Biochemical Evaluation of Glycoprotein Components, Lysosomal Enzymes and Marker Enzymes upon Kalpaamruthaa Administration in Experimental Mammary Carcinoma Rats

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Tumour markers correlate strongly with prognosis on tumour burden. Glycoprotein components and lysosomal hydrolases play an important role in carcinogenesis. Hence, this study was launched to evaluate the effect of Kalpaamruthaa (KA), a modified Siddha preparation, on the changes in glycoprotein components, lysosomal enzymes and marker enzymes in control and mammary carcinoma bearing rats. A significant increase in the activities of alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and 5′ nucleotidase (5′-NT) in plasma, liver and kidney were observed in animals with mammary carcinoma. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly reduced in the liver and kidney whereas increased in plasma of cancerous animals. On administration of KA, these changes were reverted back to near normal levels. The increased levels of glycoprotein components (hexose, hexosamine and sialic acid) and in the activities of lysosomal enzymes such as acid phosphatase (ACP), β-D-Glucuronidase, β-D-Galactosidase, *N*-acetyl-β-D-glucosaminidase and Cathepsin-D (CD) found in mammary carcinoma were also significantly decreased in KA treated animals. In all these studies, simultaneous KA administration proved more efficacious than post KA treatment, thus depicting the effective control of KA against the development of mammary carcinoma.

Key words —— Mammary carcinoma, glycoprotein, lysosomal enzyme

INTRODUCTION

Glycoproteins are organic compounds, composed of both protein and carbohydrate monosaccharides, usually hexose, hexosamine, fucose and sialic acid that are covalently linked to the polypeptide chain. Glycoproteins play a key role in mediating cell surface function, cell–cell recognition, cellular adhesion, binding and clearance of serum glycoproteins and metabolic transfer among others.[1)] Sialylated glycoconjugates also seem to be involved in tumour biology, since aberrant glycosylation patterns, are very common in human and animal neoplasia.[2, 3)] These tumour-associated antigens or tumour markers are of prognostic value, since their expression frequently correlates with invasiveness, metastasis and the tumour grade.[4)]

Glycosidases; the predominant lysosomal enzymes, are acid hydrolases mainly involved in the catabolism of glycoproteins, glycolipids and glycosaminoglycans. Alkaline phosphatase (ALP) is an enzyme associated with the plasma membrane of cells. Ciatto *et al.*,[5)] have hypothesized that in human breast cancer ALP plays a role in the de-phosphorylation of the estrogen receptor (ER) molecule and consequently modulating its binding capabilities.

Cathepsin-D (CD) plays a proteolytic role in the digestion of extracellular matrix (ECM) com-

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ponents and is implicated in tumour invasion and metastasis. The direct role of CD in cancer metastasis was first demonstrated in rat tumour cells in which transfection-induced CD over-expression increased their metastatic potential *in vivo*.[6] CD over-expression stimulates tumourigenicity and metastasis.

In breast cancer affected patients, blood-based tumour marker systems are extremely useful to correlate with the response to anticancer treatment and disease recurrence. Among the most sensitive and widely used of these liver enzymes are the aminotransferases that include: aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). Of the many tumour markers measured every 3 months after mastectomy in patients with apparently localised primary breast cancer, plasma levels of ALP and gamma glutamyl transferase (GGT) levels were the most useful in detecting metastatic disease.[7] All these enzymes were found to be greatly increased in overt mammary metastases.

Lactate dehydrogenase (LDH) catalyzes the Nicotinamide adenine di phosphate (NAD^+)-dependent conversion of lactate to pyruvate during anaerobic glycolysis. There are five major tetrameric LDH complexes, of which the LDH-A form increases during mammary gland tumourigenesis.[8] LDH activity is also elevated in breast tumour condition compared to normal mammary tissues.[9]

5′-Nucleotidase (5′-NT) that catalyses the hydrolysis of 5′-nucleotides to nucleosides inorganic phosphate, is found to be elevated in the sera of 90% breast cancer patients before treatment.[10] In many cases, a successful response to treatment was associated with a decrease of plasma 5′-NT levels. This has led to the conclusion that breast cancer cells are also a source of plasma 5′-NT and sequential determinations in breast cancer patients may have a possible prognostic relevance.

