ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 9, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

# ANTICANCER EFFECT OF METHANOLIC EXTRACT OF *ANNONA SQUAMOSA* ON MAMMARY CARCINOMA IN RATS WITH REFERENCE TO GLYCOPROTEIN COMPONENTS, LYSOSOMAL, AND MARKER ENZYMES

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***Received: 16 February 2017, Revised and Accepted: 25 May 2017***

**ABSTRACT**

**Objectives:** The aim is to evaluate the anticancer potential of *Annona squamosa* (AS) against 7, 12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary carcinoma in rats.

**Methods:** The tumor was induced in Sprague-Dawley rats by gastric intubation of 25 mg DMBA in 1 ml olive oil. After 3 months of induction period, the methanolic extract of AS at different doses of 100, 150, 200, 250, and 300 mg/kg body weight were administered orally a dose per day to mammary carcinoma-bearing rats for 45 days. The serum and tissue levels of glycoprotein components as well as the activities of marker enzymes and lysosomal enzymes were measured in DMBA-induced mammary carcinoma-bearing rats.

**Results:** Administration of AS resulted in decrease in the levels of marker and lysosomal enzymes and also alterations in the body weight and tumor volume were also restored to near normalcy in a dose-dependent manner. The results of the present study indicate that AS has anticancer effect and it exhibits its potential effect at the dosage of 200 mg/kg body weight in mammary carcinoma-bearing rats.

**Conclusions:** Based on the results obtained, it can be concluded that the methanol extract of AS possesses anticancer properties. Further study is needed to isolate the active principle of this extract responsible for anticancer activity to develop the future pharmaceuticals.

**Keywords:** *Annona squamosa*, 7, 12-dimethylbenz(*a*)anthracene, Glycoproteins, Lysosomal enzymes, Marker enzymes, Mammary carcinoma.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.17836

## INTRODUCTION

Cancer is based on genetic and epigenetic alterations that affect the regulation and function of genes. These pathologic changes are selected during progression to yield cells that multiply aggressively beyond the limits normally set by their intrinsic proliferative capacity, neighboring cells, limited mitogens, and blood supply [1]. Breast cancer is the most common malignant disease affecting women worldwide [2]. It accounts for about one-fourth of all cancers in Indian women with high morbidity and high mortality rate [3]. It is estimated that by 2030, the number of new cases of breast cancer in India will reach just fewer than 200,000/year. The Indian National Cancer Registry Programme reported that the burden of breast cancer patients will be increased to 123,634 by the year 2020 [4].

In addition to the therapeutic strategies, development of target-specific drugs to treat metastatic breast cancer [5]. The earliest adjuvant chemotherapy, with single-agent alkylating and antimetabolic drugs, has now been replaced by combination therapy, as it was demonstrated that simultaneous combination of two or more agents provided better results [6].

*Annona squamosa* Linn. (AS), *Annonaceae*, is popularly known as custard apple. It has been cultivated all over India and traditionally used for the treatment of dysentery, cardiac problems, fainting, worm infections, constipation, hemorrhage, dysuria, fever, thirst, malignant tumors, ulcer, and also an abortifacient [7,8]. The white creamy fruit pulp is soft, edible, so it is employed in preparing cool drinks, and flavor ice puddings. Unripe fruits are dried and powdered to treat ulcer, diarrhea, dysentery, and atonic dyspepsia. It is mixed with gram flour and it has been used to destroy vermin. Ripe fruit made into paste with betel leaves hasten suppuration in tumors [9]. Recently, AS peel extract is reported to have acaricidal, insecticidal, and larvicidal and also it is used for the biosynthesis of palladium and silver nanoparticles [10]. In our pervious study, we have analyzed the phytoconstituents of methanolic and aqueous extract of AS qualitatively and the results showed number of phenolic and flavonoidal compounds. Among these two extracts, methanolic extract of AS showed better radical scavenging activity than the aqueous extract [11].

The present study was designed to study the therapeutic efficacy of methanolic extract of AS on 7, 12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary carcinoma-bearing rats. The status of glycoprotein components, lysosomal enzymes, and tumor marker enzymes in the plasma, liver, and mammary tissues was evaluated in control and experimental rats.

