Biomedical & Pharmacology Journal Vol. **1**(1), 227-232 (2008)

Agglutination pattern of erythrocytes of normal subjects and cancer patients with lectins of *Tridax procumbans*

ARCHANA P. RAMTEKE and MANDAKINI B. PATIL*

*University Department of Biochemistry, RTM Nagpur University, Nagpur - 440 033 (India)

(Received: May 20, 2008; Accepted: June 21, 2008)

ABSTRACT

Tridax procumbans leaves stem and calyx lectins were found to agglutinate human erythrocytes. The hemagglutination patterns of the erythrocytes of breast cancer patients (Stage I to IV) were compared with the hemagglutination patterns of erythrocytes of normal persons. A gradual decrease in the hemagglutination units was observed with increase in the stages of cancer.

Key words: Breast cancer, Lectins, *Tridax procumbans*.

INTRODUCTION

Scientists have been investigating the nature of the cell membrane. It is well established that cellular adhesion and interactions are largely dependent on the properties of the surface of erythrocytes.

The nature of the neoplastic erythrocyte surface structure was established with the observation that the erythrocytes of normal person and neoplastic patients differ from each other. The phenomenon of invasion and proliferation of cancer cells may well depend largely on the structure of malignant cell membrane[1-5]. It has been observed that the surface sites containing N – acetyl – D – galactosamine residues interact with the agglutinin[6].

Plant lectins were used to detect the alteration in the erythrocytes as they have the ability to recognize membrane glycoconjugates present on the cell surface, and by manifesting significant changes in hemagglutination patterns reflecting the alteration in the erythrocyte membrane. Brooks and co – workers (1991) found that lectins were extremely specific for identifying cells with the improperly assembled membrane glycoproteins of the breast cancer erythrocytes as compared with the erythrocytes of normal person[7].

The present paper describes the results of hemagglutination pattern of normal erythrocytes compared with the erythrocytes of breast cancer patients (Stage I to IV), treated with stem, leaf and calyx lectins of *T. procumbans* Linn.

MATERIAL AND METHODS

Extracts of leaves, stem and calyx of the plant *T. procumbans* L (Family - Compositeae) were used as the source of lectin for the present study [8]. Papain, bovine serum albumin, guar – gum, D – galactose were obtained from Sigma Chemicals St. Louis Mo, USA. Other chemicals were of analytical grade.

Blood samples of normal persons and cancer patients (Stage I to IV) were collected in oxalate bulbs from blood bank, IGGMC and Mayo Hospital, and Rashtra Sant Tukdoji Cancer Hospital, Nagpur.

Preparation of lectins

Leaves, stem and calyx of 45 days old plants were collected from the plants grown in the garden of University Department of Biochemistry, RTM Nagpur University Nagpur, washed four to five times under the tap water and twice with distilled water and soaked between the folds of filter paper and homogenized separately for extraction of lectins as described by Ramteke and Patil, (2005) [9]. Lectins were purified by affinity chromatography by the method of Dixon, 1953 [10]. The purified lectin from leaves was designated at TPL-L, lectin from stem was said to be TPL-S and the lectin of calyx was identified to be TPL-C and characterized as described earlier [9].

Protein estimation

Protein estimation was carried out by the method of Lowry *et al.,* 1951 using BSA as standard protiens[11].

Preparation of erythrocyte suspension

The blood samples were washed three times with PBS. The washed erythrocytes were suspended in PBS to prepare a 2% suspension [12].

Agglutination assay

Hemagglutination assay was carried out using 2% suspension of papain treated erythrocytes of normal subjects and cancer patients. Agglutination inhibition assay was carried out using the method described by Deshpande and Patil, (2002) [13]. The titre strength of the lectin was determined as Hemagglutination Units (HAU) taking the reciprocal of the last dilution showing detectable agglutination [14].

