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Research Article

ANTICANCER EFFECT OF SHEMAMRUTHAA (A PHYTOCHEMICAL FORMULATION) ON 7, 12-DIMETHYLBENZ(A)ANTHRACENE INDUCED MAMMARY CARCINOMA IN RATS

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ABSTRACT

Increase in scientific investigations into indigenous wealth of herbal medicines gives ample evidence of medicinal plants as sources of drugs. The present study was therefore designed to evaluate the anticancer potential of Shemamruthaa (SM) - a phytochemical combination against 7,12-dimethylbenz(*a*)anthracene (DMBA) induced mammary carcinoma in rats. The tumour was induced in 8-week-old Sprague-Dawley rats by gastric intubation of 25 mg DMBA in 1ml olive oil. After 90 days of induction period, the rats were orally administered with different doses of SM (100,200, 300, 400, 500 and 600 mg/kg body weight) for 14 days. The serum and tissue levels of glycoconjugates, lysosomal and marker enzymes were analysed in control and DMBA induced rats. Upon administration of the SM, the levels of the above enzymes and the changes in the body weights were significantly normalized in a dose dependant manner. The results of the present study indicate that the phytochemical formulation Shemamruthaa possesses strong anticancer effects through its role in modulating glycoprotein components and the activities of lysosomal and marker enzymes. This drug exerted a strong anticancer effect at the dosage of 400 mg/kg body weight.

Keywords: *Emblica officinalis* Gaertn., flavonoids, glycoproteins, *Hibiscus rosa-sinensis* L., Lysosomal and marker enzymes, mammary carcinoma, Shemamruthaa (SM).

INTRODUCTION

Globally, breast cancer is the principal cause of cancer-related death in women, accounts for about 3,27,000 deaths per year and one in 10 of all new cancers diagnosed worldwide each year [1,2]. It is the second leading cause of cancer death among women in India [3]. The development of breast cancer is associated with alterations of the delicate balance between cell proliferation and apoptotic cell death, cellular redox status, deregulation of cellular differentiation and endocrine derangement[4,5].

Despite advances in understanding the molecular basis, diagnosis and treatment of this fatal disease over the past decades, this malignancy remains elusive. Therefore, the identification of new and efficient anticancer drugs has always been a focal point in cancer research [6,7].

Hibiscus rosa-sinensis L. (family: Malvaceae), commonly known as China rose is a potent herb in traditional system of medicine [8,9]. It is being used against cough, fever, dysentery, venereal diseases and cancerous swellings [10]. Investigations of the pharmacological properties have shown immense antioxidant [11,12], antidiabetic [13], hepatoprotective[14,15], cardioprotective[16], antipyretic and anti-inflammatory [17,18] and neuroprotective[8] activities in models. In an elegant study, Sonia Sharma and Sarwat Sultana [19,20] showed that the *Hibiscus* flower extract exerts a protective effect against hyperproliferation and oxidative damage caused by benzoyl peroxide and ultraviolet radiations in mouse skin. The constituents present in the extract include flavonoids, cyanidine, quercetin, hibiscetin, glycosides, riboflavin, niacin, carotene, taraxeryl acetate, β-sitosterol, campesterol, stigmasterol, ergosterol, citric, tartaric and oxalic acids, cyclopropenoids and anthocyanin pigments[10,21,22]. Most of these components have a protective action especially against oxidant-induced stress. Therefore, *H. rosa sinensis* was used as one of the phytochemical constituents in the present study.

The fruits of *Emblica officinalis* Gaertn. (*Phyllanthus emblica* L.; family: Euphorbiaceae) are widely consumed raw or cooked, but they are also principal constituents of many Ayurvedic preparations. The fruit extract has many pharmacological activities: it inhibits clastogenicity and mutagenicity induced by heavy metals and protects against radiations[23,24], possesses antidiabetic[25,26], cytoprotective and immunomodulating [27,28], analgesic, antipyretic, anti inflammatory[29], antioxidant[30-32] and hepatoprotective activities[33,34].Other studies confirmed the potential antitumour, anticarcinogenic and chemopreventive action of *P. emblica* extracts on various *in vivo* and *in vitro* models [35-38]. Phytochemical investigation of the plant revealed the presence of tannins , ellagic acid, flavonoids like quercetin, hydrolysable tannins (Emblicannin-A, Emblicannin-B, Punigluconin, Pedunculagin), Gallo-ellagitannoids, flavonoid (rutin), trigalloyl glucose and phyllemblic acid [39,40,41].

