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Shemamruthaa, a herbal formulation modulates xenobiotic and carbohydrate metabolizing enzymes in 7,12-dimethylbenz[a]anthracene induced breast cancer in rats

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Abstract

There is an increasing interest in identifying naturally occurring potent preventive and therapeutic agents for cancer. *Shemamruthaa*, a phytochemical formulation was evaluated for the first time with a view to potentiate more intense anticancer property. Adult female Sprague-Dawley rats (8-weeks-old) were induced for mammary carcinoma and treated with *Shemamruthaa* (SM) orally by gastric intubation for 14 days after 3 months of induction period (Group III). The status xenobiotic metabolizing enzymes, glycolytic and gluconeogenic enzymes were analysed in control and experimental rats. Our findings revealed that the SM formulation has potential to induce Phase-II enzyme activities, associated mainly with carcinogen detoxification and inhibit the Phase I enzyme activities. The activities of glycolytic and gluconeogenic enzymes were significantly brought back to near normal levels in SM treated animals. The results demonstrated unequivocally the effect of SM on inhibition of tumor progression by altering xenobiotic metabolizing enzymes and restoring energy metabolism.

Keywords: Mammary carcinoma, *Shemamruthaa* (SM), Xenobiotic-metabolizing enzymes, Glycolytic enzymes

Introduction

Breast cancer is the most frequently diagnosed cancer and the foremost cause of cancer death in females worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 (Ahmedin Jemal et al. 2011). Human breast cancer is associated with a number of factors, including ionizing radiation, socioeconomic status, hormonal factors, diet, genetics and polycyclic aromatic hydrocarbons (PAHs), the ubiquitous environmental pollutants and air pollution (Rundle et al. 2000).

The initiation of many tumors results from damage to DNA by electrophilic carcinogen metabolites, or by reactive oxygen species (ROS) that arise during carcinogen metabolism or endogenous cellular processes. Mammalian cells have evolved multiple and elaborate mechanisms for protection against such toxic insults. Phase II enzymes (e.g. glutathione-s- transferases (GST), UDP-Glucuronyl transferase, NAD(P)H: quinone reductase (QR), epoxide hydrolase, aldehyde reductase, and others) and high cellular levels of glutathione are the primary lines of defense against these reactive chemical species. These protective mechanisms disarm and facilitate the disposal of reactive electrophiles and oxygen species (Rundle et al. 2000).

Much recent evidences indicate that elevation of Phase II enzymes levels by inducers results in protection against chemical carcinogens. Indeed, modulation of the metabolism of carcinogens is one of the most effective and well-established strategies for protecting animals and their cells against the toxic and neoplastic effects of carcinogens. Consequently, specific modification of the human diet to increase the consumption of phytochemicals that induce Phase II enzymes is an attractive, safe and promising strategy for decreasing the risk of developing cancer (Bishayee et al. 2000, Wang and Leung 2010). Implementation of this strategy requires: (i) identification of edible plants rich in inducer activity; (ii) demonstration that such plants can raise Phase II enzymes when administered to animals; and (iii) ultimate demonstration of risk reduction in developing cancer.

The chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only dysregulation of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division. Under aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria; under anaerobic conditions, glycolysis is favoured and relatively little pyruvate is dispatched to the oxygen-consuming mitochondria

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(Hanahan and Weinberg 2011). Otto Warburg first observed an anomalous characteristic of cancer cell energy metabolism (Warburg 1956): even in the presence of oxygen, cancer cells can reprogram their glucose metabolism and thus, their energy production, by limiting their energy metabolism largely to glycolysis. The existence of this metabolic switch in cancer cells has been substantiated in the ensuing decades. Such reprogramming of energy metabolism is seemingly counterintuitive, in that, cancer cells must compensate for the ~18-fold lower efficiency of ATP production afforded by glycolysis relative to mitochondrial oxidative phosphorylation. They do so in part by upregulating glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm (Hsu and Sabatini 2008, DeBerardinis et al. 2008). An enhanced rate of the glycolysis is essential to keep pace with rapid cell division and membrane biosynthesis during tumor growth.

