ananas comosus* (L.) Merr. (Bromeliaceae) is well known in ethnomedicine and tradition systems of medicine for the treatment and management of various ailments such as malaria and typhoid. The juice from the fruit is used in treating certain types of cancer, while the stems are good in treating arthritis, swellings, wounds and strains, blood clots, indigestion, respiratory tract infections and reported to boost immune system (Okafor *et al.*, 2011). Hence, considering the significance of *A. comosus*, an attempt has been made to identify the protective effect of its fruits' peel in 7, 12 dimethyl(α)benzanthracene (DMBA) induced mammary carcinogenesis in Wistar albino rats.

Materials and methods

Collection of Plant material

Fresh pineapple plant was collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. P. Sathyanarayanan, Botanical Survey of India (BSI), Tamilnadu Agricultural University (TNAU) Campus, Coimbatore (India) and the voucher specimen number is BSI/SRC/5/23/2011/Tech-515. Fresh peel was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

Sample Extraction

Dried plant powder (100g) was extracted in 500ml of ethanol in a water shaker for 72hrs. Extraction was done repeatedly with the same solvent till a clear colorless solvent was obtained. The extract obtained was evaporated to dryness by using a rotary vacuum evaporator at 40-50°C and stored at 4°C in an air tight container.

Chemicals

7, 12 dimethylbenz(α)anthracene, oxidized glutathione and reduced glutathione were purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade.

Animals

Female Sprague Dawley rats weighing 180 ± 10 g were purchased from Karpagam University, Coimbatore and housed in plastic cages. The animals were maintained under controlled environmental condition on alternative 12h dark/light cycle. Commercial pelleted feed and water were given to animals. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by IAEC, Government of India for the use of Sprague Dawley rats as an animal model for cancer activity.

Experimental design

The animals were divided in to five groups of 6 numbers each. Group 1 animals served as control, Group 2 animals were administration with 25 mg/rat of DMBA in 1.0 ml of olive oil by gastric incubation, to induce breast cancer. After 90 days of tumor induction Group 3 and Group 4 animals were treated with ethanolic extract of *A. comosus* peel (250 mg/kg body weight) and standard drug tamoxifen (20 mg/kg body weight) for 30 days. In Group 5, animals were treated with ethanolic extract of the plant extract alone for 30 days.

Collection of blood and tissues from the animals

After the experimental period of 120 days, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by decapitation and serum was separated by centrifugation for 10 min at 1500 rpm and used for the estimation of various biochemical estimations.

Preparation of tissue homogenate

A 10% homogenate of the washed mammary tissue was prepared with 0.1 M Tris-HCl buffer pH 7.4 at 4°C in a potter homogenizer, fitted with a Teflon plunger at 600 rpm for 3 min. The filtrate was used for further biochemical analysis. The homogenates were used to assay the enzymes activities. A section of mammary tissue were preserved in 10% formalin and used for histopathological studies.

Biochemical analysis

Mammary tissue homogenates and serum were used to analyse the liver function marker enzymes like Aspartate transaminase and Alanine transaminase following Reitman & Frankel (1957), Alkaline phosphatase (King & Armstrong, 1943), Lactate dehydrogenase (King, 1965b),

5'Nucleotidase (Campbell, 1962), γ -glutamyl transferase (Persijn & Vander Slik, 1976) and lysosomal enzymes such as Acid Phosphatase (King, 1965a), β -glucuronidase and β -galactosidase (Kawai & Anno, 1971), Cathepsin D (Sapolsky *et al.*, 1973) and Cathepsin B following the method of Barrett (1972).

Statistical analysis

The results were expressed as mean \pm standard deviation (S.D). Difference between the groups was assessed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS 10.0 version software package for windows. The values were considered statistically significant if p value was less than 0.05 ($p < 0.05$) (Dunn, 1974).