The mixture of several crude extracts, when used in formulation enhances the beneficial effects through synergistic amplification and diminishes any possible adverse effects and offers advantage over a single isolated ingredient[42,43]. To the best of our knowledge there were no studies on the combined effects of the aforementioned plants against tumour suppression. Hence, an effort was made to formulate the drug "Shemamruthaa"(SM) with the combination of *Hibiscus rosa-sinensis* flowers, fruits of *Phyllanthus emblica* and pure honey in definite ratio.

The present study was designed to study the therapeutic efficacy of Shemamruthaa on DMBA-induced mammary carcinoma bearing rats. The status of glycoprotein components, lysosomal enzymes and tumour marker enzymes in the plasma, liver and mammary tissues was evaluated in control and experimental rats. From this study the effective dose of SM was determined in mammary carcinoma bearing rats.

MATERIALS AND METHODS

Collection of plant material and preparation of SM Drug

The flowers of *Hibiscus rosasinensis* and fruits of *Emblica officinalis* were obtained from southern part of India (Kanchipuram District, Tamil Nadu) and the pharmacognostic authentication was done by Department of Plant Sciences, University of Madras, Chennai-600 025. The flowers and deseeded fruits were air dried under shade, pulverized and mixed with pure honey in definite ratio. Honey was added to the drug, in order to promote intellect and prevent senility and for longevity [44].

Chemicals and Reagents

7,12-Dimethylbenz(*a*)anthracene (DMBA), Nicotinamide Adenine Dinucleotide (NAD^+), adenosine-5'-monophosphate and N-acetyl muramic acid and other fine chemicals were purchased from Sigma Chemical Co. (St. Louis, MO,USA). All other chemicals and solvents used were of analytical grade and highest purity.

Animals

Adult female rats of Sprague-Dawley strain weighing 195-205g 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 °C) and light (12 h light/dark). They were fed with standard rat pellet diet and water *ad libitum*. The study has got the approval from the Institutional Animal Ethical Committee (IAEC), regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests (Animal Welfare Division), Government of India [No. 01/030/2010].

Experimental design

The rats were divided into eight groups with six animals in each group and were given the following dose regimen. Normal healthy controls at the age of 8 weeks received a single dose of olive oil (1 ml) by gastric intubation (Group I). Mammary carcinoma was induced in Group II rats by gastric intubation of 25 mg DMBA (Sigma, MO, USA) in 1ml olive oil. Mammary carcinoma was confirmed by palpation and histopathological examination. Mammary carcinoma was induced in Group III, IV, V, VI, VII and VIII rats also (as in Group II) but after 3 months, treatment was started orally with different doses of SM (100, 200, 300, 400, 500 and 600 mg/kg body weight respectively) and continued for 14 days. After the investigation period (3 months after induction + 14 days treatment), the animals were sacrificed. The body weight, organs weight and the mean tumour volume was calculated according to the formula $v= 4/3\pi r_1^2 r_2$ (radius $r_1< r_2$; r= tumor diameter in mm/2)[45]. Liver and mammary gland were washed well with ice-cold saline and homogenized in Tris-HCl buffer (0.1 M, pH 7.4). Blood was also collected for further analyses.