Kalpaamruthaa (KA) is a modified Siddha formulation which contains *Semecarpus anacardium* Linn. nut milk extract (SA), dried fruit powder of *Emblica officinalis* (EO) and honey. The component study of KA reveals the presence of flavonoids, ascorbic acid, polyphenols, tannins, sugars, sterols, ellagic acid, *etc*. Dose dependant study of KA suggested the effective dosage level at 300 mg/kg body weight.[11] SA (Family: anacardiaceae) is commonly called as marking nut.[12] A number of pharmacological properties such as anticancer,[13–15] antirheumatic effects[16, 17] and effectiveness against hepatocellular carcinoma[18] have been reported on SA. EO (syn: Phyllanthus emblica; family: Euphorbiaceae) commonly known as amla have been proven to possess strong anti-cancer property. Mahmud *et al.*,[19] reported that the supplementation of EO combats cytotoxicity and has protective effect upon chemical carcinogenesis. Ascorbic acid, an important constituent of EO has been shown to prevent cancer.[20] Honey has been reported to promote cell death and inhibit cancer cell growth,[21] possibly due to chrysin- a key component of honey, which has been proved to induce malignant cell apoptosis and cell cycle arrest.[22] The present study has been ventured to investigate the effect of KA on the alterations in glycoprotein components, lysosomal enzymes and marker enzymes in experimental mammary carcinoma in rats.

MATERIALS AND METHODS

Animals —— Female albino Sprague-Dawley rats, 50–55 days, were obtained from the National Institute of Nutrition, Hyderabad, and maintained at 25°C (approx.) on a 12 ± 1 hr day-night rhythm. The rats were fed with commercial rat feed supplied by Hindustan lever Ltd., Mumbai, India under the trade name Gold Mohur rat feed. Food and water were given *ad libitum*.

Animal Model —— The rats were divided into five groups with six animals in each group that were given the following dose regimen. Group I: Control animals. Group II: Breast cancer was induced in overnight fasted animals by a single dose of 7,12-dimethyl benz(a)anthracene (DMBA) in olive oil (25 mg/kg body weight) by gastric intubation. After six weeks mammary carcinoma was confirmed by histological examination. Group III: Breast cancer induced animals (as in Group II) but in addition, after three months, treatment was started orally with KA (300 mg/kg body weight/day) dissolved in 0.5 ml of olive oil and continued for 48 days daily by gastric intubation. Group IV: Mammary carcinoma was induced (as in group II) but in addition, treatment was started from the day of induction orally with KA (300 mg/kg body weight/day) dissolved in 0.5 ml of olive oil and continued for 48 days daily by gastric intubation. Group V: Drug control, the drug KA (300 mg/kg body weight) was administered to control animals for 48 days by gastric intubation.

The study has got the ethical committee clearance and the experimental animals were handled ac-

cording to the University and Institutional Legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

All the animals were maintained under the same time period and sacrificed by cervical decapitation on the same day after an overnight fast. Breast tissues, liver and kidney were immediately excised from the animals and 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.

Hexose was estimated by the method of Neibes.[23] Hexosamine and sialic acid were estimated by the methods of Warren[24] and Wagner,[25] respectively. The values are expressed as mg/g of dry defatted tissue. Acid phosphatase was assayed by the method of King.[26] The enzyme activity is expressed as μmoles of phenol liberated per min/mg protein. CD activity was estimated by the method of Sapolsky *et al.*,[27] and its activity is expressed as μmoles of tyrosine liberated per min/mg protein at 37°C. *β*-D-Glucuronidase and *β*-D-Galactosidase were assayed by the method of Kawai and Anno.[28] *N*-acetyl-*β*-D-glucosaminidase was assayed by the method of Marhur.[29] The enzyme activities are expressed as μmoles of *p*-nitrophenol formed per min/mg protein.