In the last decades, phytochemicals have attracted a growing attention as anti-cancer agents due to their ability to modulate apoptosis signalling pathways (Tosetti et al. 2009). The mixture of several crude extracts, when used in formulation, enhance the beneficial effects through synergistic amplification and diminishing any possible adverse effects and offer advantage over a single isolated ingredient (Koshimizu 1988). In this perspective, the study of herbal formulations from traditional medicine represents a challenging research field, since it has been applied for the treatment of cancers for many years (Hsiao and Liu 2010).

In our previous studies, we have reported that SM formulation (constituted dried flowers of *Hibiscus rosasinensis*, fruits of *Emblica officinalis* and honey) exerts profound anticancer activity through its role in reinstating the normal levels of glycoprotein components, revitalizing the lysosomal membrane stability (Purushothaman et al. 2012a), restoring antioxidant status (Purushothaman et al. 2012b), marker enzymes and TCA cycle enzymes (Purushothaman et al. 2013) in DMBA-induced mammary carcinoma bearing rats. In the present study, we show that SM interferes with progression of in breast cancer in rat models through induction of Phase II enzymes and inhibit the Phase I enzyme activities. SM also restores altered energy metabolism without affecting other cellular functions and body weight. Most importantly, we also provide evidence that SM consumption can delay tumorigenesis in rats.

Materials and Methods

Preparation of SM

The flowers of *Hibiscus rosasinensis* were collected from a local garden in southern part of India (Kanchepuram District, Tamil Nadu) and the pharmacognostic authentication was done by Department of Plant Sciences, University of Madras, Chennai-600 025. The fruits of *Emblica* (*P. emblica* L.), at the mature stage, were purchased commercially from the market and the rinds were carefully removed from the seeds. The flowers of *Hibiscus rosasinensis* and the rinds were air dried under shade, pulverized to fine powder using a cutting mill and mixed with pure honey in definite ratio.

Animals and Experimental Design

Adult female albino rats of Sprague-Dawley strain weighing 180±10g were provided from Central Animal House facility, University of Madras, Taramani Campus, Chennai-600 113, Tamil Nadu, India. The animals were maintained under standard conditions of humidity, temperature (25±2 ºC) and light (12 h light/dark). They were fed with standard rat pellet diet manufactured by M/s. Pranav Agro Industries Ltd., India under the trade name "Amrut" rat/mice feed and water *ad libitum*. The experiments were performed in accordance with the Institutional Animal Ethics Committee Guidelines.

The animals (female Sprague-Dawley rats, 8 weeks old) were randomly divided into four groups of six animals each: Group I served as control animals. Group II, breast cancer induced in overnight-fasted animals by a single dose of DMBA in olive oil (25 mg) by gastric intubation (DMBA-induced). Group III, mammary carcinoma was induced as in Group II, in addition, after three months; animals were treated with SM at the dose of 400 mg/ kg body weight and continued for fourteen days by gastric intubation (DMBA+ SM). Group IV, SM-treated control (400 mg/kg body wt) administered to control animals for 14 days by gastric intubation (SM control).

Gross observations and Tumor volume

The tumor incidence and changes in body weight, liver and kidney weights were measured and the weights were recorded in g. During the experimental period, i.e., Prior to sacrificing the animals, the animals were weighed, explored by inspection and palpation and the two major and perpendicular diameters of each tumor were measured with a Vanier calliper. The tumor volume (v) was measured as described by Escrich et al. (2004).

$v = 4/3\ \pi\ (d_1/2) \times (d_2/2)^2$, where d_1 and d_2 are the two diameters of the tumor ($d_1 > d_2$)

After sacrificing, the volume of each tumor was calculated using its three diameters:

$v = 4/3\ \pi\ (d_1/2) \times (d_2/2) \times (d_3/2)$; ($d_1 > d_2 > d_3$).

