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Research article

ANTICANCER EFFECTS OF *CARICA PAPAYA* IN EXPERIMENTAL INDUCED MAMMARY TUMORS IN RATS

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ABSTRACT

Objective: To evaluate the anticancer effect of *Carica papaya* in DMBA induced mammary tumors in rats. **Methods:** Wistar rats were divided in to five groups (n=6), Group-I (Normal control) administered vehicle olive oil, Group-II, Group-III, Group-IV and V induced mammary tumors by administering single dose of DMBA (7,12 Dimethyl benz(A)anthracene) orally 65 mg/kg. Group-III was administered aqueous leaf extract of *Carica papaya* (ALQECP) in a dose of 200 mg/kg body wt for a period of 3 months, group-IV has given ALQECP 200 mg/kg body wt for a period of 21 days post 3 months of tumor induction, group-V rats were administered a small dose of *Carica papaya* extract intra tumor locally in the region of tumor. **Results:** Values of CA15-3 were increased in group-II rats (tumor control) significantly, whereas in group-III (prevention group) the levels of CA15-3 were found to be reduced substantially and the P value < 0.001. Similarly, CA-15-3 levels were reduced significantly in group-IV (treatment group) and P<0.005. The levels of LDH were seen to be increased in group-II, where as in group-III LDH levels were decreased and P<0.001. Similarly group-IV LDH levels also reduced significantly but not to the level of group-III. **Conclusion:** Among the various markers for the detection of cancer antigen-15(CA15-3) and lactate dehydrogenase (LDH) are important biochemical parameters that give a clear understanding of the progression and proliferation of cancer cells. In this study it was found that there is increase in the levels of markers such as CA15-3 and LDH and also the tumor volume in tumor control, these marker levels were decreased by the administration of aqueous leaf extract of *Carica papaya* in a dose of 200 mg/kg body wt. ALQECP not only prevented the progression of cancer growth but also has significant effect in reducing the both CA15-3 and LDH levels in treatment group.

Keywords: *Carica papaya*, DMBA, Wistar rats

INTRODUCTION

Breast cancer is one of the principal cause of cancer related deaths in women worldwide and it accounts to the tune of 3,27,000 deaths every year and one in every 10 newly detected cancer cases each year^[1,2]. In India breast cancer is second leading cause of cancer deaths among women's^[3]. Breast cancer development

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]. In India breast cancer is second leading cause of cancer deaths among women.

Carica papaya is known by many other names such as *papaya*, papaw, pawpaw, mamao and melon tree^[8]. It is cultivated for its young leaves, shoots and fruits which are cooked as a vegetable or for its ripe fruit which is consumed as a beverage^[9]. Literature reported that, papaya had positive effect against bacterial various infections^[10]. Treatment with *carica papaya* improved efficiency of phagocytic cells that destroy bacteria^[11]. In vitro studies conducted on extracts from skin, flesh, and seeds of both ripe and unripe *Carica papaya* gave antibacterial activities against various microorganisms including *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Shigella Flexner*^[12]. Papain which is the unique enzyme found in *Carica papaya* is effective natural medicine in controlling both inflammation and edema associated with surgical procedures^[13]. It also produced therapeutic beneficial effects in patients with inflammatory disorders of liver, intestine and eye^[14]. It was reported that some diseases which have inflammatory conditions such as rheumatism, asthma and arthritis wound healing can be treated by using extracts from leaves of *Carica papaya*^[15]. An aqueous extract of *Carica papaya* was also reported for its effect on growth of various tumor cell lines and on human lymphocytes and have shown positive significant results^[16]. The results also showed drastic growth inhibitory activity of *Carica papaya* extract on tumor cell lines^[17]. Thus, literature search we did not found any in vivo study for its anticancer activity.

The present study was designed to study the therapeutic efficacy of *Carica papaya* on DMBA-induced mammary carcinoma bearing rats. The status of tumour marker enzymes such as cancer antigen 15 (CA-15) and LDH in the plasma, was evaluated in control and experimental rats.

MATERIALS AND METHODS

Study design: Experimental animal based study

Ethics approval: The study was approved by the Institutional animal Ethics Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Collection of plant material: Plant The leaves of *Carica papaya* were obtained from farms of Bijapur district Karnataka India and the pharmacognostic authentication was done by Department of Botany KCP Sciences, College Bijapur. Aqueous leaf extract of the *Carica papaya* was prepared by standard extraction procedures^[18].

Chemicals and reagents: 7,12-Dimethylbenz(a)anthracene (DMBA), 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.

Animal: Adult female rats of Albino wistar strain weighing 195-205g were procured from Central Animal House, BLDEU's Sri BM Patil Medical College Hospital & Research Center, Bijapur, India, were used in the study. They were housed in the quarantine room individually in polypropylene cages for one week before the experiment was started. 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.

Experimental design

The rats were divided into five groups with six animals in each group and were given the following dose regimen.

Grouping of animals

Group-I: Normal control-vehicle treated (olive oil 1 ml) by gastric intubation.

Group-II: Tumor control-DMBA (65mg/kg orally single dose in olive oil)

Group-III: Single dose DMBA and ALQECP 200mg/kg orally for 3 months

Group-IV: Single dose DMBA and ALQECP 200mg/kg orally for 21 days post 3 months of DMBA

Group-V: Single dose of DMBA and ALQECP locally at tumor for 21 days post 3 months of DMBA

Mammary carcinoma was confirmed by palpation of tumor and tumor volume was measured by calliper once they have attained measurable size^[19]. At the end of study period blood samples were collected from retro orbital plexus by using heparinised capillary tubes under ketamine anaesthesia for biochemical

investigations (post 3 months+21 days treatment) and the end animals were sacrificed by cervical dislocation.