Biochemical estimations

The levels of glycoprotein components namely hexose, hexosamine and sialic acid in plasma, liver and mammary gland were estimated by the method of Niebes, Wagner and Warren respectively[46,47,48]. The values are expressed as mg/dl for plasma and mg/g of defatted tissue for tissue. Protein was estimated by the method of Lowry *et al* [49]. Acid phosphatase, Cathepsin-D enzyme activities were measured by the method of King[50] and Sapolsky[51] and their values are expressed as µmoles of phenol liberated per min/mg protein, µmoles of tyrosine liberated min/mg protein respectively. β-D-glucuronidase was assayed by the method of Kawai and Anno[52] and the activity was expressed as µmoles of p-nitrophenol formed per min/mg protein.

The assay of gamma glutamyl transferase was carried out according to Rosalki and Rau [53]. The activity was expressed as IU/l for plasma and µmoles of *p*-nitroaniline liberated per minute/mg of protein for tissue.

Lactate dehydrogenase was assayed by the method of King [54] and its activity is expressed as IU/l for plasma and µmoles of pyruvate liberated per minute/mg of protein for tissue. The activity of 5'-nucleotidase was determined by the method of Luly *et al* [55] and expressed as µmoles of phosphorus liberated per minute/mg of protein. The amount of phosphorus liberated was estimated by Fiske and Subbarow method[56].

Statistical analysis

Values are given as the mean ± S.D of six rats. The results were statistically evaluated using Student's *t*-test using SPSS 16 (Statistical Package for Social Sciences) software and one-way analysis of variance (ANOVA). The differences between the groups were considered as significant at $*p<0.05$

RESULTS

Gross observations

Table 1 represents the changes in the whole body weight and organs weight in control, cancer induced and drug treated rats. The body weight and organ weights such as liver and kidneys were significantly decreased ($p< 0.05$) in mammary carcinoma bearing rats when compared to control. Oral administration of SM with different doses (100-600 mg of SM/kg body weight per day) significantly recouped the body weight and organ weights in a dose dependent manner.

Glycoprotein components

Table 2 depicts the levels of glycocomponents of glycoproteins in plasma, liver and mammary tissue of control, cancer induced and SM drug treated rats. Elevated levels of hexose, hexosamine and sialic acid in plasma, liver and mammary tisssue were observed in cancer induced rats when compared to the controls. Upon drug administration, the animals responded better to SM and the levels of those biochemical parameters were significantly decreased in a dose dependent manner when compared to untreated rats. The drug treatment at the doses of 400, 500 and 600 mg of SM/kg body weight showed a highly significant effect ($p<0.05$) when compared with cancer induced animals.

Lysosomal enzymes

Figure 1-3 depicts the activities of lysosomal enzymes such as acid phosphatase, β-D-glucuronidase and cathepsin-D in plasma, liver and mammary tissue of control and experimental animals respectively. The activities of lysosomal enzymes were found to be significantly increased ($p<0.05$) in carcinoma induced animals than in control animals. On drug treatment, the enzyme activities were significantly reverted to near normal levels in a dose dependent manner, showing a favourable change in rats treated with 400mg of SM/kg body weight.

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

Parameters	Control	Induced	Induced + Treated with SM at different doses					
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Body weight (g)								
Initial	198.4±5.9	197.6±5.7	201.4±5.9	202.7±5.6	200.3±6.3	201.4±6.2	199.6±5.8	202.7±6.5
Final	234.8±13.6	208.4±11.8^{*a}	213.7±11.6$^{\#}$	215.3±12.4$^{\#}$	219.6±12.9$^{\#}$	226.3±11.5$^{\#a}$	226.9±12.4$^{\#a}$	225.6±13.9$^{\#a}$
Organs weight (g)								
Liver	8.5±0.76	6.2±0.56^{*a}	6.4±0.44$^{\#}$	6.7±0.47$^{\#}$	7.1±0.51$^{\#}$	7.8±0.55$^{\#a}$	7.9±0.48$^{\#a}$	7.9±0.58$^{\#a}$
Kidneys	1.85±0.17	1.26±0.13^{*a}	1.30±0.14$^{\#}$	1.38±0.17$^{\#}$	1.41±0.12$^{\#}$	1.70±0.16$^{\#a}$	1.73±0.17$^{\#a}$	1.76±0.15$^{\#a}$
Tumour volume(mm^3)	---	4705±412^{*a}	4312±416$^{\#}$	4039±397$^{\#}$	3941±369$^{\#}$	3436±312$^{\#a}$	3300±300$^{\#a}$	3257±336$^{\#a}$

Values are expressed as mean±SD; n= 6

Group I Control, Group II Mammary carcinoma induced, Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weight/day respectively.