Alanine aminotransferase and aspartate aminotransferase were assayed by the method of King.[30] The enzyme activity is expressed as μmoles of pyruvate liberated per min/mg protein. ALP was assayed by the method of King.[26] The enzyme activity is expressed as IU/l for plasma and μmoles of phenol liberated per min/mg protein for tissue. LDH was assayed according to the method of King[31] and its activity is expressed as IU/l for plasma and μmoles of pyruvate liberated per min/mg protein for tissue. The assay of GGT was carried out by the method of Rosalki and Rau.[32] The enzyme activity is expressed as IU/l for plasma and μmoles of *p*-nitroaniline liberated per min/mg protein for tissue. 5′-NT was assayed by the method of Luly *et al.*,[33] and expressed as μmoles of phosphorus per min/mg protein, where the phosphorus liberated was estimated by the method of Fiske and Subbarow.[34] Total protein was estimated by the method of Lowry *et al.*[35] Protein concentration is expressed as mg/g wet tissue for homogenates.

Statistical Analysis —— Results were presented as mean ± S.D. of six rats. The results were statistically analysed using one-way analysis of variance (ANOVA) followed by the post hoc Student's Neuman Keuls test for multiple comparisons using Statistical Package for Social Sciences (SPSS) computer package. Values of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Figures 1–3 show the levels of glycoprotein components namely, hexose, hexosamine and sialic acid in the liver, breast tissue and plasma of the control and mammary carcinoma bearing animals, re-

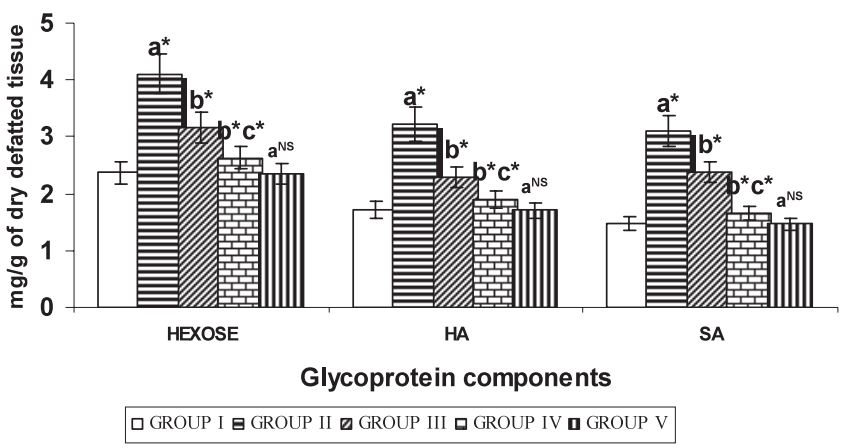

Fig. 1. Effect of KA on the Glycoprotein Components in Liver of Control and Mammary Carcinoma Bearing Animals

Glycoprotein Components (mg/g): Hexose, Hexosamine (HA), Sialic Acid (SA). Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

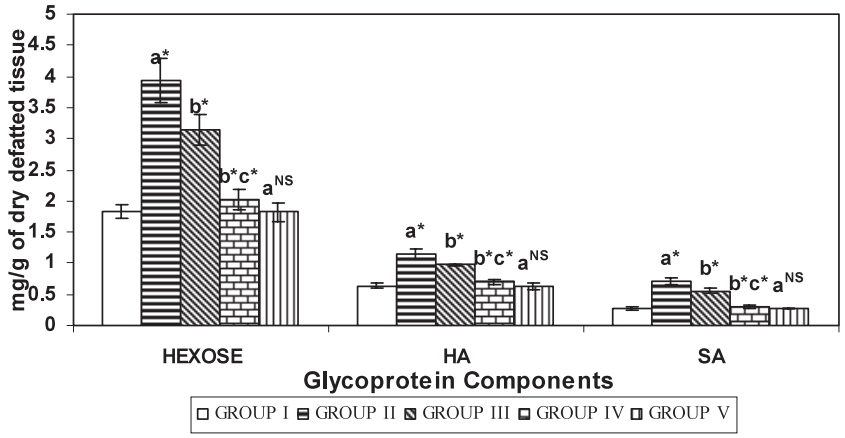

Fig. 2. Effect of KA on the Glycoprotein Components in Breast Tissue of Control and Mammary Carcinoma Bearing Animals