Preparation of homogenates and microsomal fractions

A 10% tissue homogenate was prepared in 0.01 M Tris-HCl buffer, pH 7.4 by means of a Potter Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 9000×g for 20 min and the supernatant was further centrifuged at 105,000×g for 60 min at 4 ºC to obtain the microsomal fraction Hanioka et al. 1997. The protein content was estimated by the method of Lowry et al. (1951).

Assay of Phase I and phase II enzymes activities

Cytochrome P_{450} and Cytochrome b_5 were estimated by the method of Omura and Sato, (1964). Microsomes suspended in phosphate buffer were reduced by a few milligrams of solid sodium dithionate. Then 1 ml of water saturated with carbon monoxide was added. The absorbances of the samples were measured at 400-500 nm. The level of cytochrome P_{450} was expressed as nmol/mg protein. Cytochrome b_5 was expressed as nmol/mg protein. NADPH-cytochrome P_{450} reductase was estimated according to the method of Omura and Takesue, (1970), glutathione-*S*-transferase, GST was estimated using Habig et al. (1974) and UDP-glucuronyl transferase, UDP-GT according to Hollmann and Touster, (1962), were also assayed in microsomal fractions of liver and mammary tissues of control and experimental rats.

Assay of carbohydrate metabolizing enzymes

Hexokinase was assayed by the method of Brandstrup et al. 1957. Phosphogluco isomerase was assayed by the method of Horrocks et al. (1963). Aldolase was estimated by the method of King (1965). Glucose-6-phosphatase was assayed according to the method of Gancedo and Gancedo (1971).

Histopathology

The tissues were immediately fixed in 10% neutral buffered formalin, embedded in paraffin, processed by means of routine histological techniques, and stained with Hematoxylin-Eosin and observed under light microscope (*Nikon, Eclipse Ti, Japan*) for histopathological analysis.

Statistical Analysis

The values are expressed as mean ± standard deviation (SD). The results were computed statistically using Statistical Package for Social Sciences (SPSS software package, Version 16) and one-way analysis of variance (ANOVA). Post hoc testing was performed for inter-group comparison using Student-Newman-Kuel multiple comparison test. Values of $p< 0.05$ were considered statistically significant.

Results and Discussion

Gross observations

The body weight and organ weights such as liver and kidneys were significantly decreased ($p< 0.05$) in DMBA-induced mammary carcinoma bearing rats when compared to control animals. Oral administration of SM with different doses significantly recouped the body weight and organ weights in a dose dependent manner. The SM treated rats showed a significant reduction in tumor volume when compared to DMBA-induced mammary carcinoma rats (Table 1, Figure 1).

Table 1: Effect of SM on changes in body weight, organs weight and tumour volume in control and experimental animals

Parameters	Control	DMBA-induced	DMBA+ SM treated	SM control
Final Body weight *(g)*	274.8±13.6	232.4± 11^{a,*}	254.9± 12^{b,*}	276.6± 12^{c,NS}
Liver weight *(g/100 g b. wt.)*	4.2± 0.76	2.6± 0.56^{a,*}	3.3± 0.55^{b,*}	4.1 ± 0.02^{c,NS}
Kidney weight *(g/100 g b. wt.)*	0.85± 0.17	0.64± 0.13^{a,*}	0.79± 0.16^{b,*}	0.8± 0.2^{c,NS}
Tumour volume *(cm^3)*	—	98.4± 7.52^{a,*}	66.3± 9.5^{b,*}	—

Values are expressed as mean ± SD of six animals in each group.
Comparison is made as
[a]DMBA-induced vs. Control
[b]DMBA-induced vs. DMBA+ SM treated
[c]Control vs. SM control
Statistical significance: $^{*}p< 0.05$. ^{NS}Non Significant.