Statistical analysis

The hemagglutination activity of erythrocytes of normal persons and breast cancer cases of different cancer stages (Stage I to IV) is compared to student's t test. The results were statistically analysed {Standard Deviation (Sd), Critical Difference (CD), and Correlation Coefficient (CC)} using the formula as suggested by Persons (1947), [15].

$$Sd = \sqrt{\Sigma \frac{(d-\bar{d})^2}{n-1}}$$

where

Sd = Standard Deviation

$d - \bar{d}$ = The bias (mean difference, ,)

n = number of samples analysed

$$t = \frac{\left[\bar{d_a} - \bar{d_b}\right]}{\left[\frac{(n_a-1)Sd_a^2+(n_a-2)Sd_b^2}{n_a+n_b-2}\right]\left[\frac{1}{n_a}+\frac{1}{n_b}\right]}$$

where

t = "Students" t Test

d_a = mean difference (series1)

d_b = mean difference (series2)

Sd_a = Standard Deviation (series1)

Sd_b = Standard Deviation (series 2)

n_a = number of samples analysed (series1)

n_b = number of samples analysed (series2)

The difference in control group and cancer cases were considered significant if $p < 0.01$ [15]. The carcinoma was detected under the guidance of Oncologist by necessary clinical laboratory investigation [16].

RESULTS AND DISCUSSION

Tridax procumbans L, a wild medicinal plant was found to contain galactose specific lectin in leaves, stem and calyx. The lectins were purified to homogeneity by affinity chromatography and were found to have molecular weight of 23kD, 20kD, and 23kD for leaves, stem and calyx respectively. TPL-L TPL-S and TPL-C were able to agglutinate erythrocytes of breast cancer patients with less dilution than the normal (Table 1). The results illustrated in Table 1 appear to be statistically significant [16]. These observations suggest that the receptor sites present on the erythrocytes of cancerous patients and normal persons may be different in number. David *et al., (*1978), also reported variations in lectin agglutination between erythrocytes of normal persons and cancer patients[17]. The alteration in the cell membrane depends on the molecular basis. The changes on the erythrocyte surface may be due to the glycoprotein receptor sites present on the erythrocytes of cancerous patients and normal

Table 1: Heamagglutination pattern of *Tridax procumbans* lectins with erythrocytes of normal person and breast cancer patients

Conditions And Stages	Number of cases	Average Titre		Hemagglutination units per ml				Statistical comparison of normal control v/s cancer patients					
								CC			CD		
		TPL – L	TPL – S	TPL – C	TPL – L	TPL-S	TPL-C	TPL-L	TPL-S	TPL–C	TPL-L	TPL-S	TPL-C
Control	86	37.03±11.99	18.07±9.89	37.03±11.99	2560	1280	2560	NA	NA	NA	NA	NA	NA
Breast Cancer Stage I and II	70	13.15±4.67	7.47±4.93	13.15±4.67	950	470	950	0.49	0.34	0.49	7.75*	6.46**	7.75*
Breast Cancer Stage III and IV	50	1.08±0.41	1.02±0.40	1.08±0.41	400	200	400	0.04	0.02	0.04	12.2*	8.89*	12.2*

TPL – L – *Tridax procumbans* leaf lectin, TPL – S – *Tridax procumbans* stem lectin, TPL – C – *Tridax procumbans* calyx lectin, *p=0.001, **p=0.02, ns=not significant, SD – Standard Deviation, CC – Correlation Coefficient, CD – Critical Difference, NA – Not applicable.

persons. The galactose epitopes may be increased in the erythrocytes of the breast cancer cases as compared to the normal individuals [16]. Hemagglutination studies were also carried out by Mitchell *et al.,* (1985) using *H. pomatia agglutinin* and *P. vulgaris leucoagglutinin* with erythrocytes of breast and colon cancer and reported the difference between the binding pattern in metastizing human breast and colon cancer with HPA, and PHA – L [18]. Hasija (1991) reported that the binding pattern of cancer cells differ with different lectins. He found that out of thirty-five plant lectins, sixteen plant lectins showed no difference in the titre value (first group), however a marked difference was observed in the titre value with nine plant lectins (second group), while six lectins required a higher dilutions for agglutinating the cancer cells (third group) and four lectins required a lower dilution for agglutinating the cancer cells (fourth group), as compared to the erythrocytes of normal persons [12]. Likewise *T. procumbans* lectins required a lower dilution for agglutinating the erythrocytes of breast cancer cases than the erythrocytes of normal persons. The difference between HAU due to lectin with the erythrocytes of normal and cancer patients were measured and significant results were obtained (Table 1). The results demonstrated that *T. procumbans* lectins required for agglutinating the erythrocytes of breast cancer patients were significantly more ($p = 0.001$ i.e. < 0.05 St t test for TPL – L and TPL - C) and ($p = 0.02$ i.e. less < 0.05 St t test for TPL – S) in stage I, and II as compared with control. *T. procumbans* lectins required for agglutinating the erythrocytes of breast cancer patients were still significantly more ($p = 0.001$ i.e. < 0.05 St t test for TPL – L, TPL – C, and TPL – S) in stage III and IV, as compared with control. 95% confidence limit for normal subjects was calculated by taking mean 1.96 standard error which was found to be 2560 units for TPL – L, 1280 units for TPL – S and 2560 units for TPL – C. More than 85.68% values showed their clustering towards the below upper bound limit of the normal cases. However the HAU values of the cancer patient's stage II and I were above the upper bound limit of normality range of 95% confidence limit as shown in Table 2. The Correlation Coefficient measured (Table 1) demonstrates that as the cancer stage increases the agglutination average titre decreases [16]. Durgawale *et al*., (2001), also reported similar results

