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Jack Fruit Lectin Binding Pattern in Benign and Malignant Lesions of the Breast

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Abstract. *N-acetyl D-galactosamine specific lectin was isolated from Jack fruit* (Artocarpus integrifolia) *and conjugated to horse radish peroxidase type VI. The purified conjugate was used for the study of tissue binding properties on benign and malignant lesions of the breast using diaminobenzidine as substrate on dewaxed tissue sections. Forty mammary carcinomas, 10 cystic hyperplasias of the breast and 10 normal breast tissues were used for the study. Neoplastic cells showed increased affinity to the lectin. The lectin binding was focally strong in neoplastic cells compared to the normal as well-as the hyperplastic tissues. The stroma of the cancer tissues showed an intense strong binding where elastosis was present. The use of the lectin as a histochemical reagent is discussed.*

Lectins have been used in membrane studies of normal and malignant cells (1, 2). The difference in carbohydrate structures on cell surface have been studied by the use of plant lectin mainly from Peanut Soya bean and *Helix pomatia (2, 3)*. Neoplastic transformation is accopmanied by a variety of changes on cell membrane, most of them involving glycoproteins (4), indicating the invasive potential of tumours and the risk of recurrence after primary tumour (5, 6).

Peanut lectin (PNL) binding to breast epithelium has been studied in detail and was reported to be of use as a marker for mammary differentiation (7, 8). The radio labelled PNL has been used to demonstrate the presence of T antigens on mammary carcinoma cells in cultures (9). Stanley *et al (10)* could not find any correlation of PNL binding with estrogen receptor content in breast carcinomas.

Horse radish peroxidase (HRP) conjugated crude and purified Jack fruit lectin (JFL) has been used for the study of cell membranes in tissues of murine and human origin (11, 12). Recently, Vijayan *et al* (13) have shown that the HRP conjugated JFL may be of use in predicting the malignant transformation of premalignant lesions. So far no attempts have been made to study the JFL binding pattern in breast tissues. Hence the present study was undertaken to evaluate the staining pattern of JFL on benign and malignant lesions of the breast.

Materials and Methods

Forty mammary carcinomas, ten cystic hypersplasias of the breast and ten normal breast tissues were taken up for the study. Wax embedded tissues were cut into 3-5 u size, dewaxed using xylene and rehydrated with graded alcohol. The JFL was prepared as reported earlier by Vijayakumar and Forrester (14) and the lectin was conjugated to HRP type VI and used for the staining as described by Vijayakumar *et al* (12) using diaminobenzidine dihydrochloride [DAB] as substrate. After staining, the slides were mounted and examined under a light microscope. Depending upon the intensity of staining, slides were graded + to + + +. The HRP was purchased from Sigma chemical company, USA and DAB from BDH, England.

Results

The results of the JFL binding in normal, benign and malignant breast tissues are given in Table I and Figures 1 to 5. The neoplastic cells showed increased affinity to the JFL. The JFL binding pattern is focally strong in neoplastic cells when compared with normal and hyperplasia cells. In most cases (70%) the membrane binding seems more intense than cytoplasmic binding, the intensity being much stronger when the tumour cells show an adenomatous pattern. Normal and hyperplastic epithelium showed weak or moderate binding. Apocrine cells showed strong membrane binding with weak cytoplasmic binding. The intensity of staining of carcinomatous cells varied in different cases but all the tumour cells were stained. Only a very few percent of normal epithelial

Abbreviations. PBS. Phosphate buffered saline; JFL: Jack fruit lectin; PNL: Pea nut lectin; HRP: Horse radish peroxidase.

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Table I. *HL binding patterns in benign and malignant lesions of breast.*

Type of tissues	Carcinomatous cells		Epithelial cells		Stromal cells	Apocrine cells
	Membrane	Cytoplasm	Membrane	Cytoplasm		
Mammary carcinoma [n=40]	+ + + [24/40] + + [7/40] + [9/40]	+ + + [13/40] + + [18/40] + [9/40]	+ + [13/40] + [27/40]	+ + [2/40] + [10/40] – [28/40]	+ + + [13/40] + + [7/40] + [20/40]	–
Cystic hyperplasia [n=10]	–	–	+ + + [4/10] + + [5/10] + [1/10]	+ + + [7/10] + + [3/10]	+ + + [9/10] + + [1/10]	+ + + [9/10] + [1/10]
Normal [n=10]	–	–	+ + + [6/10] + + [4/10]	+ + + [7/10] + + [3/10]	+ + + [8/10] + + [2/10]	+ + + [8/10] + + [2/10]