## METHODS

### Plant material

Fresh AS fruits were collected from a local market in Chennai, Tamil Nadu, India. The fruits were identified with reference to the Flora of the Presidency of Madras by Gamble [12].

### Extraction procedure

Methanolic extract of AS has exhibited good radical scavenging activity and has revealed the presence of phenols and flavonoids than the aqueous extract [11]. Further research work was carried using the methanol extracts of AS. About 50 g of AS fruit pulp was weighed accurately and soaked in 50 ml of methanol and kept in a dark place

for 3 days in a shaker. Carbon dioxide was released frequently. After 3 days, sample was filtered and the filtrate was kept in a water bath at about 40°C to concentrate them. The concentrated filtrate obtained was used for further studies to determine the effective dose at different concentrations.

**Chemicals and reagents**

DMBA, nicotinamide adenine dinucleotide+, adenosine 5'-monophosphate, and N-acetylmuramic 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 195-205 g were provided by the Central Animal House facility, University of Madras, Taramani Campus, Chennai - 600 113, Tamil Nadu, India. The animals were maintained under the standard conditions of humidity, temperature (25±2°C), and light (12 hrs light/dark). They were fed with standard rat pellet diet and water *ad libitum*. This study has got approval from the Institutional Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests (Animal Welfare Division), Government of India (No. 01/030/2011).

**Experimental design**

The rats were divided into seven groups with six animals in each group and were given the dose regimen as given below.

- Group I: Control animals.
- Group II: Breast cancer was induced in overnight-fasted animals by a single dose of DMBA in olive oil (25 mg/kg body weight) by gastric intubation.
- Group III: Breast cancer-induced animals (as in Group II) were treated with the drug, methanol extract of AS (100 mg/kg body weight/day) in olive oil orally by gastric intubation for 45 days.
- Group IV: Breast cancer-induced animals were treated with the drug, methanol extract of AS (150 mg/kg body weight/day) in olive oil orally by gastric intubation for 45 days.
- Group V: Breast cancer-induced animals were treated with the drug, methanol extract of AS (200 mg/kg body weight/day) in olive oil orally by gastric intubation for 45 days.
- Group VI: Breast cancer-induced animals were treated with the drug, methanol extract of AS (250 mg/kg body weight/day) in olive oil orally by gastric intubation for 45 days.
- Group VII: Breast cancer-induced animals (as in Group II) were treated with the drug, methanol extract of AS (300 mg/kg body weight/day) in olive oil orally by gastric intubation for 45 days.

Group II animals induced with breast cancer were latter treated with the drugs of methanol extracts of AS. The body weight, organs weight, and mean tumor volume were 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) [13]. 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 analysis.

**Enzyme assays**

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 [14-16]. The values are expressed as mg/dl for plasma and mg/g of defatted tissue. Protein was estimated by the method of Lowry *et al.* [17]. Acid phosphatase and Cathepsin D (CAT-D) enzyme activities were measured by the method of King and Sapolsky [18,19] and their values are expressed as μmoles of phenol liberated per min/mg protein, μmoles of tyrosine liberated min/mg protein, respectively. αD-glucuronidase was assayed by the method of Kawai and Anno [20] and the activity was expressed as μmoles of p-nitrophenol formed per min/mg protein.

The assay of γ-glutamyl transferase (γ-GT) was carried out according to the Rosalki and Rau [21]. The activity was expressed as IU/L for plasma and μmoles of p-nitroaniline liberated per minute/mg of protein for tissue.

Lactate dehydrogenase (LDH) was assayed by the method of King [22] 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 (ND) was determined by the method of Luly *et al.* [23] and expressed as μmoles of phosphorus liberated per minute/mg of protein. The amount of phosphorus liberated was estimated by Fiske and Subbarow method [24].