Table 2: Hemagglutination activity (units / ml) and distribution of 95% Confidence limit of *Tridax procumbans* lectins with erythrocytes of normal persons and breast cancer patients

Conditions and Stages	Number of cases	Below upper bound limit			Above lower bound limit		
		TPL-L	TPL-S	TPL-C	TPL- L	TPL-S	TPL-C
Control	86	44 (91.7%)	44 (81.2%)	44 (91.7%)	26 (8.3%)	26 (18.2%)	26 (8.3%)
Breast Cancer Stage I and II	70	50 (91.08%)	50 (45%)	50 (91.08%)	20 (8.92%)	20 (55%)	20 (8.92%)
Breast Cancer Stage III and IV	50	35 (95%)	35 (71%)	35 (95%)	15 (5%)	15 (28%)	15 (5%)

TPL-L- *Tridax procumbans* leaf lectin, TPL- S- *Tridax procumbans* stem lectin, TPL-C - *Tridax procumbans* calyx lectin

with *Synadenium root* (Hook F) lectin and stated that more receptor sites were available on the surface of cancerous cells [16]. Hemagglutination pattern of *T. procumbans* lectins also showed that binding sites differ on erythrocyte membrane in different stages of cancer (Stage I to IV). Similarly David *et al.,* (1978), also reported that binding sites differ in different stages resulting in cell membrane changes and hemagglutination pattern with lectins [17].

Lectins are capable of binding exogenous carbohydrate - containing molecules present on the surface of tumor cells and internalize them by endocytosis. Lectins can be potentially used in cancer treatment strategies for instance wheat germ agglutinin as reported by Lotan, (1983) [19]. Lectins are known to be good markers and have been used for detection of the development of breast cancer causing carbohydrate alterations, which is due to the incomplete glycosylation in the cancer cells as reported by Hull *et. al*., (1991)[20]

Breast Cancer is very common in women in developed countries and more than 40% of all breast cancer cases were reported in developing countries [21]. There was a significant decrease in the amount of TPL –L, TPL – S and TPL – C, required for agglutinating the cancer cells (Stage I to IV). The lectins in *T. procumbans* leaves, stems and calyx were able to recognize the altered glycosylation on cancer cells appearing to make itself remarkable proteins that can be used in the detection of erythrocytes, during the stages (I to IV) of breast cancer. These results suggest that lectins of *T. procumbans* and can be used as a diagnostic tool to differentiate cancer cells from normal erythrocytes and maybe helpful in the design of novel therapeutics [22, 23].

ACKNOWLEDGEMENTS

Authors thank the Director, Rashtra Sant Tukdoji Cancer Hospital, Nagpur, and staff, Blood Bank, Indira Gandhi Medical College and Mayo Hospital, Nagpur for their kind co-operation and grateful to Head, Department of Biochemistry, Nagpur University, Nagpur for encouragement.