Results

Effect of *A. comosus* on marker enzymes in control and experimental animals

The analysis of marker enzymes can be used as an indication of neoplastic condition and therapy. The effect

of *A. comosus* peel extract on the levels of transaminases, alkaline phosphatase, lactate dehydrogenase, 5'-nucleotidase and γ -glutamyl transpeptidase in serum and mammary tissue of control and experimental animals are presented in Tables 1 and 2. There was a significant ($p < 0.05$) increase in levels of marker enzymes in serum and decrease of those levels in tissues were observed in Group 2 cancer bearing animals when compared to Group 1 control animals. Conversely, these levels were significantly reverted back to near normal in Group 3 and Group 4. *A. comosus* and tamoxifen treated animals and was comparable to Group 2 cancer bearing animals ($p < 0.05$). However, there was no significant changes in the activity of marker enzymes were observed in Group 5 plant extract alone treated animals when compared to the Group 1 control animals.

Effect of *A. comosus* on lysosomal enzymes in control and experimental animals

The effect of ethanolic extract of *A. comosus* peel on lysosomal enzyme activities in serum, liver, kidney and mammary tissues of control and experimental animals are illustrated in the Tables 3 and 4. The activities of lysosomal

Table 1. Effect of *Ananus comosus* on the activities of marker enzymes in serum of control and experimental animals

Groups	AST	ALT	ALP	LDH	5'NT	γ -GT
Group 1	45.35 \pm 2.38 ^a	24.3 \pm 1.99 ^a	45.19 \pm 1.29 ^a	72.19 \pm 1.41 ^a	1.57 \pm 0.59 ^a	3.20 \pm 1.24 ^a
Group 2	66.91 \pm 1.77 ^c	54.41 \pm 0.12 ^c	136.85 \pm 1.56 ^c	109.47 \pm 1.02 ^c	5.18 \pm 0.51 ^c	4.57 \pm 1.78 ^c
Group 3	52.53 \pm 1.76 ^b	41.62 \pm 1.02 ^b	93.60 \pm 1.62 ^b	90.91 \pm 1.05 ^b	4.28 \pm 0.55 ^b	3.80 \pm 1.65 ^{bc}
Group 4	48.94 \pm 2.02 ^{ab}	43.81 \pm 1.30 ^b	80.04 \pm 1.06 ^b	83.07 \pm 1.20 ^b	3.87 \pm 0.86 ^b	3.60 \pm 1.69 ^b
Group 5	45.17 \pm 2.68 ^a	24.74 \pm 1.81 ^a	44.54 \pm 1.16 ^a	72.68 \pm 1.55 ^a	1.50 \pm 0.71 ^a	3.00 \pm 1.82 ^a

Values are expressed as Mean \pm SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT). Units: AST, ALT, LDH- μ moles of pyruvate liberated/L; ALP- μ moles of phenol liberated/L; 5'NT - μ mol of phosphorus liberated/ L; γ -GT - μ moles of *p*-nitroanilide liberated/ L.

Table 2. Effect of *Ananus comosus* on the activities of marker enzymes in mammary tissue of control and experimental animals

Groups	AST	ALT	ALP	LDH	5'NT	γ -GT
Group 1	25.46 \pm 1.66 ^a	26.82 \pm 0.71 ^a	32.91 \pm 1.69 ^a	67.90 \pm 1.29 ^a	2.52 \pm 0.19 ^{ab}	4.39 \pm 1.52 ^a
Group 2	16.62 \pm 1.33 ^c	17.33 \pm 0.69 ^c	20.76 \pm 1.35 ^c	43.74 \pm 1.83 ^c	1.09 \pm 0.09 ^b	2.98 \pm 1.82 ^b
Group 3	19.25 \pm 0.07 ^{bc}	19.18 \pm 0.99 ^{bc}	24.72 \pm 1.40 ^b	50.23 \pm 1.90 ^b	1.45 \pm 0.21 ^a	3.36 \pm 0.70 ^{ab}
Group 4	24.34 \pm 1.86 ^b	25.71 \pm 1.11 ^b	28.37 \pm 0.68 ^b	58.93 \pm 1.94 ^b	1.98 \pm 0.14 ^a	4.05 \pm 1.52 ^a
Group 5	25.39 \pm 1.78 ^a	26.76 \pm 0.99 ^a	32.52 \pm 1.74 ^a	67.71 \pm 1.69 ^a	2.46 \pm 0.15 ^{ab}	4.40 \pm 1.51 ^a