**Statistical analysis**

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

## RESULTS

The body weight and organ weights such of liver and kidneys were significantly decreased ($p<0.05$), whereas their volume was found to be enormously elevated ($p<0.05$) in mammary carcinoma-bearing rats. On treatment with AS at different concentrations, the body weight, organ weights, and tumor volumes were significantly recoupled back to near normal conditions in a dose-dependent manner. AS at a concentration of 200 mg/kg body weight was found to be the effective dosage (Table 1).

The levels of glycoproteins in plasma, liver, and mammary tissue of control and experimental animals were studied. Elevated levels of protein-bound carbohydrate components such as hexose, hexosamine, and sialic acid in plasma, liver, and mammary tissue were observed in cancer-induced rats when compared to the control rats. After treatment with AS, the glycoprotein levels were significantly decreased in drug-treated animals in a dose-dependent manner when compared to mammary carcinoma-bearing rats. The drug treatment at the concentration of 200 and 250 mg/kg showed a highly significant effect ($p<0.05$) when compared with cancer-induced animals (Fig. 1 and Table 2).

Lysosomal enzymes, namely, acid phosphatase, CAT-D were studied in plasma, liver, and mammary tissue of control and experimental rats. 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 levels were significantly decreased in a dose-dependent manner showing a favorable change in groups treated with 200 mg/kg of the drug AS (Figs. 2-4).

The levels of marker enzymes such as γ-GT, LDH, and ND in plasma and liver of control and experimental animals are depicted in Table 3. The 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 AS-treated groups with a higher significance in 200 mg/kg body weight treatment.

## DISCUSSION

More than 13,000 plants have been studied during the past 5-year period for the development of new chemotherapeutic agents [25]. Use of herbal products has grown dramatically in the Western world. With the narrow therapeutic range associated with most anticancer drugs, there is an increasing need for understanding possible adverse drug interactions in medical oncology [24]. The present study was carried out to estimate the anticancer potential of the AS on DMBA-induced mammary carcinoma in rats. The results showed that administration of AS at the dosage of 200 mg/kg body weight exhibited enhanced anticancer effect.

It is well known that in cancer condition, excessive energy expenditure of the host, ultimately contribute to mechanisms that promote weight

**Table 1: Effect of AS on body weight, organs weight, and tumor volume in the experimental rats**

| Parameters | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|---|---|---|---|---|---|---|---|
| Body weight (g) | | | | | | | |
| Initial | 183.5±10.6 | 185.43±9.54 | 181.85±5.43 | 183.28±7.34 | 184.75±6.7 | 185.89±7.32 | 184.34±9.45 |
| Final | 249.91±8.3 | 204.81±8.54$^{a*}$ | 217.6±9.48$^{b}$ | 224.8±11.2$^{b*}$ | 251.28±10.4$^{b*}$ | 254.56±8.56$^{b*}$ | 253.87±19.21$^{b*}$ |
| Organ weight (g) | | | | | | | |
| Liver | 8.2±0.59 | 6.51±0.45$^{a*}$ | 6.59±0.51$^{b}$ | 7.21±0.54$^{b}$ | 7.65±0.62$^{b*}$ | 7.72±0.59$^{b*}$ | 7.69±0.61$^{b*}$ |
| Kidneys | 1.96±0.14 | 1.32±0.11$^{a*}$ | 1.34±0.12$^{b}$ | 1.52±0.14$^{b}$ | 1.88±0.17$^{b*}$ | 1.89±0.17$^{b*}$ | 1.88±0.18$^{b*}$ |
| Tumor volume ($mm^3$) | - | 5210±437$^{a*}$ | 4865±386$^{b}$ | 44478±312$^{b*}$ | 4054±389$^{b*}$ | 4010±356$^{b*}$ | 4085±348$^{b*}$ |

Values are expressed as mean±standard deviation; n=6. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI and VII - Mammary carcinoma induced and treated with AS at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. $^{a}$When compared with Group I; $^{b}$when compared with Group II; Statistical significance: *p<0.05. AS: *Annona squamosa*, SD: Standard deviation