– = no staining
+ = weak staining
+ + = strong staining
+ + + = strong intense staining
* = luminal binding prominant, elastosis present

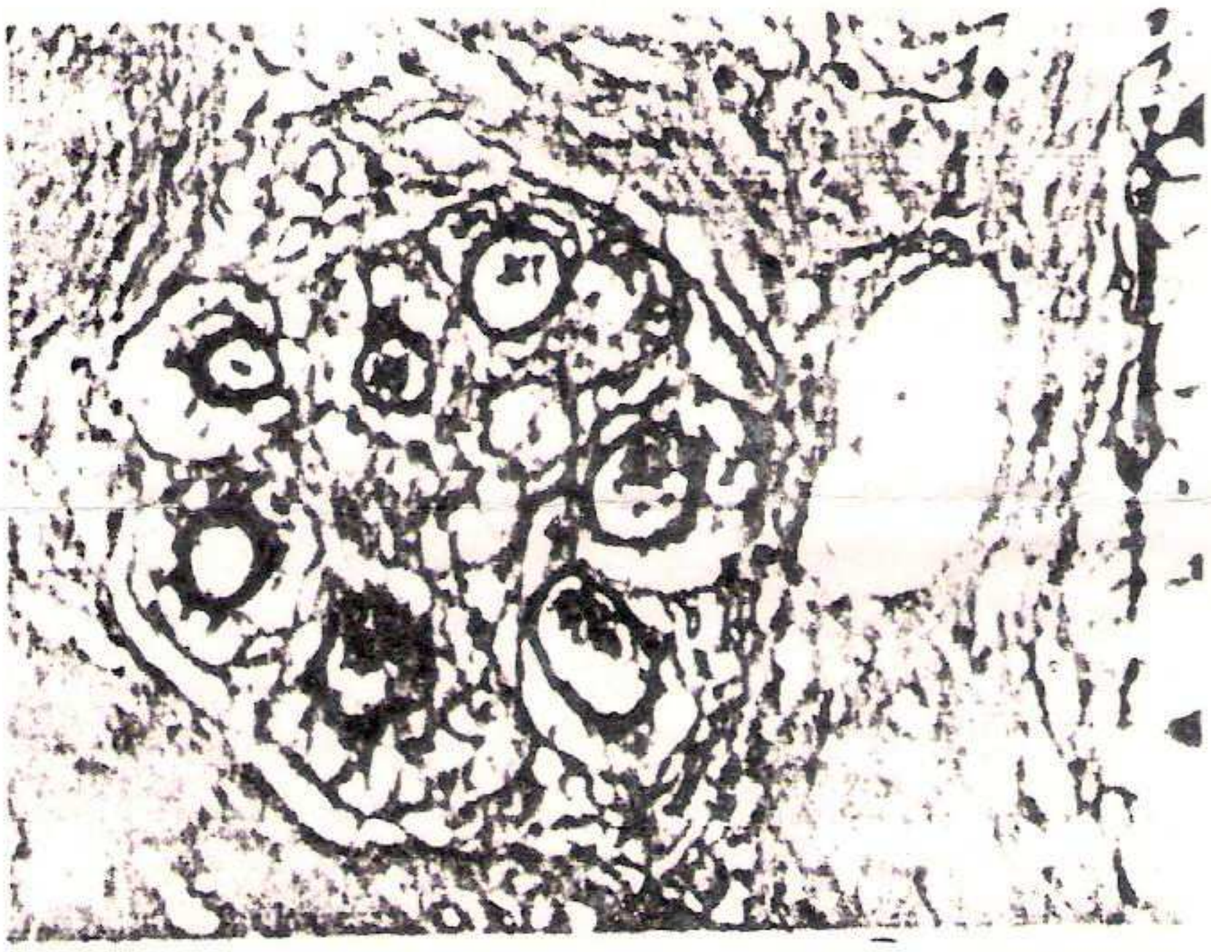

Figure 1. *Dense luminal binding in hyperplastic acinar glands.*

Figure 3. *Dense irregular membrane binding of carcinomatous cells.*

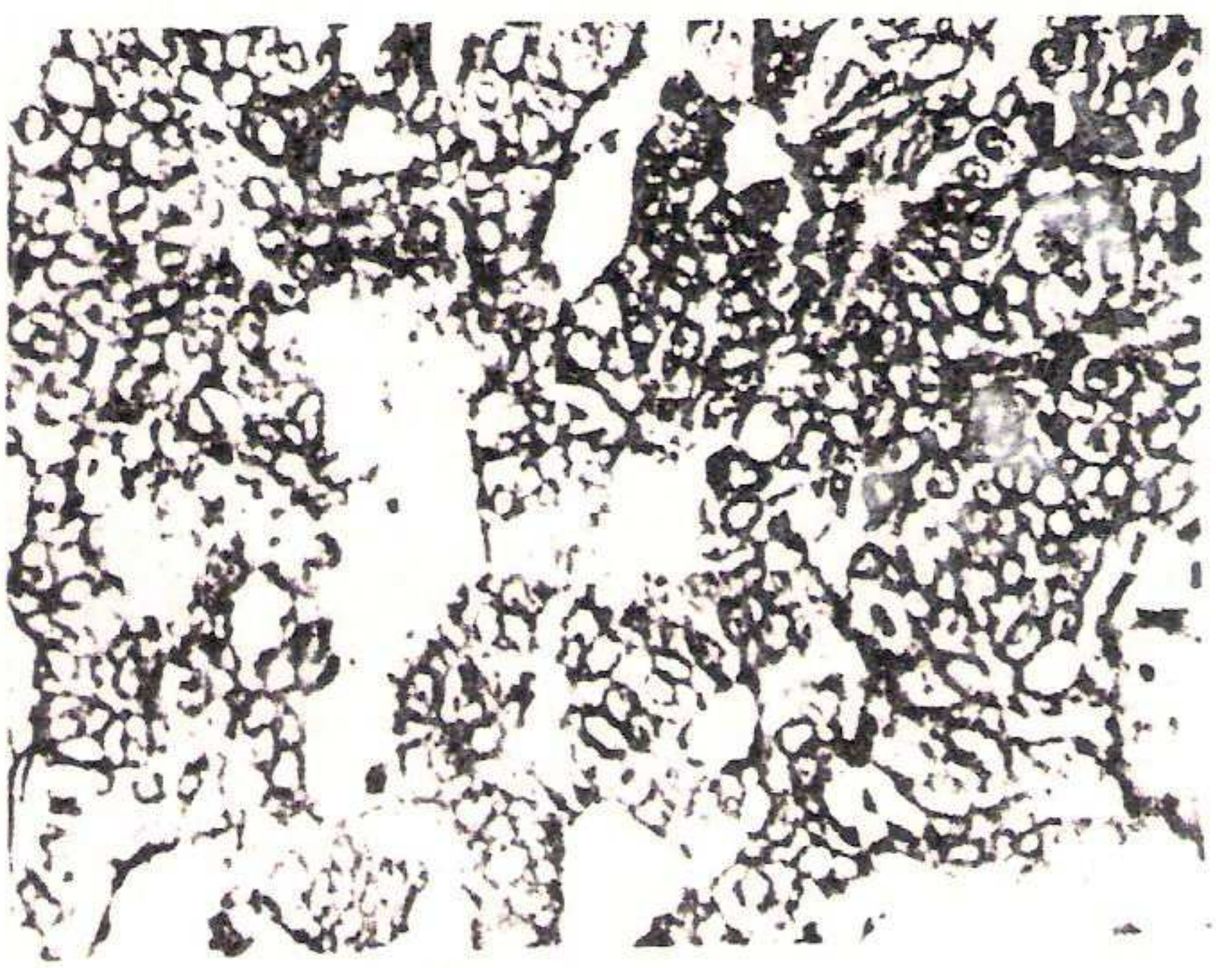

Figure 2. *Carcinomatous cells showing focal cytoplasmic binding*

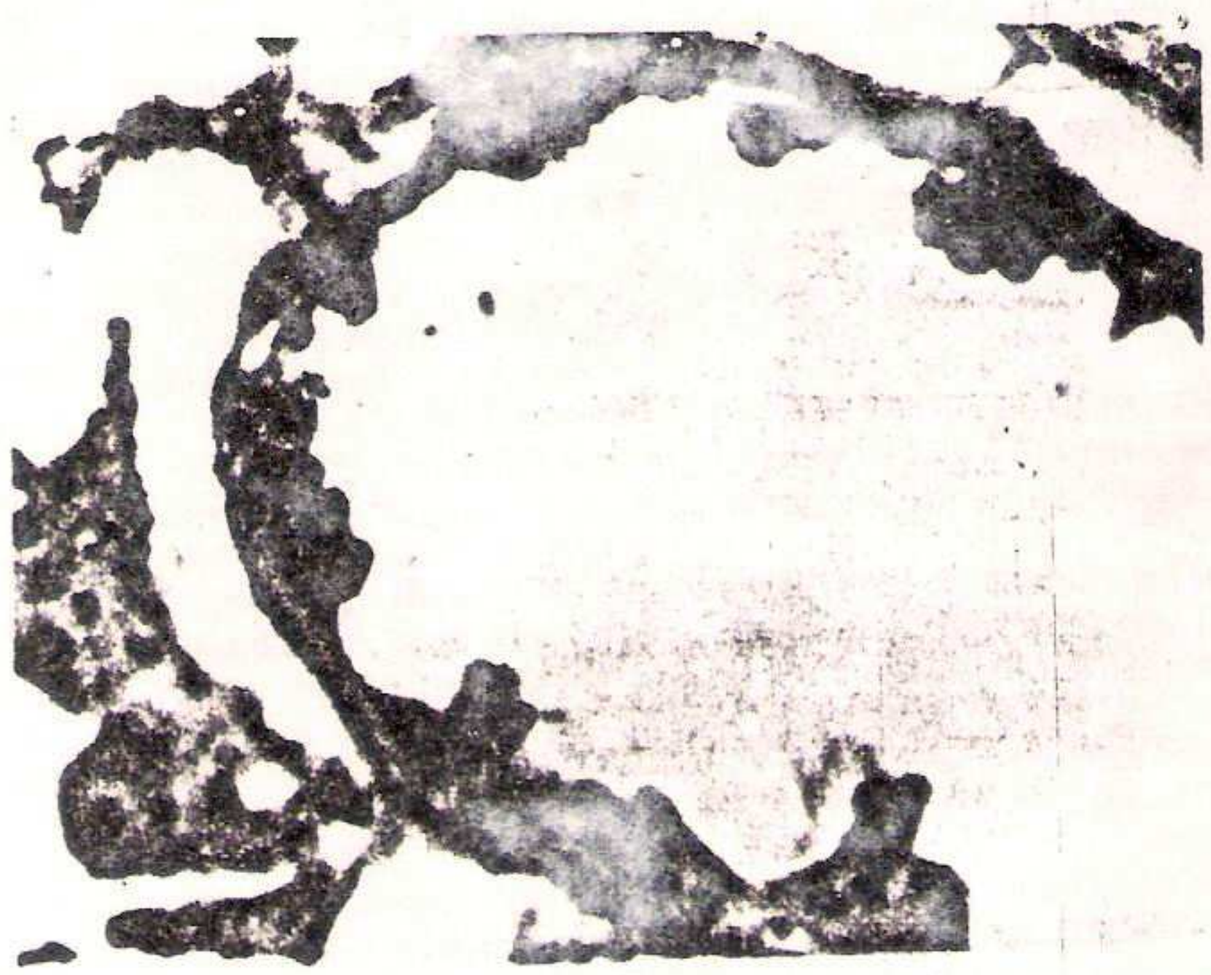

Figure 4. *Apocrine cells showing intense membrane binding (Hyperplasia).*

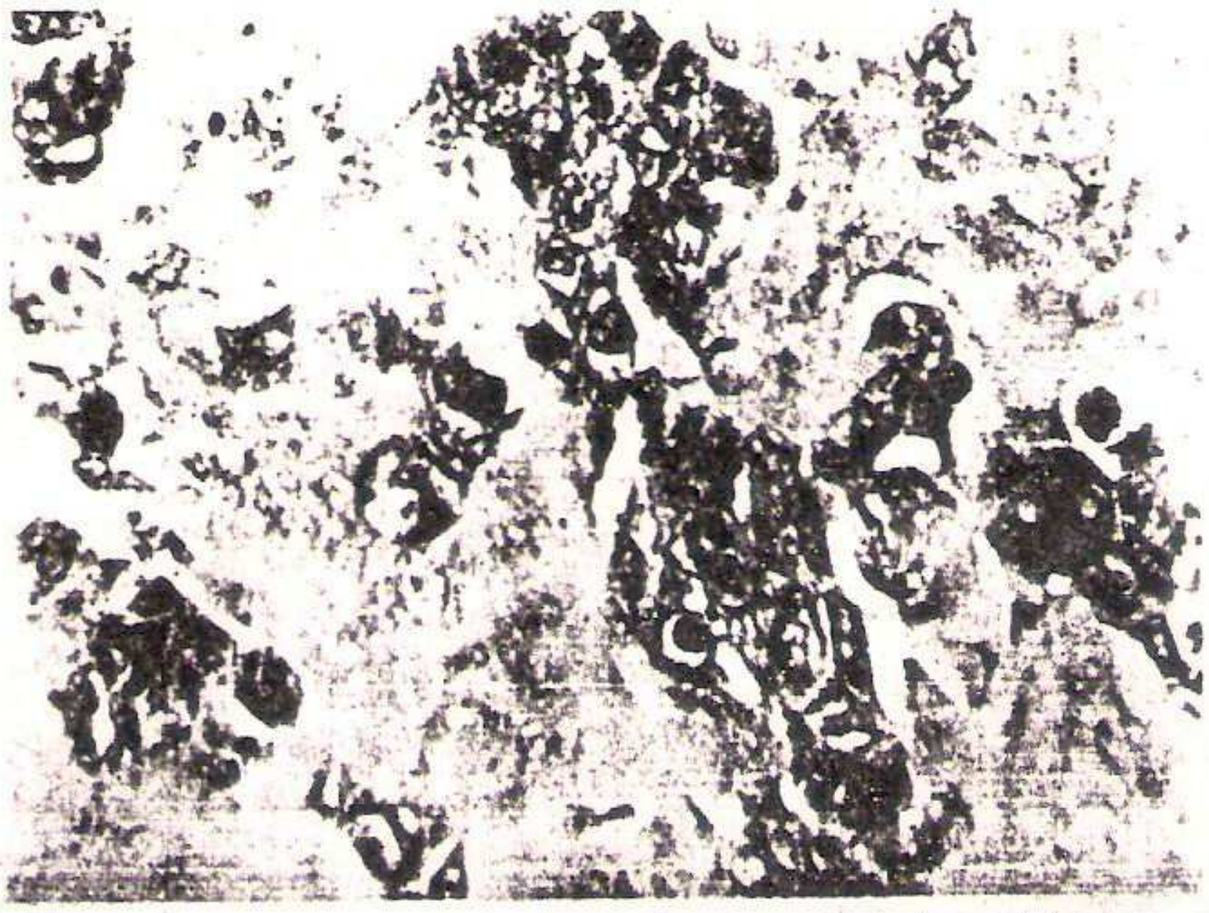

Figure 5. *Some of the cells showing dense cytoplasmic positivity in carcinoma.*

cells seen along with the cancer tissue showed significant membrane and cytoplasmic binding. The pattern was similar in benign proliferative lesions in the breast which showed membrane and cytoplasmic binding.