Glycoprotein Components (mg/g): Hexose, Hexosamine (HA), Sialic Acid (SA). Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

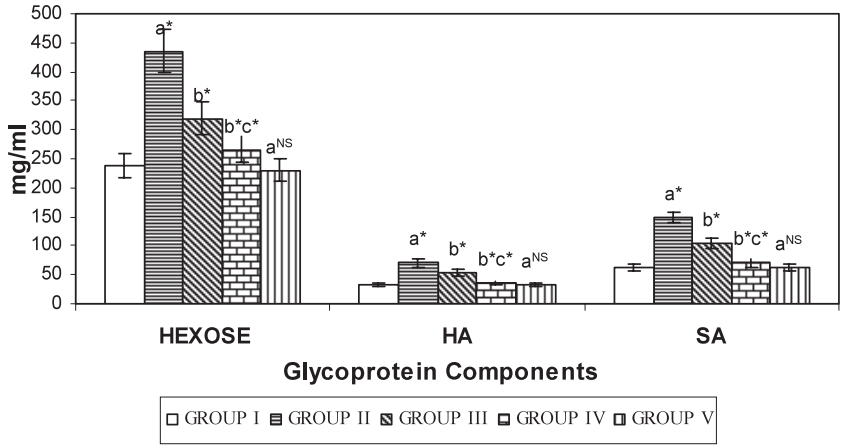

Fig. 3. Effect of KA on the Glycoprotein Components in Plasma of Control and Mammary Carcinoma Bearing Animals

Glycoprotein Components (mg/ml): Hexose, Hexosamine (HA), Sialic Acid (SA). Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

spectively. The levels of these glycoprotein components were significantly ($p < 0.05$) increased in liver, breast tissue and plasma of the mammary carcinoma bearing animals when compared to the control animals. Upon drug administration, the animals under simultaneous treatment responded better to KA, when compared to the animals under post-treatment. The levels of hexose, hexosamine and sialic acid of Group III and IV animals were significantly (liver, plasma $p < 0.05$) decreased when compared to untreated animals. Drug control animals did not show any significant changes when compared to normal healthy control animals.

Carbohydrates are recognized as differentiation markers and as antigenic determinants. Modified carbohydrates and oligosaccharides have the ability to interfere with carbohydrate–protein interactions and therefore, inhibit the cell–cell recognition and adhesion processes that play an important role in cancer growth and progression.[36] Elevations of glycoprotein contents are thus a useful indicator of carcinogenic process and these changes alter the rigidity of cell membrane.[37] Thus, the combined evaluation of these carbohydrate residues of glycoproteins might help to establish a useful aid in strengthening the diagnosis and treatment monitoring of mammary cancer patients.[38] The increased level of glycoprotein components in cancer may be due to the increased synthesis by sequential addition of monosaccharides to parent protein molecule

catalysed by glycosyl transferase. The increased serum sialic acid level in tumour patients has been explained by a spontaneous release (shedding) of aberrant sialic acid-containing cell surface glycoconjugates.[39]

On drug treatment, glycoprotein component levels were reverted back to near normal levels. This could be due to the cytostabilizing property of KA as it contains SA and EO as its principle components.[11,18] EO; previously proven to combat cytotoxicity,[19] has also been shown to confer protective effect upon chemical carcinogenesis, and has been used in the treatment of cancer.

Flavonoids and isoflavones markedly decrease the activity of glycoproteins.[40] Flavonoids and ascorbic acid have been proved to possess inhibitory action against carcinogenesis.[21,41] The drug therefore possibly alters cell membrane glycoprotein synthesis and structure, which in turn indicates its potent antitumour property, contributed by the additive effects of SA, (EO) and honey.