*When compared with Group I, $^{\#}$ when compared with Group II, Statistical significance: $^{a}p < 0.05$

Table 2: Effect of SM on glycoprotein components in plasma, Liver and mammary tissues in control and experimental rats.

Parameters	Control	Induced	Induced + Treated with SM at different doses					
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Plasma (mg/dl)								
Hexose	144.65±8.2	198.69±11.21*a	194.61±10.42#	191.53±9.64#	188.95±8.72#	153.77±8.23#a	150.25±9.13#a	147.68±9.78#a
Hexosamine	36.45±3.6	51.36±4.6*a	50.68±4.2#	48.27±3.6#	46.39±3.76#a	43.87±4.1#a	42.66±3.9#a	41.95±3.8#a
Sialic acid	56.67±4.8	128.65±9.62*a	125.28±8.65#	121.34±8.51#	119.32±9.2#	94.56±7.61#a	93.67±7.80#a	92.91±8.32#a
Liver (mg/g of defatted tissue)								
Hexose	3.78±0.28	9.69±0.75*a	8.55±0.89#	6.38±0.59#	5.69±0.54#	4.17±0.37#a	4.11±0.39#a	4.16±0.4#a
Hexosamine	3.52±0.27	10.25±1.14*a	9.63±0.67#	7.87±0.64#	6.48±0.56#	4.44±0.36#a	4.51±0.38#a	4.37±0.41#a
Sialic acid	2.94±0.27	6.32±0.46*a	5.89±0.48#	5.24±0.51#	4.85±0.39#a	3.78±0.36#a	3.69±0.38#a	3.55±0.31#a
Mammary tissue (mg/g of defatted tissue)								
Hexose	1.78±0.75	4.23±0.35*a	4.11±0.38#	3.81±0.24#	3.39±0.25#	2.13±0.17#a	2.08±0.16#a	1.97±0.17#a
Hexosamine	0.69±0.04	1.48±0.13*a	1.36±0.13#	1.29±0.10#	1.23±0.09#	0.94±0.06#a	0.92±0.06#a	0.89±0.06#a
Sialic acid	0.26±0.01	0.79±0.04*a	0.77±0.05#	0.74±0.04#	0.68±0.04#	0.32±0.02#a	0.30±0.02#a	0.31±0.03#a

Values are expressed as mean±SD; n= 6
Group I Control, Group II Mammary carcinoma induced, Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weight/day respectively.
*** when compared with Group I; # when compared with Group II; Statistical significance: [a] $p < 0.05$**

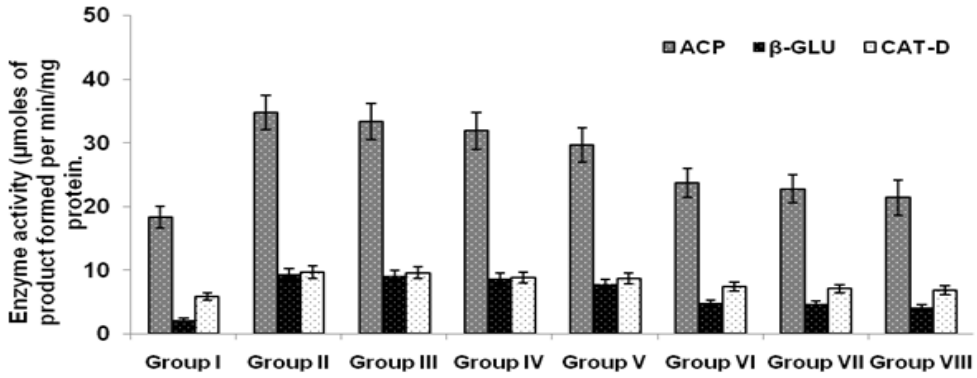

Figure 1: The levels of acid phosphatase (ACP), beta glucuronidase (β-GLU) and cathepsin-D (CAT-D) activities in the plasma of control and experimental animals (mean±SD; n=6).
Group I Control, Group II Mammary carcinoma induced , Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weight/day respectively

