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Interaction pattern of *Tridax procumbans* lectins with erythrocytes of normal and diabetic patients

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ABSTRACT

The results of interaction between lectins of *Tridax procumbans L* and erythrocytes from the normal and diabetic patients show that as the level of blood glucose increases the hemagglutination titre decreases.

Key words: *Diabetes mellitus*, Lectins, *Tridax procumbans*.

INTRODUCTION

Most of the diseases are known to be associated with changes in the glycosylation profiles of membranes proteins. The glycosylation patterns reflect the internal and external environment of the cells. The glycosylated proteins act as sensitive indicators of alterations in cell function brought about by the disease. Changes in the glycosylation are therefore being widely used as markers both for initial diagnosis and for following the disease progress [1, 2].

Lectins or hemagglutinins have the ability to recognize and bind with specific carbohydrate residue on cell surface membrane. This specific property of these proteins has been widely employed in biochemical research [3, 4]. Lectins bind to the specific terminal of carbohydrate residues of glycoconjugate on the cell surface and have therefore been used as specific agents to study cell surface structure and structural component of cell membrane [5]. Plant lectins have the ability to recognize complex glycoconjugates present on the cell surface and can be used to detect the alteration in the erythrocytes by manifesting significant changes in the hemagglutination titre. Noticeable changes in hemagglutination patterns, glycosylation profiles of normal and diseased cells are reported by Goldstein and Hayes (1978) [5].

In the present paper the hemagglutination pattern of erythrocytes of insulin dependent and non - insulin dependent *Diabetes mellitus* patients has been compared with the hemagglutination pattern of erythrocytes of normal persons using the lectins of *T. procumbans L*.

MATERIAL AND METHODS

Leaves, stem and calyx of the plant *T. procumbans L* (*Family - Compositeae*) were used as the source of lectin [6]. 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 diabetic patients were collected in citrate bulbs from blood bank, and clinical biochemistry laboratory of local hospital, Nagpur.

Isolation of lectins

Leaves, stem and calyx of 45 days old plants were collected from 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) [7]. Lectins were purified to homogeneity by affinity chromatography by the method of Dixon, (1953), [8]. The lectins were characterized as described earlier[9].

Protein estimation

Protein estimation was carried out by the method of Lowry *et al.,* (1951) using B. S. A. as standard protein[10].

Agglutination assay

The method of Deshpande and Patil (2002) was used to perform agglutination assay, using 2% suspension of papain treated erythrocytes of normal and diabetic patients [9]. The hemagglutination titre was determined by serial dilution in 96 U well plates. The reciprocal of the last dilution showing detectable agglutination was taken as the titre strength of the lectin and expressed as Hemagglutination Units (HAU) [11].

Evaluation

The hemagglutination activity of erythrocytes of normal persons and diabetic patients (NIDDM, IDDM) is compared to student's t test. The results were analysed with suitable statistical analysis (Standard Deviation, Critical Difference, and Correlation Coefficient) by the method of Persons 1947, using the following formula [12].

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

where

Sd= Standard Deviation

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

n = number of samples analysed

where

t = "Students' " t Test

= mean difference (series1)

= 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 diabetic cases were considered significant if $p < 0.01$ [12, 13].

RESULTS AND DISCUSSION

Tridax procumbans L 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 respectively. Results presented in Table 1 and 2 show that there is a gradual significant reduction in the hemagglutination titre with the duration of diabetic condition in NIDDM patients, whereas erythrocytes of the IDDM patients show a significant drastic decline in the titre as compared to the erythrocytes of normal individuals. Changes in the hormones and insulin deficiency are known to effect macromolecular synthesis. Membrane proteins get glycosylated during diabetic conditions and undergo complex changes [14].