Tables 1–3 show the activities of lysosomal enzymes in liver, plasma and breast tissue of the control and mammary carcinoma bearing animals, respectively. In mammary carcinoma bearing rats, the activities of these lysosomal enzymes were significantly (liver, plasma, breast tissue $p < 0.05$) increased when compared to control animals. Administration of KA showed significant (liver and plasma $p < 0.05$) decrease in the activities of these lysosomal enzymes when compared to untreated animals. Simultaneous KA treated animals showed better response against cancer progression when compared to post KA treated animals. Drug control animals did not show any significant changes when compared to control animals.

The activities of lysosomal enzymes are linked to progression and regression of mammary tumours.[42] Degradation of basement membrane (BM) and ECM plays a crucial role in tumour invasion and metastasis. ECM degradation by proteases is involved not only in local invasion, but also in several stages of metastatic cascade, including angiogenesis, intravasation and extravasation.[43] CD plays a proteolytic role in the digestion of ECM components and plays a crucial role in tumour metastasis. In most breast tumours, CD is over expressed from 2- to 50-fold compared to its concentration in other cell types such as fibroblasts or normal mammary glands.[44] This is an independent prognostic parameter correlated with the incidence of metastasis, shorter survival times and poor prognosis of mammary cancer.[45] Several reports have indicated that CD also plays an essential role in stimulating cancer cell proliferation,[46] β-glucuronidase is shown to be a sensitive marker of lysosomal integrity.[47] Increased serum glycosidase levels found in cancerous conditions may thus be

Table 1. Effect of KA on the Activities of Lysosomal Enzymes in Liver of Control and Mammary Carcinoma Bearing Animals

Parameters	Group I (Control)	Group II (Tumour Induced)	Group III (Tumour Induced + Post KA Treatment)	Group IV (Tumour Induced + Simultaneous KA Treatment)	Group V (Control + Drug)
Acid Phosphatase (ACP) (μmol of phenol liberated per min/mg protein)	5.98 ± 0.54	7.61 ± 0.71^{a*}	7.17 ± 0.64^{b*}	6.64 ± 0.61^{b*,c*}	5.96 ± 0.51^{a,NS}
β-glucuronidase (μmol of *p*-nitrophenol formed per min/mg protein)	24.17 ± 2.10	39.81 ± 3.34^{a*}	32.22 ± 2.90^{b*}	26.85 ± 2.16^{b*,c*}	24.14 ± 2.21^{a,NS}
β-galactosidase (μmol of *p*-nitrophenol formed per min/mg protein)	16.52 ± 1.31	27.83 ± 2.50^{a*}	22.05 ± 2.13^{b*}	18.35 ± 1.16^{b*,c*}	16.50 ± 1.1^{a,NS}
N-acetyl β-D-glucosaminidase (μmol of *p*-nitrophenol formed per min/mg protein)	34.18 ± 3.30	47.27 ± 4.10^{a*}	45.56 ± 4.17^{b*}	37.96 ± 3.14^{b*,c*}	34.19 ± 3.24^{a,NS}
Cathepsin-D (CD) (μmol of tyrosine liberated per min/mg protein)	33.52 ± 3.21	49.95 ± 4.80^{a*}	44.69 ± 4.16^{b*}	37.24 ± 3.61^{b*,c*}	33.49 ± 3.04^{a,NS}

Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

Table 2. Effect of KA on the Activities of Lysosomal Enzymes in Plasma of Control and Mammary Carcinoma Bearing Animals