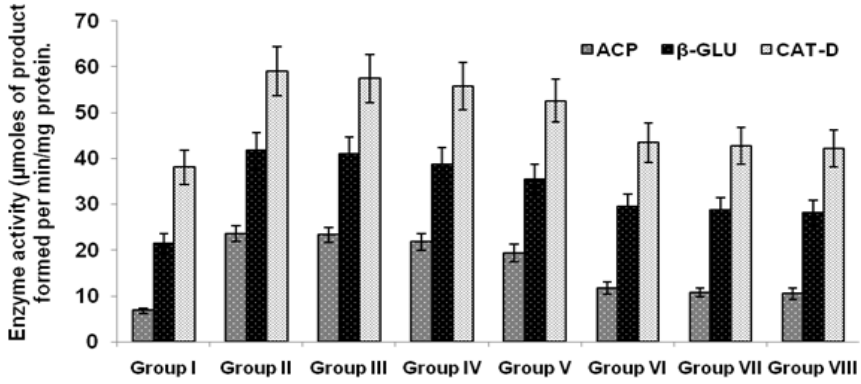

Figure 2: The levels of acid phosphatase (ACP), beta glucuronidase (β-GLU) and cathepsin-D (CAT-D) activities in the liver of control and experimental animals (mean±SD; n=6).
Group I Control, Group II Mammary carcinoma induced, Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weight/day respectively.

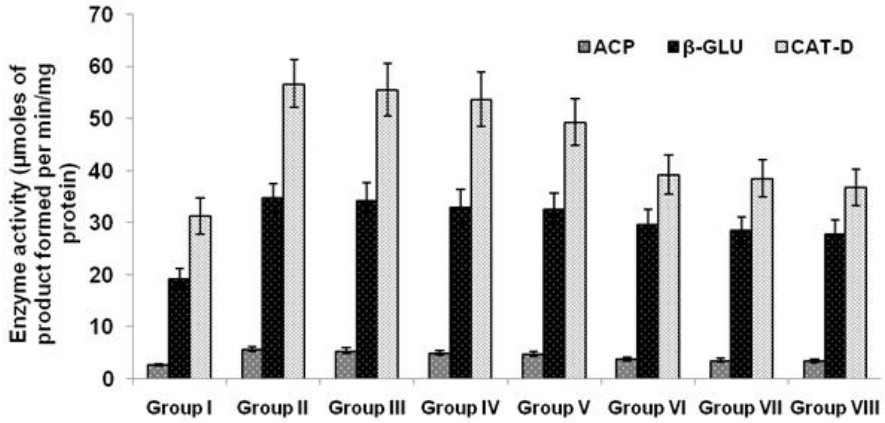

Figure 3: The levels of acid phosphatase (ACP), beta glucuronidase (β-GLU) and cathepsin-D (CAT-D) activities in the mammary gland of control and experimental animals (mean±SD; n=6).
Group I Control, Group II Mammary carcinoma induced, Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weights respectively.

Tumour marker enzymes

Table 3 shows the activities of marker enzymes such as gamma glutamyl transferase, lactate dehydrogenase and 5'- nucleotidase in plasma and liver respectively. Activities of marker enzymes were found to be significantly increased in mammary carcinoma bearing rats when compared to control rats. There was a significant decrease in the levels of these enzymes in SM treated groups with a higher significance in 400 mg/kg body weight treatment.

DISCUSSION

More than 13,000 plants have been studied during the last five year period for the development of new chemotherapeutic agents [57]. The present study was carried out to evaluate the anticancer potential of the SM drug on DMBA induced mammary carcinoma in rats. The results showed that administration of SM at the dosage of 400 mg/kg body weight exhibited enhanced anticancer effect.