Values are expressed as Mean \pm SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT). Units: AST, ALT, LDH- μ moles of pyruvate liberated/min/mg protein; ALP- μ moles of phenol liberated/ min/mg protein; 5'NT - μ mol of phosphorus liberated/ min/mg protein; γ -GT - μ moles of *p*-nitroanilide liberated/min/mg protein.

enzymes were significantly deprived in serum of Group 2 cancer bearing animals ($p < 0.05$) whereas in tissues those levels were increased when compared to Group 1 control animals. On the contrary, the lysosomal enzyme levels were significantly brought towards normal range ($p < 0.05$) in Group 3 *A. comosus* and Group 4 tamoxifen treated

drug tamoxifen showed many focals of regressed tumor and scattered few normal lobules (Group 4). However, rats treated with ethanolic extract alone (Group 5) showed normal morphological appearances of mammary tissues with normal lobular site when compared to Group 1 control rats.

Table 3. Effect of *Ananus comosus* on the activities of lysosomal marker enzymes in serum of control and experimental animals

Groups	ACP	β -GLU	β -GAL	CAT-D	CAT-B
Group 1	90.22 \pm 1.24 ^a	57.19 \pm 1.04 ^a	4.22 \pm 0.19 ^a	19.84 \pm 0.59 ^a	40.84 \pm 0.62 ^a
Group 2	55.74 \pm 1.58 ^c	29.53 \pm 1.09 ^c	3.30 \pm 0.16 ^{bc}	10.02 \pm 0.83 ^c	27.02 \pm 0.14 ^c
Group 3	62.34 \pm 1.80 ^b	40.15 \pm 0.37 ^b	3.54 \pm 0.32 ^b	13.67 \pm 0.19 ^b	35.67 \pm 0.52 ^b
Group 4	88.93 \pm 1.31 ^a	47.41 \pm 1.51 ^b	3.93 \pm 0.38 ^b	17.18 \pm 1.07 ^{ab}	39.18 \pm 0.43 ^{ab}
Group 5	90.13 \pm 2.24 ^a	57.06 \pm 1.30 ^a	4.20 \pm 0.47 ^a	19.79 \pm 1.16 ^a	40.79 \pm 0.71 ^a

Values are expressed as Mean \pm SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT). Units: ACP- μ moles of phenol liberated/L; β -Glu, β -Gal- μ moles of *p*-nitrophenol formed/ L; CAT-D- μ mole of tyrosine liberated/ L; Cathepsin B- μ mole of *p*-nitroaniline liberated/ L.

Table 4. Effect of *Ananus comosus* on the activities of lysosomal marker enzymes in mammary tissue of control and experimental animals.

Groups	ACP	β -GLU	β -GAL	CAT-D	CAT-B
Group 1	28.32 \pm 0.54 ^a	41.53 \pm 1.36 ^a	13.47 \pm 1.73 ^a	12.84 \pm 0.89 ^a	20.23 \pm 1.17 ^a
Group 2	39.97 \pm 0.73 ^c	77.46 \pm 1.64 ^c	22.72 \pm 0.72 ^b	21.02 \pm 0.53 ^c	29.73 \pm 0.52 ^c
Group 3	34.19 \pm 0.58 ^{bc}	61.45 \pm 1.85 ^b	16.96 \pm 0.69 ^a	18.67 \pm 0.49 ^b	26.40 \pm 0.48 ^b
Group 4	30.36 \pm 0.55 ^{ab}	49.67 \pm 1.51 ^a	16.56 \pm 1.11 ^a	16.18 \pm 1.27 ^{ab}	24.52 \pm 0.69 ^b
Group 5	28.29 \pm 1.75 ^a	39.008 \pm 1.77 ^a	13.43 \pm 1.82 ^a	12.36 \pm 1.46 ^a	20.27 \pm 1.09 ^a