Parameters	Group I (Control)	Group II (Tumour Induced)	Group III (Tumour Induced + Post KA Treatment)	Group IV (Tumour Induced + Simultaneous KA Treatment)	Group V (Control + Drug)
Acid Phosphatase (ACP) (μmol of phenol liberated per min/mg protein)	21.27 ± 2.15	38.15 ± 3.30$^{a^*}$	28.36 ± 2.24$^{b^*}$	23.63 ± 2.17$^{b^*,c^*}$	21.25 ± 2.05^{a,NS}
β-glucuronidase (μmol of *p*-nitrophenol formed per min/mg protein)	1.87 ± 0.15	8.33 ± 0.63$^{a^*}$	4.49 ± 0.41$^{b^*}$	2.07 ± 0.15$^{b^*,c^*}$	1.87 ± 0.16^{a,NS}
β-galactosidase (μmol of *p*-nitrophenol formed per min/mg protein)	1.32 ± 0.09	5.95 ± 0.38$^{a^*}$	3.76 ± 0.31$^{b^*}$	1.46 ± 0.11$^{b^*,c^*}$	1.31 ± 0.10^{a,NS}
N-acetyl β-D-glucosaminidase (μmol of *p*-nitrophenol formed per min/mg protein)	1.42 ± 0.11	5.33 ± 0.47$^{a^*}$	2.89 ± 0.22$^{b^*}$	1.57 ± 0.11$^{b^*,c^*}$	1.43 ± 0.09^{a,NS}
Cathepsin-D (CD) (μmol of tyrosine liberated per min/mg protein)	6.31 ± 0.55	11.01 ± 1.04$^{a^*}$	9.41 ± 0.88$^{b^*}$	7.01 ± 0.64$^{b^*,c^*}$	6.31 ± 0.59^{a,NS}

Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

Table 3. Effect of KA on the Activities of Lysosomal Enzymes in Breast Tissue of Control and Mammary Carcinoma Bearing Animals

Parameters	Group I (Control)	Group II (Tumour Induced)	Group III (Tumour Induced + Post KA Treatment)	Group IV (Tumour Induced + Simultaneous KA Treatment)	Group V (Control + Drug)
Acid Phosphatase (ACP) (μmol of phenol liberated per min/mg protein)	3.14 ± 0.29	5.96 ± 0.51$^{a^*}$	4.18 ± 0.37$^{b^*}$	3.48 ± 0.30$^{b^*,c^*}$	3.16 ± 0.27^{a,NS}
β-glucuronidase (μmol of *p*-nitrophenol formed per min/mg protein)	17.48 ± 1.70	31.64 ± 2.94$^{a^*}$	26.30 ± 2.38$^{b^*}$	19.42 ± 1.73$^{b^*,c^*}$	17.46 ± 1.65^{a,NS}
β-galactosidase (μmol of *p*-nitrophenol formed per min/mg protein)	14.27 ± 1.35	22.68 ± 2.17$^{a^*}$	19.02 ± 1.50$^{b^*}$	15.85 ± 1.18$^{b^*,c^*}$	14.26 ± 1.10^{a,NS}
N-acetyl β-D-glucosaminidase (μmol of *p*-nitrophenol formed per min/mg protein)	23.46 ± 2.30	34.62 ± 3.31$^{a^*}$	31.28 ± 3.01$^{b^*}$	26.06 ± 2.16$^{b^*,c^*}$	23.47 ± 2.10^{a,NS}
Cathepsin-D (CD) (μmol of tyrosine liberated per min/mg protein)	27.41 ± 2.60	51.22 ± 4.93$^{a^*}$	36.54 ± 3.49$^{b^*}$	30.45 ± 2.94$^{b^*,c^*}$	27.42 ± 2.70^{a,NS}

Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

associated with structural changes in the enzymes which make the liver cell unable to recognize them, thereby preventing their clearance from the blood. The levels of glycan moieties and the activities of glycosidases can be used as diagnostic markers to assess the stage of cancer and can be used as prognostic markers during therapy.[48)]

The decreased enzyme activities observed upon KA treatment could be an evidence for its ability to significantly reduce the leakage of enzymes most likely via stabilizing the membrane archi-

Table 4. Effect of KA on the Activities of Marker Enzymes in Liver of Control and Mammary Carcinoma Bearing Animals