In the present study, oral administration of SM to DMBA-induced mammary carcinoma rats significantly recouped the body weight and organ weights in a dose dependent manner. There was a sharp fall in the body weight and organs (liver and kidneys) weight in mammary carcinoma induced animals. This may be due to tumor cachexia, characterized by weakness, lethargy, anorexia, depletion of host components, tissue wasting and a progressive waning of vital functions (Argiles and Azcon-Bieto 1988). The SM treated animals showed a gradual increase in their body weights indicating the counteractive property of SM. Many studies in different cell lines, animal models and human epidemiological trials have shown the potential of dietary polyphenols as anticarcinogenic agents (Yang et al. 2001). The polyphenolic compounds, flavonoids, ascorbic acid, β-carotene and other bioactive components present in the SM might influence the growth due to their antioxidant, immunopotentiating and anti-inflammatory properties.

The SM treated rats showed a significant reduction in tumor volume when compared to DMBA-induced mammary carcinoma rats. This might be due to the protective effect of flavonoids present in the SM since, flavonoids are known to display a vast array of cellular events, they can control the overall process of carcinogenesis by several mechanisms including modulation of survival/proliferation pathways, activation of caspases, down-regulation of Bcl-2 and Bcl-xL expression and enhanced expression of Bax and Bak and modulation of nuclear factor κB might be responsible for tumor regression in SM treated animals (Shimizu et al. 2005, Gong et al. 2003).

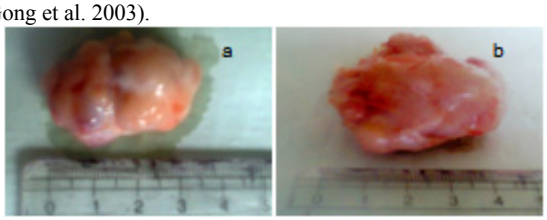

Figure 1: Photographs showing A- DMBA- induced rat mammary gland with tumour formation. B- DMBA-induced + SM (400 mg/kg body weight) treated rat mammary gland with tumour regression.

Phase I and phase II enzymes activities

Table 2 and 3 depict the influence of administration of SM on the levels of Phase I (cytochrome P_{450}, cytochrome b_5 and NADPH cytochtrome P_{450} reductase) and Phase II (Glutathione-S-transferase and UDP-Glucuronyl transferase) biotransformation enzymes in liver and mammary gland of control and experimental animals, respectively. The activities

Table 2: Effect of SM on the activities of Phase I and Phase II biotransformation enzymes in liver of control and experimental animals.

Parameters	Control	DMBA-induced	DMBA + SM treated	SM control
Cytochrome P_{450}	0.87 ± 0.04	1.13± 0.07^{a,*}	0.68 ± 0.03^{b,*}	0.90 ± 0.03^{c,NS}
Cytochrome b_5	0.31 ± 0.02	0.67 ± 0.04^{a,*}	0.43 ± 0.03^{b,*}	0.34 ± 0.02^{c,NS}
NADPH-cytochrome P_{450} reductase	0.48 ± 0.03	1.24 ± 0.08^{a,*}	0.53 ± 0.04^{b,*}	0.46 ± 0.03^{c,NS}
Glutathione-S-transferase	124.9± 8.61	78.65 ± 7.52^{a,*}	108.3 ± 9.56^{b,*}	125.4 ± 6.2^{c,NS}
UDP-Glucuronyl transferase	34.77 ± 1.86	23.65 ± 1.36^{a,*}	27.52 ± 1.88^{b,*}	32.89 ± 1.37^{c,NS}

Values are expressed as mean ± SD of six animals in each group.
Comparison is made as
[a]DMBA-induced vs. Control
[b]DMBA-induced vs. DMBA+ SM treated
[c]Control vs. SM control
Statistical significance: $^{*}p< 0.05$. ^{NS}Non Significant.

of cytochrome P_{450}, cytochrome b_5 and NADPH cytochrome P_{450} reductase were significantly increased, whereas the activities of glutathione-S-transferase and UDP-glucuronyl

transferase were decreased in mammary cancer bearing animals when compared to control animals. Administration of SM showed ($p< 0.05$) significantly reduced activities of these Phase I enzymes with significant increase in the activities of phase II enzymes.