**Table 2: Effect of AS on the levels of glycoprotein components in experimental rats**

| Parameters (mg/g of defatted tissue) | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
|---|---|---|---|---|---|---|---|
| Liver | | | | | | | |
| Hexose | 4.01±0.37 | 11.54±0.93$^{a*}$ | 9.59±0.78$^{b}$ | 6.44±0.61$^{b*}$ | 4.89±0.44$^{b*}$ | 4.76±0.41$^{b*}$ | 4.79±0.39$^{b*}$ |
| Hexosamine | 3.56±0.28 | 9.04±0.89$^{a*}$ | 7.49±0.66$^{b}$ | 4.23±0.37$^{b*}$ | 4.19±0.38$^{b*}$ | 4.23±0.37$^{b*}$ | 4.15±0.36$^{b*}$ |
| Sialic acid | 3.06±0.24 | 7.15±0.71$^{a*}$ | 6.33±0.45$^{b}$ | 4.78±0.41$^{b*}$ | 3.52±0.33$^{b*}$ | 3.61±0.35$^{b*}$ | 3.51±0.32$^{b*}$ |
| Mammary tissue | | | | | | | |
| Hexose | 2.04±0.19 | 4.98±0.29$^{a*}$ | 4.33±0.4$^{b}$ | 3.98±0.32$^{b*}$ | 2.56±0.19$^{b*}$ | 2.43±0.23$^{b*}$ | 2.46±0.23$^{b*}$ |
| Hexosamine | 0.89±0.06 | 1.78±0.09$^{a*}$ | 1.53±0.11$^{b}$ | 1.33±0.12$^{b*}$ | 1.06±0.09$^{b*}$ | 1.02±0.08$^{b*}$ | 1.08±0.11$^{b*}$ |
| Sialic acid | 0.22±0.02 | 0.74±0.06$^{a*}$ | 0.69±0.05$^{b}$ | 0.51±0.04$^{b*}$ | 0.29±0.02$^{b*}$ | 0.29±0.02$^{b*}$ | 0.28±0.03$^{b*}$ |

Values are expressed as mean±SD; n=6. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI and VII - Mammary carcinoma induced and treated with AS at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. $^{a}$When compared with Group I, $^{b}$when compared with Group II; Statistical significance: *p<0.05. AS: *Annona squamosa*, SD: Standard deviation

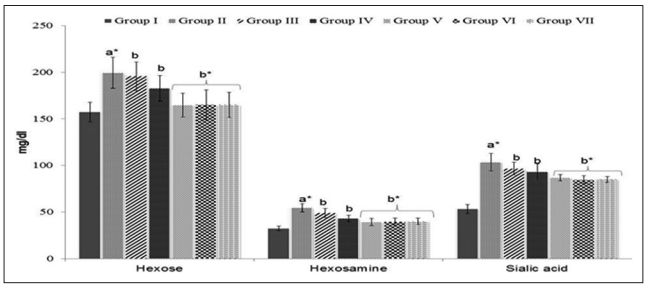


**Fig. 1: Effect of *Annona squamosa* (AS) on the levels of glycoprotein components in plasma of experimental rats. Values are expressed as mean±standard deviation; n=6. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI, and VII - Mammary carcinoma induced and treated with AS at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. $^{a}$When compared with Group I; $^{b}$when compared with Group II; Statistical significance: *p<0.05**

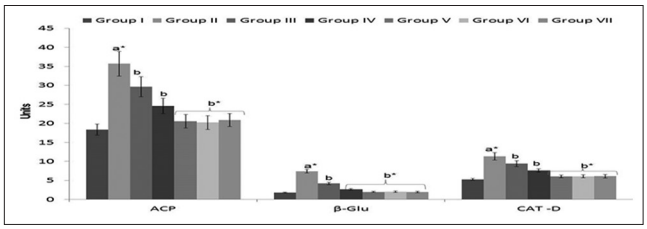


**Fig. 2: The levels of acid phosphatase (ACP), β-glucuronidase (β-GLU), and cathepsin-D (CAT-D) activities in the plasma of experimental rats. Values are expressed as mean±standard deviation; n=6. Units: ACP - µmole of phenol liberated per min/mg protein; β-GLU - µmole of p-nitrophenol formed per min/mg protein; CAT-D - µmole of tyrosine liberated per min/mg protein. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI, and VII - Mammary carcinoma induced and treated with *Annona squamosa* at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. $^{a}$When compared with Group I; $^{b}$when compared with Group II; Statistical significance: *p<0.05**