Parameters	Group I (Control)	Group II (Tumour Induced)	Group III (Tumour Induced + Post KA Treatment)	Group IV (Tumour Induced + Simultaneous KA Treatment)	Group V (Control + Drug)
Aspartate transaminase (AST) (μmol of pyruvate liberated per min/mg protein)	27.93 ± 2.10	12.27 ± 1.02^{a*}	20.94 ± 1.90^{b*}	25.13 ± 2.14^{b*,c*}	27.95 ± 2.19^{a,NS}
Alanine transaminase (ALT) (μmol of pyruvate liberated per min/mg protein)	12.34 ± 1.16	6.81 ± 0.62^{a*}	8.25 ± 0.79^{b*}	11.106 ± 1.02^{b*,c*}	12.35 ± 1.10^{a,NS}
Alkaline Phosphatase (ALP) (μmol of phenol liberated per min/mg protein)	1.27 ± 0.10	3.35 ± 0.29^{a*}	1.69 ± 0.12^{b*}	1.41 ± 0.10^{b*,c*}	1.25 ± 0.09^{a,NS}
Gamma Glutamyl Transferase (GGT) (μmol of *p*-nitroaniline liberated per min/mg protein)	3.72 ± 0.32	7.05 ± 0.64^{a*}	4.96 ± 0.41^{b*}	4.13 ± 36^{b*,c*}	3.70 ± 28^{a,NS}
Lactate Dehydrogenase (LDH) (μmol of pyruvate liberated per min/mg protein)	2.68 ± 0.22	4.39 ± 0.41^{a*}	3.57 ± 0.31^{b*}	2.97 ± 0.24^{b*,c*}	2.67 ± 0.22^{a,NS}
5′-Nucleotidase (5′-NT) (μmol of Pi per min/mg protein)	3.61 ± 0.32	5.16 ± 0.43^{a*}	4.81 ± 0.39^{b*}	4.01 ± 0.33^{b*,c*}	3.60 ± 0.27^{a,NS}

Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinoma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

tecture. This could be attributed to the presence of flavonoids that have an inhibiting property on lysosomal membranes.[49] Havsteen *et al.*,[50] have reported the inhibition of acid hydrolases by flavonoids. Decharneux *et al.*[51] proposed a more direct action of flavonoids on lysosomal membranes. Additionally, the localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation as documented by some investigators.[52,53] The combined effects of such flavonoids rich SA, together with EO and honey hence contribute strongly to the therapeutic efficacy of KA. KA has thus been found to stabilize lysosomal integrity, resumption and retrieval of the normal functioning of lysosomes.[54]

Tables 4 and 5 show the activities of marker enzymes in control and mammary carcinoma bearing animals, respectively. In mammary carcinoma bearing animals, the activities of the AST and ALT were significantly (liver $p < 0.05$) decreased, whereas ALP, GGT, LDH and 5′-NT were found to be significantly (liver $p < 0.05$) increased when compared to the control animals. All the marker enzymes were found to be significantly increased in the plasma of tumour bearing animals when compared to normal control animals. On drug treatment, the activities of all these enzymes were significantly brought back to near normal levels. Animals under simultaneous treatment seemed to respond more effectively to KA when compared to the animals under post treatment.

Aminotransferases are important class of enzymes linking carbohydrate and amino acid metabolism. The activities of liver marker enzymes are proportional to the extent of malignancy and can thus be used as indicators for the diagnosis and prognosis of disease. The abnormal variation in the marker enzymes reflect the overall change in metabolism that occurs during malignancy.[55] The elevation in the activities of these marker enzymes in the plasma of the mammary carcinoma bearing animals could be attributed to the destruction of neoplastic tissue.

Tissue damage is the sensitive feature in cancerous conditions; therefore such a deterioration or destruction of the membrane can lead to the leakage of these enzymes from the tissues. Hence, elevation of these liver specific enzymes observed in breast cancer condition is a possible indicator of the progression of tumour growth.[56] ALP, an orthophosphoric monoester phosphorylase, is found to be the most sensitive marker in metastasis.[57] Stieber *et al.*,[58] have shown that determination of ALP is a rapid

Table 5. Effect of KA on the Activities of Marker Enzymes in Plasma of Control and Mammary Carcinoma Bearing Animals