Values are expressed as Mean \pm SD for six animals. Values not sharing common Superscript letters (a-c) differ significantly at $p < 0.05$ (DMRT). Units: ACP- μ moles of phenol liberated/min/mg protein; β -Glu, β -Gal- μ moles of *p*-nitrophenol formed/min/mg protein; CAT-D- μ mole of tyrosine liberated/min/mg protein; Cathepsin B- μ mole of *p*-nitroaniline liberated/min/mg protein.

animals when compared with Group 2 cancer bearing rats. Whereas, no significant alterations were observed in Group 5 ethanolic extract alone treated animals when compared to Group 1 control animals.

Histopathological observation

The histopathological changes in mammary tissues of control and experimental animals were shown in Fig 1. The histopathological observation of mammary tissue in control Group (Group 1) showed normal architecture of tissues with more number of muscle bundles. Group 2 breast cancer bearing rats showed pleomorphism and hyperchromatic nuclei. The cancer induced rats were treated with ethanolic extract of *A. comosus*, the Group 3 showed hyperchromatic nuclei and few sites of regressed tumor. Cancer induced rats were treated with standard

Discussion

Effect of A. comosus on marker enzymes in control and experimental animals

Biochemical marker enzymes are used to screen particularly cancer conditions for differential diagnosis, prognosis, monitoring the progress and for assessing the response to therapy (Mc-Intrye & Rosalki, 1992). These enzymes are more inimitable and changes in their activities reflect the effect of proliferation of cells with growth potential and its metabolic turnover. The rise in their activities is shown to be a good correlation with the number of transformed cells in cancer conditions (Kamdem *et al.*, 1982). In cancer conditions, there will be a disturbance in the transport function carried out by cell organelles including hepatocytes, resulting in the leakage