loss [26]. The weight loss indicates poor diagnosis and tinier survival time for cancer patients [27]. On drug treatment, the body weight was increased. This indicates the curative property of the AS. Medicinal herbs have been shown to improve appetite, food intake, malnutrition, fatigue, and sensation of well-being which could elicit body weight gain [28]. In AS-treated Groups III-VII, the tumor did not disappear totally, but a significant regression was found when compared to induced Group II showing the inhibitory action of the drug on tumor growth. Many studies in different cell lines, animal models, and human epidemiological trials have shown the potential of dietary polyphenols acting as potential anticarcinogenic agents [29]. Flavonoids are reported to have inhibitory action on various stages of tumor development in animal studies [30].

On drug administration, the body weight was significantly increased. It indicates the antineoplastic activity of the drug. This might be due to the presence of various polyphenols, flavonoids, and catechins in the methanolic extract of AS [31]. The inhibiting property may be due to the presence of flavonoids, as they have been reported to import antiproliferative active action on several cancer cell.

Carbohydrates are familiar as differentiation markers and as antigenic determinations. Modified carbohydrates and oligosaccharides have the ability to impede with carbohydrate–protein interactions and therefore, inhibit the cell – cell recognition and adhesion processes that play an important role in cancer growth and metastasize [32]. Tumor metastasis results in death since various treatment strategies have developed [33]. Alterations in the glycoprotein metabolism have been demoralized for the diagnosis of growth [34]. Sialic acid, glycopeptides present in the surface of malignant cell lines [35]. The combined evaluation of these carbohydrate residues of glycoproteins might help to establish potential beneficial effect in the diagnosis and treatment monitoring of mammary cancer patients [36].

**Table 3: Effect of AS on marker enzymes in plasma, liver, and mammary tissues in experimental rats**

| Parameters | Control | Induced | Induced+treated with AS at different dose | | | | |
|---|---|---|---|---|---|---|---|
| | Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII |
| Plasma | | | | | | | |
| γ-GT (IU/L) | 1.56±0.13 | 2.89±0.26[a]* | 2.68±0.24[b] | 2.36±0.23[b] | 1.82±0.16[b]* | 1.85±0.18[b]* | 1.84±0.17[b]* |
| LDH (IU/L) | 0.42±0.04 | 1.66±0.11[a]* | 1.53±0.13[b] | 1.11±0.09[b] | 0.78±0.05[b]* | 0.75±0.06[b]* | 0.78±0.06[b]* |
| 5'-ND (µmol of Pi liberated/min/mg protein) | 3.86±0.28 | 5.44±0.41[a]* | 4.89±0.43[b] | 4.42±0.38[b] | 3.94±0.33[b]* | 4.10±0.37[b]* | 3.91±0.35[b]* |
| Liver | | | | | | | |
| γ-GT (µmoles of p-nitroaniline liberated per minute/mg of protein) | 4.11±0.39 | 7.43±0.64[a]* | 6.78±0.54[b] | 5.44±0.43[b] | 4.56±0.34[b]* | 4.62±0.44[b]* | 4.59±0.41[b]* |
| LDH (µmoles of pyruvate liberated per minute/mg of protein) | 2.65±0.22 | 4.63±0.39[a]* | 4.12±0.31[b] | 3.76±0.33[b] | 2.98±0.24[b]* | 2.86±0.21[b]* | 2.83±0.24[b]* |
| 5'-ND (µmol of Pi liberated/min/mg protein) | 3.54±0.31 | 8.44±0.65[a]* | 7.23±0.56[b] | 5.44±0.42[b] | 4.01±0.29[b]* | 4.32±0.38[b]* | 4.26±0.41[b]* |
| Mammary tissue | | | | | | | |
| γ-GT (µmoles of p-nitroaniline liberated per min/mg of protein) | 3.48±0.31 | 5.82±0.57 | 5.22±0.29[b] | 4.58±0.43[b]* | 3.71±0.54 | 3.73±0.42 | 3.74±0.62 |
| LDH (µmoles of pyruvate liberated per minute/mg of protein) | 2.57±0.25 | 4.35±0.39[a]* | 3.99±0.62[b] | 3.62±0.35[b]* | 2.96±0.29[b]* | 3.02±0.58[b]* | 3.09±0.33[b]* |
| 5'-ND (µmol of Pi liberated/min/mg protein) | 2.62±0.36 | 3.94±0.18 | 3.64±0.40[b] | 3.42±0.31[b] | 2.83±0.30[b]* | 2.94±0.22[b]* | 2.87±0.42[b]* |