Gross observations

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 tumour cachexia, characterized by weakness, lethargy, anorexia, depletion of host components, tissue wasting and a progressive waning of vital functions [58,59]. The drug treated animals showed a gradual increase in their body weights indicates the counteractive property of the drug. Many studies in different cell lines, animal models and human epidemiological trials have shown the potential of dietary polyphenols as anticarcinogenic agents [60,61]. The polyphenolic acids, 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 drug treated rats showed a significant reduction in tumour volume when compared to DMBA-induced mammary carcinoma rats. The flavonoids display a vast array of cellular effects, they can affect the overall process of carcinogenesis by several mechanisms including modulation of survival/proliferation pathways[62,63], activation of caspases[62], down-regulation of Bcl-2 and Bcl-xL expression and enhanced expression of Bax and Bak[64,65], and modulation of nuclear factor κB [66] might be responsible for growth inhibition and regression of tumour in drug treated animals.

Glycoprotein components

In the malignant tumour of the breast, the activities of glycosidases were increased 2-3 times as compared to normal tissue [67]. The presence of cancer-specific sialic acid-rich glycopeptides was first demonstrated in proteolytic digests derived from the surface of malignant cell lines [68]. Thus, the combined evaluation of hexose, hexosamine and sialic acid residues of glycoproteins might help to establish a useful aid in strengthening the diagnosis and treatment monitoring of mammary cancer patients [69,70].

The increased levels of plasma glycoprotein components in cancer condition may be due to the leakage of the disturbed membrane components from either disintegrating or dying neoplastic cells or as a consequent shedding of plasma membrane and due to increased synthesis by sequential addition of monosaccharide units to parent protein molecule catalysed by multiple glycosyltransferases such as sialyltransferase (NeuAc-T), galactosyltransferase (Gal-T), fucosyltransferases (Fuc-T A and Fuc-T B)[71-73]. An increased expression of glycoprotein components in malignant mammary tissue when compared to normal rats observed in our investigation is in line with previous reports.

On drug treatment, glycoprotein components levels were reverted back to near normal levels. This could be due to the cytostabilising property of the drug. Limtrakul *et al.*[74] showed that the flavonoids possess inhibitory action against carcinogenesis. Thus the flavonoids, alkaloids and other bioactive components of the drug may significantly alter the expression of glycosyltransferases thereby modulate glycoprotein synthesis and protected the structural integrity of cell surface and membrane, indicating its potent anticancer property.

Lysosomal enzymes

Malignant tumours contain high levels of lysosomal enzymes which may have local and systemic effects when released from the tumour. This enzyme release may occur either because of the tumour lysosomes are inherently more labile than normal or because of hypoxic conditions within the tumour mass, a known stimulus for lysosomal enzyme release. The progression to malignancy is often associated with deregulation of the normal mechanisms regulating proteolysis, resulting in numerous proteases having dramatically altered (significantly up regulated) in cancer [75].

Elevations in acid phosphatase activities in untreated tumour bearing animals correlate with the previous studies, which reported an increase in the activity of hydrolytic enzymes with optimum activity between pH 4 and 6 in the serum of cancerous patients [76, 77]. In the malignant tumours of the breast, the levels of glycosidases are increased 2-3 times when compared to normal tissue. Studies on cultured normal and virus transformed fibroblasts have revealed that the activity of lysosomal enzymes namely beta-N-acetylglucosaminidase, beta-D-glucosidase, beta-D-galactosidase and beta-N-acetyl galactosaminidase were increased[78]. An over expression of cathepsin D in breast cancer cells is associated with increased risk of metastasis [79].

Table 3: Effect of SM on marker enzymes in plasma and liver tissues of control and experimental rats.