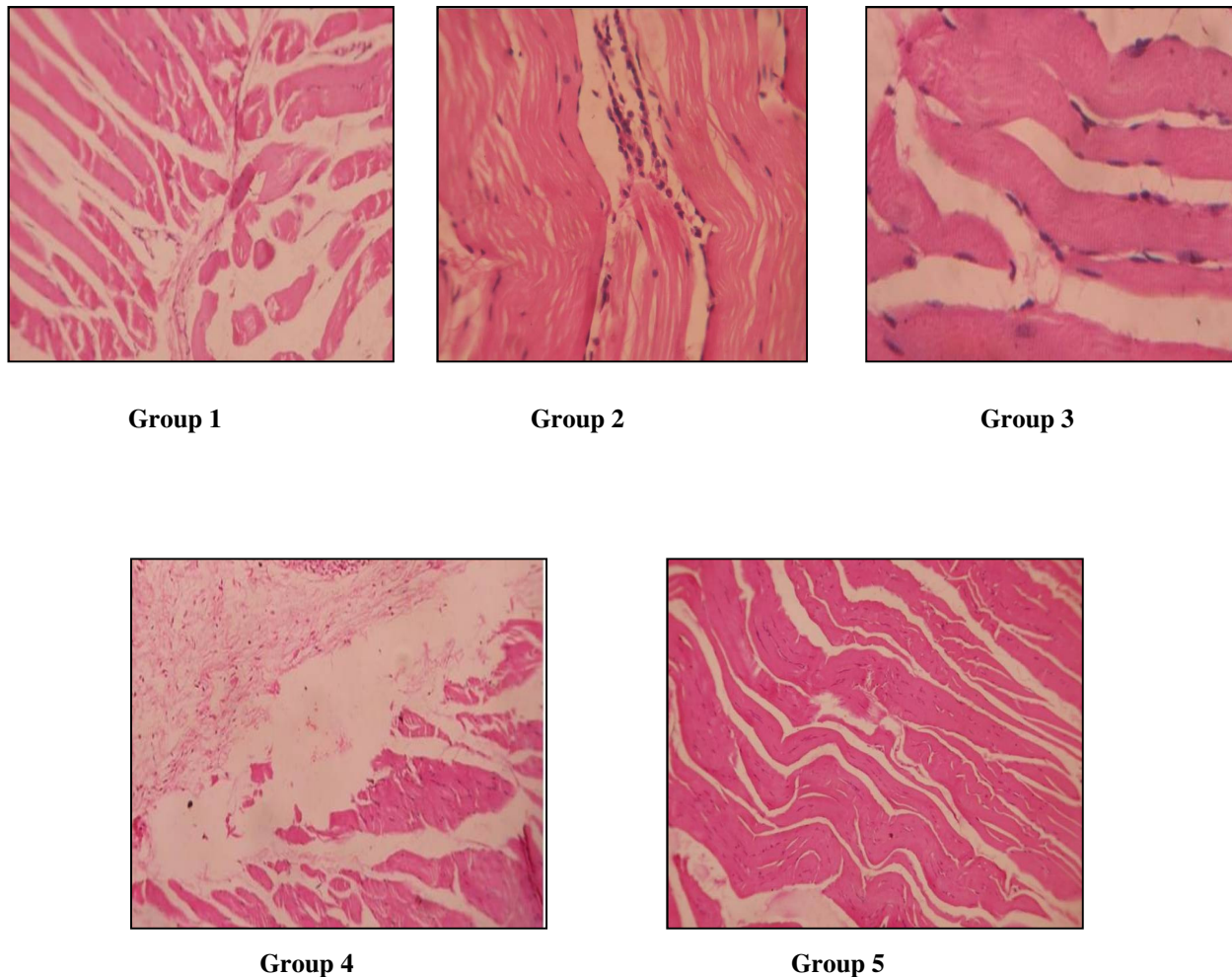


Fig 1. Histopathological examination of Mammary tissue (X100 Magnification)

of enzymes due to altered permeability of plasma membrane and thereby causing a decreased level of these marker enzymes in the cells and increased level in serum. The structural integrity of the cells has been reported to be damaged in toxicity induced animals and this results in cytoplasmic leakage of enzyme into the blood stream (El-Beshbihiy, 2005).

The role of transaminases in biological system is well known. These enzymes serve as the index of tissues, cell injury and can be used to identify or confirm the carcinogenic conditions. Both ALT and aspartate transaminase (AST) constitute a Group of enzymes that catalyze the interconversion of amino acids and α -ketoacids by transfer of amino groups. The α -ketoglutarate or L-glutamate couple serves as an amino Group acceptor and donor pair in amino-transfer reactions. ALT catalyses the transfer of the amino Group from alanine to α -ketoglutarate to form glutamate and pyruvate, while AST catalyses the transfer of the amino Group from aspartate

to α -ketoglutarate to form glutamate and oxaloacetate.

Increased levels of enzyme activity in the extracellular fluid or plasma is a sensitive indicator of even minor cellular damage since the levels of these enzymes within the cell exceed those in the extracellular fluids by more than three orders of magnitude. In this connection, the measurement of enzyme activities in the serum and tissues is frequently used as a diagnostic tool in human medicine. Most research on the use of serum transaminase activities as an indicator of tissue damage has, therefore, been performed on human, since both ALT and AST activities are of great clinical significance. In the present investigation, the elevated and decline levels of transaminases such as aspartate aminotransaminase (AST) and alanine transaminases (ALT) were observed in serum and in tissues of cancer bearing animals. The increased activities of these enzymes in serum may be due to leakage of enzymes from the neoplastic cells into the blood or may be due to the release of enzyme from normal tissue by

tumor or may be due to the possible effect of tumor on remote tissue leading to the loss of its enzyme and release into the blood (Moss *et al.*, 1986).