Values are expressed as mean±SD; n=6. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI, and VII - Mammary carcinoma induced and treated with AS at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. [a]When compared with Group I, [b]when compared with Group II; Statistical significance: *p<0.05. γ-GT: γ-glutamyl transferase, LDH: Lactate dehydrogenase, 5'-ND: 5'-Nucleotidase

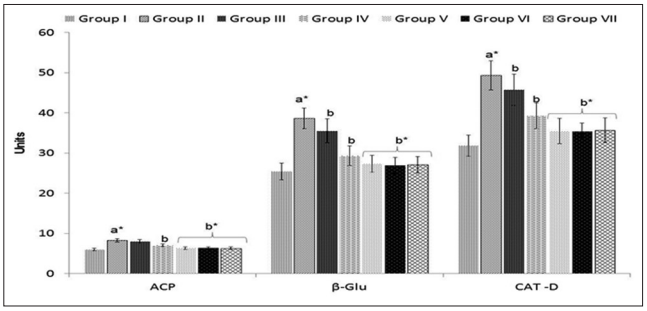


**Fig. 3: The levels of acid phosphatase (ACP), β-glucuronidase (β-GLU), and cathepsin-D (CAT-D) activities in the liver of experimental rats. Values are expressed as mean±standard deviation; n=6. Units: ACP - µmole of phenol liberated per min/mg protein; β-GLU - µmole of p-nitrophenol formed per min/mg protein; CAT-D - µmole of tyrosine liberated per min/mg protein. Group I - Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI, and VII - Mammary carcinoma induced and treated with *Annona squamosa* at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. [a]When compared with Group I; [b]when compared with Group II; Statistical significance: *p<0.05**

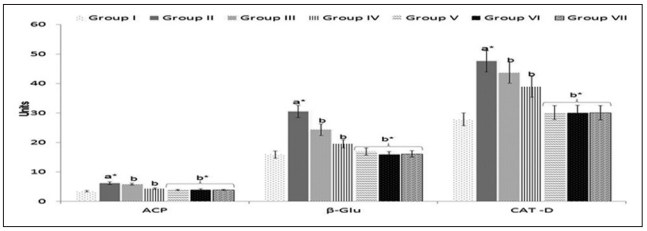


**Fig. 4: The levels of acid phosphatase (ACP), β-glucuronidase (β-GLU), and cathepsin-D (CAT-D) activities in experimental rat. Values are expressed as mean±standard deviation; n=6. Units: ACP - µmole of phenol liberated per min/mg protein; β-GLU - µmole of p-nitrophenol formed per min/mg protein; CAT-D - µmole of tyrosine liberated per min/mg protein. Group I – Control, Group II - Mammary carcinoma induced, Groups III, IV, V, VI, and VII - Mammary carcinoma induced and treated with *Annona squamosa* at the dosage of 100, 150, 200, 250, and 300 mg/kg body weight/day, respectively. [a]When compared with Group I; [b]when compared with Group II; Statistical significance: *p<0.05**

The glycoprotein components levels were reverted to near normal levels on the AS treatment condition, due to the cytostabilizing property of AS fruit. Isoflavones and flavonoids prominently decrease the activity of glycoproteins levels [37]. Flavonoids have been proved to possess inhibitory action against carcinogenesis [38]. Flavonoids acts as inducer of apoptosis and acts as a potent antiproliferative agent and this strategy suggest their potential use in cancer control [39]. Thus the flavonoids, alkaloids, and other bioactive components of the drug might have significantly altered the expression of glycosyltransferases and thereby modulated glycoprotein synthesis and protected the structural reliability of cell surface and membrane, indicating its potent anticancer property.