The metabolic activation and detoxification of DMBA *in vivo* are known to occur primarily in the liver and also in a variety of other organs including the mammary gland. The metabolism of DMBA in the liver often quantitatively predominates over organ-specific metabolism. Although the liver is not a target organ for DMBA-induced carcinogenesis, both proximate and ultimate metabolites of DMBA are formed in liver cells that can be transported to mammary glands resulting in DMBA-DNA adducts formation. In view of the acknowledged importance of the liver as a major organ responsible for DMBA activation and detoxification, an analysis of representative hepatic phase I and II xenobiotic metabolizing enzymes would provide adequate mechanistic information regarding the systemic effect of SM on DMBA metabolism and its overall impact on the eventual occurrence of mammary cancer.

Table 3: Effect of SM on the activities of Phase I and Phase II biotransformation enzymes in mammary tissue of control and experimental animals.

Parameters	Control	DMBA-induced	DMBA + SM treated	SM control
Cytochrome P_{450}	0.23 ± 0.01	0.50 ± 0.022 ^{a,*}	0.32 ± 0.017^{b,*}	0.21 ± 0.012^{c,NS}
Cytochrome b_5	0.17 ± 0.006	0.38 ± 0.018 ^{a,*}	0.28 ± 0.013^{b,*}	0.16 ± 0.004^{c,NS}
NADPH-cytochrome P_{450} reductase	0.74 ± 0.031	1.36 ± 0.12 ^{a,*}	0.93 ± 0.042^{b,*}	0.76 ± 0.03^{c,NS}
Glutathione-S-transferase	2.74 ± 0.13	4.16 ± 0.22^{a,*}	5.17 ± 0.28^{b,*}	2.69 ± 0.12^{c,NS}
UDP-Glucuronyl transferase	7.28 ± 0.32	4.35 ± 0.24 ^{a,*}	6.48 ± 0.47^{b,*}	7.43 ± 0.51^{c,NS}

Values are expressed as mean ± SD of six animals in each group.
Comparison is made as
[a]DMBA-induced vs. Control
[b]DMBA-induced vs. DMBA+ SM treated
[c]Control vs. SM control
Statistical significance: $^{*}p< 0.05$. ^{NS}Non Significant.

Units: Cytochrome P_{450} and Cytochrome b_5 are expressed as nmoles/mg of microsomal protein. NADPH-cytochrome P_{450} reductase is expressed as µmoles of NADPH oxidized/min/mg of microsomal protein. Glutathione-S-transferase is expressed as µmoles of CDNB-GSH conjugate formed/h/mg of microsomal protein**.** UDP-Glucuronyl transferase is expressed as µmoless/min/mg of microsomal protein.

The metabolic activation and detoxification of DMBA *in vivo* are known to occur primarily in the liver and also in a variety of other organs including the mammary gland. The metabolism of DMBA in the liver often quantitatively predominates over organ-specific metabolism. Although the liver is not a target organ for DMBA-induced carcinogenesis, both proximate and ultimate metabolites of DMBA are formed in liver cells that can be transported to mammary glands resulting in DMBA-DNA adducts formation. In view of the acknowledged importance of the liver as a major organ responsible for DMBA activation and detoxification, an analysis of representative hepatic phase I and II xenobiotic metabolizing enzymes would provide adequate mechanistic information regarding the systemic effect of SM on DMBA metabolism and its overall impact on the eventual occurrence of mammary cancer.