Estimation of tumor volume: Tumor volume from all the rats was calculated using below formula

$$TV=(w(2) \times L) / 2 \quad \{TV=Tumor \text{ volume, } W=Tumor \text{ width, } L=Tumor \text{ length}\}$$

Biochemical estimations: The levels of cancer antigen (CA15-3) were estimated by ELISA kit sandwich method and lactate dehydrogenase (LDH) levels were estimated by colorimetric absorbance method.

Statistical analysis: Values are given as the mean \pm 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

Values of CA15-3 were increased in group-II rats (tumor control) significantly, whereas in group-III (prevention group) the levels of CA15-3 were found to be reduced substantially and the P value < 0.001 . Similarly, CA-15-3 levels were reduced significantly in group-IV (treatment group) and $P < 0.005$. The levels of LDH were seen to be increased in group-II, where as in group-III LDH levels were decreased and $P < 0.001$. Similarly group-IV LDH levels also reduced significantly but not to the level of group-III. There is no significant change in group-V LDH levels when compared to group-II.

Table 1: Levels of CA-15, LDH and tumor volume

Groups	CA15-3 (IU/ml)	LDH (mU/ml)	Tumour volume (cm ³)
Group-I	7.93(0.42)	4.67(0.65)	-
Group-II	16.92(4.89)	8.87(1.98)	3.30 \pm 2.8
Group-III	13.43(1.66)*	5.73(0.63)*	2.68 \pm 56
Group-IV	14.48(0.34) #	6.38(0.57) #	2.68 \pm 40
Group-V	16.74(2.33)	7.58(0.80)	3.10 \pm 42

Data expressed as (mean \pm SD, n=6) *p-values < 0.001 when compared group-I. #P-Values < 0.05

DISCUSSION

Approximately 13,000 plants have been screened in last five years for the development of new drugs for

the treatment of cancer. This present study was carried with an aim to evaluate the anticancer potential of aqueous leaf extract of *Carica papaya* on DMBA induced mammary tumors in rats. The result showed that administration of aqueous leaf extract of *Carica papaya* at a dosage of 200 mg/kg body wt showed anticancer effect. CA15-3 is a tumor marker used to monitor specifically, breast cancer, along with other types of cancer. It is found on the surface of many types of cancer cells and shed into the blood stream. It is used to monitor advanced stage cancer. According to Keshaviah et al Elevated CA15-3 was found to be associated with an increased chance of early recurrence of breast cancer. (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^[17,20]. Numerous other reports also revealed the elevated levels of LDH in various types of cancers^[21]. 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 increased activity of LDH, 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^[22-23]. Oral administration of ALQECP drug reduced the activity of LDH enzyme to near-normal levels, which may indicate the anticancer effects of the drug.

Tumor volume shows there is significant decrease in the tumor volume in group-III animals indicating once again the anticancer effect of ALQECP in DMBA induced mammary tumors in rats. Aqueous extract of papaya leaves in an unknown composition shown to possess anticancer activity and inhibition of cell proliferation in a various cancer cell lines, this has been patented by Morimoto et al^[25]. Similarly, the ALQECP showed antitumor activity and immunomodulatory activity in tumor cell lines and it proved upregulation of immunomodulatory genes by microarray studies^[26].

Mechanism of action

At University of Florida researchers Nam Dang and his colleagues in Japan have documented papaya's powerful anticancer properties and impact various lab-grown-tumor^[26].

International team of doctors and researchers from US and Japan have discovered that enzymes present in papaya leaf tea have cancer-fighting properties against a vast category of tumors^[27].

Papaya is high Source of Enzyme Papain which Effective against Cancer. Papain is an endolytic plant cysteine protease enzyme isolated from papaya (*Carica papaya L.*) latex. (Abu-Alruz et al., 2009)^[28] It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. (Menard et al., 1990)^[29] The unique structure of papain gives its functionality that helps to understand how this Proteolytic enzyme works and it's useful for a variety of purposes. (*Carica papaya L.*) Latex, (Mitchel, 1970)^[30]. Many cancer cells having a protective coating of fibrin. That is why they go unnoticed for many years. Papain breaks fibrin coat of cancer cell wall. So ultimately it helps against the cancer. Papaya has larger stores of Cancer Fighting Lycopene. According to Stahl et al., 1992 and Knachik et al., 2002, lycopene is a member which helps in overcoming the toxic manifestations of cancer cells.

CONCLUSION

Among the various markers for the detection of cancer CA15-3 and LDH are important biochemical parameters that give a clear understanding of the progression and proliferation of cancer cells. In this study it was found that there is increase in the levels of markers such as CA15-3 and LDH and also the tumor volume in tumor control, these marker levels were decreased by the administration of aqueous leaf extract of *Carica papaya* in a dose of 200 mg/kg body wt. not only prevented the progression of cancer growth but also has significant effect in reducing the both CA15-3 and LDH levels in treatment group.

Limitation of study: However, further investigation using cell culture studies, animal studies and clinical trials are required for confirming the chemoprevention and therapeutic potential papaya leaves and check the adverse affects if any.

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