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

Table 1: Hemagglutination pattern of *Tridax procumbans* lectins with erythrocytes of normal persons and diabetic patients

Conditions and Duration	Number of cases	Fasting Blood glucose average (mg/dl)	Hemagglutination Titre			CC	CD
			TPL-L	TPL-S	TPL-C		
Control	86	98.09±5.67	16-64	8-32	16-64	NA	NA
NIDDM	177	217.3±21.68				4.96	
0-2 years	80		16-32	8-16	16-32		
2 –10 years	67		8-32	4-16	8-32		
10 years and above	30		8-16	4-8	8-16		
IDDM	73	357.2±70.14				2.55	0.36**
0 –2 years	30		4-8	2-4	4-8		
2 –10 years	35		2-8	1-4	2-8		
10 years and above	8		2-4	1-2	2-4		

*p=0.002, **p=0.05, SD – Standard Deviation, CC – Correlation Coefficient, CD– Critical Difference, TPL – L – *Tridax procumbans* leaf lectin, TPL – S – *Tridax procumbans* stem lectin, TPL – C –*Tridax procumbans* calyx lectin, NIDDM – Non-Insulin dependent *Diabetes mellitus*, IDDM – Insulin dependent *Diabetes mellitus.*

Table 2: **Hemagglutination Units of *Tridax procumbans* lectins with erythrocytes of normal persons and diabetic patients**

Conditios	**No of cases**	**Average titre**			**Hemagglutination units per ml**			**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 ± 11.99	18.7 ± 9.89	37.3 ± 11.99	2560	1280	2560	NA	NA	NA	NA	NA	NA
NIDDM	177												
0 – 2 years	80	28± 6.45	14± 3.48	28± 6.45	1280	640	1280	0.39	0.47	0.39	3.9****	6.12**	3.9****
2–10 years	67	21.1 ± 7.93	8.83 ± 3.62	21.1 ± 7.93	1280	640	1280	0.73	0.53	0.73	5.10**	5.2****	5.10**
10years and above	30	13.4 ± 3.84	6.67 ± 1.92	13.4 ± 3.84	640	320	640	0.69	0.69	0.69	4.69*	3.59*	4.69*
IDDM0 – 2 years	**73**												
	30	5.73 ± 2.02	2.87 ± 1.01	5.73 ± 2.02	1280	640	1280	0.16	0.16	0.16	6.39***	4.86****	6.39***
2–10 years	35	5.65± 2.41	2.98 ± 1.06	5.65 ± 2.41	320	160	320	0.15	0.19	0.15	7.49****	5.52**	7.49****
10years and above	8	3.5 ± 0.33	1.75 ± 0.52	3.5± 0.33	160	320	160	0.45	0.45	0.45	1.85NS	1.34NS	1.85NS

*p<0.01, **p<0.001, ***p<0.02, ****p<0.002, NS Non – significant, SD – Standard Deviation, CC – Correlation Coefficient, CD – Critical Difference, NA – Not applicable. TPL – L – *Tridax procumbans* leaf lectin, TPL – S – *Tridax procumbans* stem lectin, TPL – C – *Tridax procumbans* calyx lectin, NIDDM – Non insulin dependent *Diabetes mellitus*, IDDM – Insulin dependent *Diabetes mellitus*

T. procumbans leaves stem and calyx lectins showed similar hemagglutination pattern with erythrocytes of insulin dependent and non - insulin dependent diabetic patients. Results illustrated in Table 1 and 2 - showed significant decrease in hemagglutination units with increase in the blood glucose. Nagada and Deshmukh (1998) reported similar results, with *P. tithymaloid* lectin. They also found a gradual decrease in hemagglutination units with the increase in the blood glucose level [13]. Similar comparative study carried out by Marques *et al*., (2000) from normal and diabetic patients also reported that diabetic disorders appear to be associated with quantitative alterations of erythrocytes then the normal controls [14]. These results (Table 1 and 2) clearly demonstrate that there is an increase in diabetic condition and decrease in the hemagglutination titre as compared to the control. Thus the leaves stem and calyx lectins of *T. procumbans L* can be used in the medicinal chemistry research, as lectins are used extensively to investigate changes in the hemagglutination pattern, allowing new insights into the biological and pathological significance of glycosylation and may help in the diagnosis and treatment therapy in Diabetes mellitus[15].

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