Transaminases, an important class of enzyme that link carbohydrate and amino acid metabolism and establish a relationship between the intermediate of TCA cycle and amino acids synthesis (Rodwell, 1996). Our investigation also showed the increased levels of ALT and AST in serum of cancer bearing animals, which indicates severity of tissue damage by the DMBA and this is well in accordance with the previous findings of Van der Oost *et al.* (2003).

Phosphatase is a Group of non-specific membrane bound enzymes, which hydrolyses esters of orthophosphates in alkaline condition (ALP) and alkaline phosphatase. In acute injury, lysosomal membrane liberates degradative enzymes followed by destruction of cell membrane. ALP's alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. This enzyme activity is used as a specific tumor marker during diagnosis and in the early detection of cancer (Kobayashi & Kawakubo, 1994). Moreover, ALP is mainly involved in the transfer of metabolites across cell membrane, in protein synthesis and glycogen metabolism. Elevated level of ALP activity in cancer bearing animals may be due to altered synthesis of certain enzymes as in other cancerous condition. ALP and AST are the most sensitive markers, which are considered as diagnostic tool in malignant diseases (Sharma *et al.*, 1995). In the present study, ethanolic peel extract of *A. comosus* mediated suppression of the elevated levels of ALP in DMBA induced rats suggest the possibility of the drug being able to stabilize the plasma membrane.

LDH is a tetrameric enzyme and is recognized as potential tumor marker especially for solid tumors in assessing the progression of proliferating malignant cells (Lippert *et al.*, 1981). It was reported that elevation and deprived levels of serum and tissue LDH activity is common in myocardial infarction, hepatitis and neoplastic disease. The elevated activity of LDH may be due to over production by tumor cells or it may be due to the release of isoenzymes from destroyed tissues. In this connection, our findings of the present study are consistent with the earlier reports of Helmes *et al.* (1998). Conversely, administration of the *A. comosus* peel extract, control LDH levels thereby decreasing permeability of the membrane and renders protection to membrane integrity.

5' nucleotidase (5'NT) is present at the bile canalicular and sinusoidal surface of plasma membrane of hepatocytes. 5'NT hydrolyze nucleotides with a phosphate Group on carbon atom of the ribose sugar and was found to be elevated in cancer bearing animals. In addition, 5'NT is also used as a diagnostic tool for liver injury (Fredericks *et al.*, 1990). In the present investigation, an increased activity of 5'NT activity was observed in breast cancer

bearing animals. The elevation of the marker enzyme may be correlated with the progression of the malignancy and also due to the hepatic cell damage, which may cause leakage of 5'nucleotidase into the circulation. In this connection, Walia *et al.* (1995) reported that higher activities of 5'NT were observed in cancer of breast tissues of humans and this is consistence with our present findings. Conversely, treatment with hesperidin significantly decreased the activity of the enzyme 5'NT. This may be due to the recouplement of the hepatic cell damage by the *A. comosus* through its free radical quenching capability.

Gamma glutamyl transpeptidase (γ -GT) is a membrane bound enzyme located on the external surface of cells exhibiting large secretory or detoxification activities (Yao *et al.*, 2000). The enzyme level is found to be raised in serum, liver condition like cholestasis and bile duct necrosis and is also considered to be one of the best indicators of liver damage. A variety of substances including xenobiotics have been reported to become a substrate of γ -GT after their conjugation to GSH, occurring mainly in the liver. The depletion of GSH may also induce hepatic γ -GT activity through an increased synthesis of its mRNA. In the present investigation, the γ -GT activity was found to increase in cancer bearing animals. This is well in accordance with the previous finding of Buckpitt *et al.* (1979) & Makpol *et al.* (1997). On the other hand, ethanolic extract normalizes the γ -GT enzyme level during treatment. This may be due to the cytoprotective nature of extract which might have helped in stabilizing the cell membrane of the hepatic and other tissue cells and also prevented the loss of functional integrity of the cell membrane.