The results presented here demonstrate conspicuously the effect of SM on the etiology of carcinogenesis by altering xenobiotic metabolizing enzymes. SM administration at the dosage of 400 mg/ kg body weight significantly reduced the multiplicity of DMBA-induced breast tumors and mean tumor burden as well as observed preneoplastic lesions. These results are consistent with the chemopreventive effects of flavonoids documented by other workers (Szliszka et al. 2011). The liver plays a central role in producing proximate mutagens that could be transported to the breast for final metabolic activation to form the ultimate DNA-reactive metabolites. Although hepatic enzymes are likely to play a role in the metabolism of potential breast carcinogens, it is probable that enzymes locally expressed in the target site also have an important influence in modulating levels of DNA-reactive species (Williams and Philips 2000). DMBA, a highly reactive polycyclic aromatic hydrocarbon (PAH), is the extensively used carcinogen to produce experimental mammary gland tumor. This fat soluble compound accumulates and persists in the adipose tissue of the mammary gland and increases the exposure of the mammary epithelium to DMBA in Group II rats. The bay region theory of PAH carcinogen predicts that DMBA will be activated to DNA binding forms via the formation of electrophilic dihydrodiol epoxides. A substantial increase of hepatic Phase I enzymes was observed in the carcinogen treated animals that was reversed following SM supplementation. Hence, the effect of SM on Phase I enzymes might be due to its flavonoid content that decrease cytochrome P_{450} activity either by increasing Vmax or decreasing Km for microsomal monoxygenase and enhancing the interaction of NADPH-cytochrome P_{450} reductase with cytochrome P_{450} binding to the catalytic site. Treatment with SM actively reduces the activity of NADPH cytochrome P_{450} reductase and cytochrome b_5 in the cancer bearing animals, indicating its overall effect on Phase I enzymes mediated by its flavonoid content.

The above experimental results have indicated that administration of SM normalizes the activities of microsomal markers and increases the DMBA detoxification enzymes in microsomes during mammary carcinoma. The enzyme modifying capability of SM might play an important role in its anticarcinogenic potency against DMBA-induced mammary cancer and to the larger extent it metabolizes the lipophilic xenobiotics.

Carbohydrate metabolizing enzymes

The effect of SM on the activities of carbohydrate metabolizing enzymes in the mammary gland and liver of control and experimental animals are presented in Table 4 The levels of Hexokinase, Phosphogluco-isomerase and Aldolase in mammary gland of cancer-bearing animals (DMBA-induced; Group II) were found to be elevated significantly ($p< 0.05$) whereas the Glucose-6-phosphatase was found to be decreased when compared to the Control (Group I) and SM control (Group IV). On the other hand, these enzyme levels were significantly brought back to near normal levels in SM treated (Group III) animals when compared to Group II cancer-bearing animals. Whereas, no significant changes were observed in sole SM treated (Group IV) animals when compared to Group I control animals. Similarly, the same pattern of results was observed in liver of control and experimental animals (shown in Table 4).

The chronic and often uncontrolled cell proliferation that represents the essence of neoplastic disease involves not only

deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division. Such reprogramming of energy metabolism is apparently counterintuitive, in that cancer cells must compensate for the ~18-fold lower efficiency of ATP production afforded by glycolysis relative to mitochondrial oxidative phosphorylation. They do so in part by upregulating glucose transporters, especially GLUT1, which substantially increases glucose import into the cytoplasm (Jones and Thompson, 2009; DeBerardinis et al. 2008; Hsu and Sabatini, 2008). Indeed, markedly increased uptake and utilization of glucose have been documented in many human tumor types. Glycolytic fueling has been shown to be associated with activated oncogenes and mutant tumor suppressors (DeBerardinis et al. 2008; Jones and Thompson, 2009), whose alterations in tumor cells have been selected primarily for their benefits in conferring the hallmark capabilities of cell proliferation, avoidance of cytostatic controls and attenuation of apoptosis. This reliance on glycolysis can be further accentuated under the hypoxic conditions that upregulate glucose transporters and multiple enzymes of the glycolytic pathway (Semenza 2010a; Jones and Thompson 2009; DeBerardinis et al. 2008). Thus, both the Ras oncoprotein and hypoxia can independently increase the levels of the HIF1a and HIF2a transcription factors, which in turn upregulate glycolysis (Semenza, 2010a, 2010b; Kroemer and Pouyssegur 2008). Vander Heiden et al. (2009) have reported that the increased glycolysis allows the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating nucleosides and amino acids; this facilitates, in turn, the biosynthesis of the macromolecules and organelles required for assembling new cells. Our results are in line with these findings.