Discussion

JFL is a non glycosylated protein isolated from the seeds of the jack fruit (*Artocarpus integrifolia*). The HRP conjugated JFL has been reported to be of diagnostic importance in carcinoma cervix and in premalignant and malignant lesions of oral cavity (11, 13). The binding of the JFL could be inhibited completely by N-acetyl D-galactosamine (12). Histochemical applications of this lectin are currently under investigation in diagnostic and prognostic pathology. All the studies carried out so far using JFL conjugate have shown that this lectin may be able to identify malignant tissues even before the clinical signs are manifested (13).

In the present investigation, the HRP-conjugated JFL was used to study the staining pattern in benign and malignant lesions of the breast. The results show that the binding is different in benign and malignant lesions. This difference in binding may be due to the fact that JFL may be able to identify incomplete non-sialylated forms of membrane glycoconjugates which may be expressed at the surface of malignant cells as reported earlier by Ross *et al* (15). Carcinoma associated antigenic alterations in breast cancer were detected by Howard *et al* (16) using lectins. Walker (17) demonstrated the selective staining of malignant cells by plant lectins.

Peanut lectin has been under trial as a histochemical reagent for the last few years (7, 9, 10, 18). Hageman *et al* (8) reported that the PNL binding may be of use to study the differentiation of mammary carcinomas, while Calafat and Janssen (19) could not find any correlation between PNL binding and histology of tumours. Studies using JFL are scanty. The earlier studies of JFL binding were carried out using either crude extract or the partially purified lectin (11, 20). Vijayakumar *et al* (21) has showed that the JFL can bind astrocytoma cells in culture. Vijayan *et al* (13) showed that the JFL can predict the malignant transformation of premalignant lesions of the oral cavity. From this study it seems that the JFL may be of use in distinguishing carcinomatous tissues from benign tissues. The ready availability, the ease of preparations in purified form and the fact that the lectin can be conjugated to diagnostic markers makes the JFL a potential histochemical reagent for exfoliative cytology.

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