Parameters	Control	Induced	Induced + Treated with SM at different doses					
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Plasma								
Gamma glutamyl transferase (IU/L)	1.38±0.11	2.56±0.24*a	2.52±0.22#	2.48±0.23#	2.37±0.19#a	1.87±0.16#a	1.89±0.18#a	1.92±0.18#a
Lactate dehydrogenase (IU/L)	0.39±0.02	1.62±0.13*a	1.61±0.14#	1.41±0.13#a	1.36±0.11#a	0.47±0.26#a	0.46±0.36#a	0.46±0.34#a
5'-Nucleotidase (µmol of Pi liberated/min/mg protein)	3.25±0.27	5.15±0.48*a	5.11±0.50#	4.84±0.46#a	4.58±0.44#a	3.12±0.28#a	3.13±0.30#a	3.10±0.28#a
Liver								
Gamma glutamyl transferase (µmoles of *p*-nitroaniline liberated per minute/ mg of protein)	4.23±0.42	7.74±0.68*a	7.72±0.69#	7.48±0.68#	6.85±0.65#a	4.78±0.45#a	4.69±0.46#a	4.79±0.46#a
Lactate dehydrogenase (µmoles of pyruvate liberated per minute/ mg of protein)	2.45±0.21	4.79±0.36*a	4.75±0.39#	4.36±0.38#	3.97±.031#a	2.86±0.23#a	2.85±0.26#a	2.83±0.25#a
5'-Nucleotidase (µmol of Pi liberated/min/mg protein)	2.83±0.27	5.26±0.51*a	5.22±0.49#	5.14±0.48#	4.77±0.45#a	3.26±0.30#a	3.28±0.31#a	3.27±0.29#a

Values are expressed as mean±SD; n= 6
Group I Control, Group II Mammary carcinoma induced, Groups III, IV, V, VI, VII and VIII Mammary carcinoma induced and treated with SM at the dosage of 100, 200, 300, 400, 500 and 600 mg/kg body weight respectively.
*** when compared with Group I, # when compared with Group II, Statistical significance: [a] $p < 0.05$**

Reversion of lysosomal enzyme levels in drug treated animals may be due to the stabilizing property of the drug on lysosomal membrane which could have been impacted by the flavonoids, as it is well established that flavonoids have inhibiting property on lysosomal membranes. The drug may modify the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and thereby preventing the discharge of acid hydrolases or by inhibiting the release of lysosomal enzymes[80]. The lysosomes can only be acidified and activated by their proton pump, and that can be inhibited by flavonoids might also be involved in experiential changes in lysosomal enzymes of drug treated rats[81].

Marker enzymes

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 [82,83]. Numerous other reports also revealed the elevated levels of LDH in various types of cancers[84]. 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. The results of the present study show that 5'- nucleotidase activity (5'-NT) was elevated in cancerous animals. Dao *et al.*[85] have reported that the increased activity of 5'-nucleotidase seems to have originated from the proliferating breast cells. Walia *et al.*[86] have reported higher activities of 5'-NT in breast cancer patients. Gamma glutamyl transferase (γ-GT), a key enzyme in glutathione metabolism provides high intracellular levels of GSH required for conjugation by glutathione-S-transferase, is involved in protecting cells against toxins and carcinogens. It has been reported that there is an increased risk of breast cancer in individuals with elevated levels of serum γ-GT [87].

The increased activity of LDH, 5'-NT and γ-GT seen in the present study substantiates the over expression of these enzymes in a wide range of malignancies including breast tumours and may be responsible for neoplastic transformation [88-90]. Oral administration of SM drug reduced the activities of these enzymes to near-normal levels, which may indicate the anticancer effects of the drug.

CONCLUSION

The biochemical alterations observed in cancer bearing animals in the present study may be due to the induction of lipid per oxidation and reduction of antioxidant levels following DMBA administration. However, oral administration of 400 mg/kg body weight of SM drug significantly reversed the alterations to near normal level in cancer-bearing animals. From these results, it can be inferred that the drug SM possesses a profound anticancer effect through its role in modulating the glycoprotein components, lysosomal membrane stability and restoration of normal activities of marker enzymes. Further investigations are under way to bring about the molecular mechanism of action of the drug.

AUTHOR DISCLOSURE STATEMENT

The authors have no competing financial interest. Conflict of Interest: NIL

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