High levels of lysosomal enzymes released from tumor cells have local and systemic effects [35]. The activities of lysosomal enzymes are linked to evolution and regression of mammary cancer [34]. Lysosomes are a group of cytoplasmic organelles present in numerous animal tissues, characterized by their control of acid hydrolases. These cytoplasmic vesicles contain hydrolytic enzymes that are capable of digesting the macromolecules such as polysaccharides, nucleic acid, and lipids [40]. Malignant tumors contain high levels of lysosomal enzymes which may have local and systemic effects when released from the tumor. Increase of lysosomal enzymes in cancerous condition, it may be due to the abnormal fragility of the lysosomes, which depends on the damage of cell membrane due to the vast production of free radicals in the cancerous condition [41]. The lysosomal aspartic protease CAT-D is overexpressed and hypersecreted by epithelial breast cancer cells. This protease is an independent marker of poor prognosis in breast cancer being correlated with the incidence of clinical metastasis. CAT-D overexpression stimulates tumorigenicity and metastasis [42].

The decreased enzymes activities observed on AS treatment could be an evidence for its ability to significantly reduce the leakage of enzymes most likely through stabilizing the membrane architecture which could have been impacted by the flavonoids.

The lysosomal enzyme levels were reversion on drug-treated animals may be due to the stabilizing property of the drug on lysosomal membrane which could have been impacted by the flavonoids. It is well established that flavonoids have inhibiting property on lysosomal membranes. The drug may amend the lysosomal membrane in such a way that it is capable of blending with the plasma membrane and thereby preventing the discharge of acid hydrolases or by inhibiting the release of lysosomal enzyme [43]. The lysosomes can 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 [44].

The analysis of marker enzymes can be used as an indication of neoplastic condition and therapy [45]. LDH is a tetrameric enzyme recognized as a marker with potential use in assessing the progression of the proliferating malignant cells. In the present study, the activities of LDH increase inbearing animals, possibly as a result of overproduction by cells [46]. LDH is a fairly sensitive marker for solid neoplasm [47]. Numerous reports reveal elevated LDH activity in various types of cancer [48]. This may 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 current study show that 5'-ND activity was elevated in cancerous animals. Veena *et al*. have reported that the increased activity of 5'-ND seems to have originated from the proliferating breast cells [49]. This elevation of the marker enzyme may be correlated with the progression of the malignancy. Walia *et al.* and Canbolat *et al.* have reported higher activities of 5'-ND in cancerous breast tissue of patients [50,51].

The γ-GT is a membrane-bound enzyme that is located on the external surface of cells that exhibit large secretory or detoxification activities [52]. The enzyme level is found to be raised in serum and liver in conditions such as 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 has 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 [53]. In the present investigation, the γ-GT activity was found to be increased in cancer-bearing animals. This is well in accordance with the previous finding of Makpol *et al*., and Fentiman and Allen [54,55].

Several studies have proven the efficacy of flavonoids on tumor inhibition in various animal models [56]. The presence of flavonoids has been shown to impart inhibitory action against carcinogenesis. Phytoestrogens are phenolic non-steroidal plant compounds with estrogen-like biological activity. Most flavonoids are non-estrogenic or weakly estrogenic; however, the isoflavones such as genistein, other flavonoids such as apigenin and kaempferol, and the polyphenolic stilbenes such as resveratrol act through estrogen receptor-mediated mechanisms and also have antiestrogenic effects [57]. Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Flavonoids have a variety of biological effects in numerous mammalian cell systems, *in vitro* as well as *in vivo.* They have been shown to exert antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-inflammatory, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet activities, as well as anticancer effect [58,59].

## CONCLUSIONS

The results of the present study indicate that methanol extract of AS possesses strong anticancer effect. AS extract exerted a strong anticancer effect in a dose-dependent manner and its pronounced effect was observed at the dosage of 200 mg/kg body weight.

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