Hexokinase levels occupy an important place in determining the glycolytic capacity of cancer cells (Parry and Pedersen 1983). In our study, a significant increase in the activity of hexokinase was observed in breast cancer-bearing (Group II) animals. This may be due to the fact that tumors catabolise large amount of glucose because glucose is the preferred substrate for most of the rapidly growing cancer cells. Phosphoglucoisomerase (PGI) acts as a catalyst in the conversion of glucose-6-phosphate to fructose-6-phosphate. Campbell and King (1962) claimed that PGI is an indicator of metastatic growth and is elevated in patients with neoplasm, especially after metastasis. Reports also indicate the elevated levels of PGI in sarcoma and in cancers of the lung, mouth, rectum and breast (Parveen et al. 1974). In the present investigation, the increased levels of phosphoglucoisomerase was found in cancer-bearing (Group II) animals, which may be due to the higher glycolytic rate in liver tissues and further leakage from the destruction of neoplastic tissues. Aldolase is also found to be elevated in the tumor bearing animals. Sibley and Fleisher (1954) have reported that the activity of aldolase was elevated in the breast cancer condition. This is also supported by the results of Hennipman et al. (1988) in the metastatic condition.

Glucose-6-phosphatase is unique among the gluconeogenic enzymes and also a marker enzyme for liver microsomal activity. The activities of gluconeogenic enzymes were significantly inhibited in tumor bearing (Group II) animals. This may be due to the higher lactic acid production of neoplastic tissues, and it has been proved that the tumor utilizes a large proportion of lactate for glycolysis and protein synthesis (Dobrosielski 1984).

The SM treated (Group III) animals showed a significant drop in the activities of glycolytic enzymes and a concomitant elevation in the levels of gluconeogenic enzymes. This modulation may be due to the antitumor activity of the drug either by inhibiting the glycolytic enzymes activities or by the suppression of tumor progression. This activity may be attributed to the presence of flavonoids in the formulation SM, which has an effective role over aerobic glycolysis and also inhibits the migration of cancer cells and malignant cell proliferation, since they inhibit several biochemical events associated with cellular growth (Kandaswami et al. 1991).

The results of present investigation inevitably prove that SM has some intervention role in the glycolytic pathway, which is evidenced in the gluconeogenesis process of our findings. Furthermore, the reason for the observed anticancer activity could be due to the inhibition of glycolytic pathway and activation of gluconeogenesis via antioxidant activity. Thus, the SM may interrupt the energy requirement of neoplastic tissues and may lead to suppression of tumor growth.

Table 4: Effect of SM on activities of glycolytic and gluconeogenic enzymes in the mammary tissue and liver of control and experimental animals.