Parameters	Group I (Control)	Group II (Tumour Induced)	Group III (Tumour Induced + Post KA Treatment)	Group IV (Tumour Induced + Simultaneous KA Treatment)	Group V (Control + Drug)
Aspartate transaminase (AST) (μmol of pyruvate liberated per min/mg protein)	5.73 ± 0.52	7.89 ± 0.74$^{a^*}$	6.29 ± 0.57$^{b^*}$	5.8 ± 0.55$^{b^*, c^*}$	5.71 ± 0.53$^{a, NS}$
Alanine transaminase (ALT) (μmol of pyruvate liberated per min/mg protein)	3.54 ± 0.29	5.32 ± 0.49$^{a^*}$	4.65 ± 0.42$^{b^*}$	3.78 ± 0.32$^{b^*, c^*}$	3.54 ± 0.30$^{a, NS}$
Alkaline Phosphatase (ALP) (IU/l)	1.35 ± 0.10	2.47 ± 0.21$^{a^*}$	1.80 ± 0.13$^{b^*}$	1.50 ± 0.11$^{b^*, c^*}$	1.34 ± 0.10$^{a, NS}$
Gamma Glutamyl Transferase (GGT) (IU/l)	1.45 ± 0.10	2.87 ± 0.22$^{a^*}$	2.13 ± 0.17$^{b^*}$	1.61 ± 0.12$^{b^*, c^*}$	1.45 ± 0.10$^{a, NS}$
Lactate Dehydrogenase (LDH) (IU/l)	0.32 ± 0.02	1.85 ± 0.11$^{a^*}$	1.42 ± 0.10$^{b^*}$	0.55 ± 0.04$^{b^*, c^*}$	0.32 ± 0.02$^{a, NS}$
5′-Nucleotidase (5′-NT) (μmol of Pi per min/mg protein)	2.63 ± 0.19	3.96 ± 0.32$^{a^*}$	3.50 ± 0.31$^{b^*}$	2.92 ± 0.24$^{b^*, c^*}$	2.61 ± 0.21$^{a, NS}$

Values are represented as mean ± S.D. of six animals. a; Denotes when compared with Control Animals. b; Denotes when compared with Mammary Carcinoma Bearing Animals. c; Denotes when compared with Mammary Carcinma+Post KA treated animals. *; Denotes $p < 0.05$. NS; Denotes the Non Significance at the level of $p < 0.05$.

method to detect progressive disease in breast cancer and serves to gauge the localization of metastatic diseases. The elevated ALP activity in mammary carcinoma is attributed to the secondary malignancies of mammary carcinoma that metastasizes to bone and liver. Increased ALP activity in breast cancer condition could strongly be associated with cell necrosis, organ dysfunction and cellular injury.[59)]

LDH is a tetrameric enzyme, recognized as a potential tumour marker in assessing the progression of the proliferating malignant cells. The elevated activity of LDH in malignant cells could be attributed to the over production of enzymes by the proliferated cells and further release of their isoenzymes from destroyed tissues.[60)] Elevated levels of glycolysis in cancerous condition may also contribute to increased LDH activity. Walia *et al.*,[61)] have reported increased activity of 5′-NT in breast cancer patients which could be due to rapid proliferation of the breast tumour cells.

The reversal to near normal levels of these marker enzymes observed upon KA administration could be principally attributed to the presence of flavonoids, in conjunction with other ingredients like EO and honey. Bhilawanols, a key constituent of SA, are mainly located in the cell membrane fraction indicating its possible roles in altering membrane permeability and influencing cellular growth.[62)] These results suggest the effectiveness of KA in re-establishing the normal activities of marker enzymes, often deranged in conditions like cancer. No appreciable side effects were observed in drug control animals. This indicates that the drug acts as a safe positive pharmacological agent. Polyphenols and their esters found in KA are found to possess inhibitory activity against mammary carcinogenesis. Sanz *et al.*,[63)] have reported that natural flavonoids have the ability to decrease serum transferases activity in intoxicated animals.

From the results of this study, it can therefore be inferred that KA possesses a profound anticarcinogenic effect through its role in modulating the glycoprotein metabolism, reviving the lysosomal membrane stability and restoring the normal activities of the tumour marker enzymes.

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