Effect of A. comosus on lysosomal enzymes in control and experimental animals

Lysosomal enzymes are implicated in tissue remodeling, which occurs during the physiological involution of the uterus, prostate gland, and the mammary gland (Halaby, 2002). Lysosomes contain digestive enzymes capable of degrading all macromolecules such as proteins, nucleic acids, lipids and carbohydrates. These enzymes can trigger apoptosis in human breast carcinoma cells as well as in rat mammary gland cells (Halaby *et al.*, 2004a). Increased production of free radicals in cancer condition led to deterioration of membrane, which resulted in the leakage of enclosed enzymes from the lysosomal sacs (Geetha, 1993). In this connection, Halaby *et al.* (2004b) are of the opinion that the increased level of lysosomal enzymes could reflect in the secretion of enzyme source by the tumor. In the present study, elevated levels of lysosomal enzymes were found in breast cancer bearing animals. Increased activity of acid phosphatase is also one of the direct or indirect reasons for elevation of lysosomal enzymes in cancer condition (Blicharski *et al.*, 1983).



β -D-glucuronidase is a sensitive indicator of lysosomal integrity (Beem *et al.*, 1987). It is released due to the presence of free radicals and as a cellular hydrolase, this enzyme has the ability to degrade cell organelles and digest cell materials. During the cancer conditions β -D-glucuronidase and β -D-galactosidase were observed in breast cancer patients (Calvo *et al.*, 1982). In the present study, the increased levels of lysosomal enzymes were observed in breast cancer bearing animals which is consistent with the earlier findings of aforesaid.

Cathepsin-D is an aspartic endoprotease which is ubiquitously expressed in lysosomes of all tissues and catalytically active at acidic pH values that vary according to substrates, but is mostly found in intracellular vesicles, lysosomes, phagosomes and late endosomes. Cathepsin-D is over-expressed and hyper-secreted by epithelial breast cancer cells, possibly through extracellular interaction with a yet-unknown cell surface membrane receptors and serves as marker for prognosis (Cavaillès *et al.*, 1993). It is also a key mediator of apoptosis induced by stimuli such as interferon (IFN) gamma, FAS or APO, TNF α , oxidative stress and DNA damaging agents. A large number of clinical studies have been associated with high cathepsin-D concentrations and increased risk of breast cancers and subsequent lead to metastasis (Liaudet-Coopman *et al.*, 2006). In the present investigation, increased level of cathepsin-D was observed in the DMBA-induced breast cancer bearing animals. This is well in accordance with the earlier findings of Rochefort *et al.* (2000). Conversely, the restoration of lysosomal enzymes upon ethanolic extract treatment in breast cancer bearing animals may be due to the membrane stabilizing property of *A. comosus* on lysosomal membranes, which protects the rapid leakage of enzymes and obstruct the rise in the enzymatic activity.

Conclusion

The ethanolic extract of *A. comosus* significantly ameliorates the changes on marker enzymes and lysosomal enzymes at the concentration of 250 mg/kg body weight. Therefore, it can be concluded that the *A. comosus* possesses antineoplastic activity by modulating the energy reservoir of the cell and also by maintaining the oxidative phosphorylation. The membrane stabilizing property of *A. comosus* on lysosomal membrane protects the rapid leakage of enzymes and obstructs the rise in the enzymatic activity and also modulates the hepatic drug-metabolizing enzymes through its antiradical capacity. In addition, the histopathological analysis of mammary tissues inevitably supports the biochemical alterations and thus proves the antineoplastic property of the *A. comosus* peel extract.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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