REFERENCES

1. Ambrose, E. J., in Biological Interactions in normal and neoplastic growth, ed. Brennan M. J., and Simpson, W. L., (little Brown and Company), 149 (1962).
2. Bervick, L., and Coman, D. R., some chemical factors in cellular adhesion and stickness. *Cancer Research.* **22**: 982-986 (1962).
3. Coman, D. R., Adhesiveness and Stickness: two independent properties of the cell surface. *Cancer Research*. **21**: 1436-1438, (1961).
4. Easty, G. C. and Motolo, V., The nature of the intracellular material of adult mammalian tissue. *Exptl. Cell. Res.* **21**: 374-385 (1960).
5. Abercrombie, M., and Ambrose, E. J., The surface properties of cancer cells: a review. Cancer Research. **22**: 525-548 (1962).
6. Morde, D. S., and Nagada, K. K., Erythrocyte surface changes in patients with cancer of G. I. tract, Ast. C. C. N. Ann. Conf. Assoc. Clin. Biochem. India, A. F. M. C. Pune, C-2, (1998).
7. Brooks, S. A. and Leathem, A. J. Prediction of lymph node involvement in breast cancer by detection of altered glycosylation in the primary tumour. *Lancet.* **33**: 71-74, (1991)
8. Ugemuge, N. R., Flora of Nagpur District, First Edition, Shri Prakashan, Nagpur, p216, (1986).
9. Ramteke, A. P., and Patil, M. B., Purification and Characterization of *Tridax procumbans* calyx lectins. *Biosci. Biotech. Res. Asia.* **3**(1): 103-110 (2005).
10. Dixon, M., Nomogram for ammonium sulfate solution. *J. Biochem.* **54**: 457-458, (1953).
11. Lowry, O. H., Rosebrough, N, J., Farr, A. L., and Randall, R, J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **193**: 265-275 (1951).

12. Hasija, K., Lectin in agglutination and its role in detection of sugars on the surface of red blood cells of normal and cancer patients. *Ind. J. Clin. Biochem.* **6**: 89-96 (1991).
13. Deshpande, K., and Patil, M., Studies on lectins of medicinal plants. *Ph. D. Thesis,* Nagpur University, Nagpur, (2002).
14. Deshpande, K., and Patil, M., *Ind. Vet. Med. J.* **25**: 385-387 (2001).
15. Sir Austin Bradford Hill, (Tenth Edition) A short textbook of medical statistics. The English Language Book Society, Hodder and Stoughton London. **86**: 173, 285, 297, 313 (1997).
16. Durgawale, P. P., Shukla, P. S., Sontakke, S. D., and Chougale, P. G., Differential erythrocytes agglutination pattern in normal and cancer patients with *Synadenium Grantii root* (Hook – F) lectin. *Ind. J. Clin. Biochem.* **16**(1): 110-112 (2001).
17. David, C., Kilpatrick, C., and Yeoman, M. M., Purification of lectins from *Datura stramonium, Biochem. J.* **175**: 1151-1153 (1978).
18. Mitchell, B. S., Brooke, S. A., Leathern, A. J., and Schumacher, U., Do HPA, and PHA – L have the same binding pattern in metastizing human breast and colon cancer? *Cancer Lett.* 16, **123**(1): 113-119 (1998).
19. Lotan, R., Differentiation associated modulation of lactoside binding lectins in cancer cells. In H. Gabius and S. Gabius (Eds) Lectins and Cancer. Springerverlag Berlin. 153-169 (1983).
20. Hull, H., Sugarman, E., Spielman, J. and Carraway, K., Biosynthetic maturation of an ascites tumour cell surface sailo-mucin. Evidence for O-glycosilation of cell surface glycoprotein by the addition of new oligosaccharide during recycling. *J. Biol. Chem.,* **266**(21): 13580-13586 (1991).
21. Sharma, B. K., and Ray, A., Breast and Prostate Cancer, *Ind. J. Clin. Biochem,* **15**: 110-117 (2000).
22. Van Damme, E. J. M., Peumans, W. J., Pusztai, A. and Bardoez, S.,Eds. Handbook of Plant Lectins: Properties and biomedical applications. John Willey and Sons. Chichester. U. K., 59-80 (1998).
23. Sherwani, A. F., Mohmood, S., Khan, R. H., and Azfer, M. A., Characterization of lectins and their specificity in carcinomas-An Appraisal. *Ind. J. Clin. Biochem.* **18**(2): 169- 180 (2003).