Sample	Parameters	Control	DMBA-induced	DMBA + SM treated	SM control
Mammary tissue	Hexokinase	12.46 ± 1.07	21.55 ± 1.17^{a,*}	16.46 ± 0.84^{b,*}	13.27 ± 0.86^{c,NS}
	Phosphoglu co-isomerase	14.35 ± 1.16	23.25 ± 1.21^{a,*}	18.52 ± 1.04^{b,*}	14.67 ± 1.76^{c,NS}
	Aldolase	9.47 ± 0.64	13.45 ± 1.03^{a,*}	10.26 ± 0.94^{b,*}	9.28 ± 1.12^{c,NS}
	Glucose-6-phosphatase	12.84 ± 1.14	8.47 ± 1.08^{a,*}	10.65 ± 1.31^{b,*}	12.44 ± 1.08^{c,NS}
Liver	Hexokinase	19.56 ± 1.21	26.86 ± 1.24^{a,*}	22.44 ± 1.14^{b,*}	18.78 ± 1.16^{c,NS}
	Phosphoglu co-isomerase	17.34 ± 1.08	23.58 ± 1.41^{a,*}	19.67 ± 1.15^{b,*}	16.52 ± 1.20^{c,NS}
	Aldolase	12.58 ± 1.26	18.68 ± 1.33^{a,*}	14.76 ± 1.18^{b,*}	12.27 ± 1.14^{c,NS}
	Glucose-6-phosphatase	32.86 ± 1.53	21.87 ± 1.62^{a,*}	27.85 ± 1.16^{b,*}	33.27 ± 1.23^{c,NS}

Values are expressed as mean ± SD of six animals in each group.
Comparison is made as
[a]DMBA-induced vs. Control
[b]DMBA-induced vs. DMBA+ SM treated
[c]Control vs. SM control
Statistical significance: $^{*}p < 0.05$. ^{NS}Non Significant.

Units: Hexokinase is expressed as nmoles of glucose-6-phosphate liberated/min/mg protein. Phosphogluco-isomerase is expressed as nmoles of fructose liberated/min/mg protein**.** Aldolase is expressed as nmoles of glyceraldehyde liberated/min/mg protein. Glucose-6-phosphatase is expressed as nmoles of Pi liberated/min/mg protein.

From the results of our study, one can propose that the SM may interrupt the energy requirement of neoplastic tissues and may lead to suppression of tumour growth. The results also demonstrated the effect of SM on the etiology of carcinogenesis by altering xenobiotic metabolizing enzymes.

Histological Findings

The histological analysis of liver tissues of control and experimental animals are shown in Figure 2 A-D. The Group I (Control) and Group IV (SM control) animals showed normal architecture of hepatocytes surrounding the portal triad with granulated cytoplasm and small uniform nuclei (Figures 2 A and 2 D). In contrast, Group II (DMBA-induced) cancer bearing animals showed loss of architecture with a tendency to spread by intra-hepatic veins, both hepatic and portal with significant abnormalities within portal vessels (Figure 2 B). On

the contrary, the Group III (DMBA-induced +SM treated) animals showed almost normal hepatocytes along with congested sinusoids, which proves the protective ability of SM (Figure 2 C).

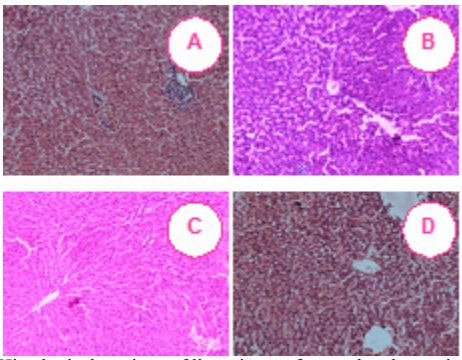

Figure 3: Histological sections of liver tissue of control and experimental rats

A. Control rats (Group I) showing normal hepatocyte architecture.
B. Mammary carcinoma bearing rats (DMBA-induced, Group II) showing the lobular disarray with inflammatory infiltrate in between hepatocytes and surrounding portal tract.
C. SM treated rats (DMBA + SM, Group III) showing decrease in hydrophic degeneration and recovering normal liver architecture.
D. SM alone treated rats showing normal liver architecture (SM control, Group IV) similar to that of Group I.

Conclusion

The results demonstrated unequivocally the effect of SM on inhibition of tumor progression by altering xenobiotic metabolizing enzymes and restoring energy metabolism. Our findings indicate that *SM* contains potential anti-cancer agents acting in synergistic manner against breast cancer cell proliferation via induction of Phase II xenobiotic enzymes and restoration of deranged energy metabolism in mammary carcinoma cells.

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