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LECTIN FROM *Datura innoxia* SEEDS: ISOLATION AND ACTIVITY IN TISSUE CULTURE

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Lectin was isolated from seeds of Indian Datura innoxia. *Certain of its physicochemical properties were determined. Its hemaglutinizing activity was demonstrated. The effect of the obtained protein on proliferation of the hybrid KML cell line from murine B-16 melanoma was studied. Its mitogenic properties were demonstrated.*

Key words: lectin, KML cell culture, proliferation, mitogen, mitotic cycle.

Lectins are carbohydrate-binding proteins that are widely distributed in nature and possess important properties for the vitality of organisms such as hemagglutinating activity, cell aggregation, adhesion, etc. [1-3]. The biological activity of lectins is connected with the mechanism of mitogenic stimulation of lymphocytes, including the binding to carbohydrate components of cell receptors that initiate the cascade of intracellular processes up to the nucleus [4, 5]. Plant lectins that are cytotoxic are known [6]. Such polyfunctionality indicates on one hand that lectins are organized into a variety of domains and, on the other, that the oligosaccharide fragments in lectins have a very large information capacity [7,8].

We studied the effect of lectin isolated from seeds of Indian *Datura innoxia* on the proliferation of murine melanoma cells in KML tissue culture.

Lectin was isolated from ground seeds of *Datura innoxia*. The total sugar content was determined by anthrone-H_2SO_4 reagent [9]. The protein:carbohydrate ratio was 2:1, respectively. The obtained protein had the ability to agglutinate human erythrocytes. Amino-acid analysis had previously revealed the presence of hydroxyproline and zinc in lectin from *Datura innoxia* [10].

Many lectins are known to influence cell proliferation [1, 4]. One of the methods for studying the functionality of lectins is their effect on cell culture. Therefore, we studied the influence of lectin from *Datura innoxia* in a culture of KML cells obtained from the murine tumor melanoma strain B-16 (skin cancer).

A total of 80,000 KML cells were used with simultaneous introduction of lectin at a dose from 100 to 0.1 μg/ml. After 24 h, ^{3}H-thymidine was added over 30 min. Labeling and rinsing of nonbonded label directly in the tube was used because the cells were not removed from the tube surface by a standard solution of versene (0.02%). The data are presented below:

Sample	Dose, μg/ml	Incorporation of ^{3}H-thymidine, %
Lectin of Indian	100.0	87.0
Datura innoxia	10.0	120.0
	0.1	102.0
Control	-	100.0

The results indicate that lectin at a dose of 100 μg/ml (for 25 μg/ml protein) slightly inhibits incorporation of the isotope. Incorporation of the label increases by 20% at a dose of 10 μg/ml (for 2.5 μg/ml protein). The protein has no effect

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on the cells at a dose of 0.1 μg/ml. Thus, *Datura innoxia* lectin in small doses stimulated KML cell proliferation. The lectin may bond to surface cell receptors and provide an additional stimulus for acelerating cell proliferation by shortening the dormancy period G_0 and G_1 (presynthetic period). Certain lectins are known not only to intensify proliferation in lymphoid cells but also to affect other cell lines such as murine fibroblasts L-939, human epithelial Hep-2 cells, murine plasmocytoma 701-BJ cells, etc. [4].

Although lectins from Solanaceae species have similar structures and a similar oligosaccharide composition, nevertheless *Datura* lectins are mitogens for human lymphocytes whereas tomato and potato lectins are not [11, 12]. We demonstrated that more mitotic entities (41%) are formed at the mitotic cell peak in the presence of Indian *Datura* lectin at a dose of 10 μg/ml than in the control (28%).

The mitotic peak starts 2-3 h earlier with *Datura innoxia* lectin.

The lectin sample used by us changed the cell adhesive properties. Thus, treatment with lectin at doses of 100, 10, and partially 1 μg/ml caused the cells to adhere to the tube surface. They were not removed by a standard solution of versene. The cells appeared rounded after 24 h in contact with lectin. This is in contrast with the control, which was flattened. A small quantity of flattened tumor cells could be observed only after 48 h. Rinsing the cell monolayer three times with nutrient medium destroyed the effect. A fibronectin domain may be present in the receptor part of *Datura innoxia* lectin that is responsible for binding with cell integrins and disorganizes the fibronectin and actin protein adhesive system that determines the internal cell structure [13].

Thus, lectin isolated from Indian *Datura* causes hemagglutination of human erythrocytes, changes the cell adhesive properties, intensifies proliferation in KML cell culture, and acts as a mitogen.

EXPERIMENTAL

Lectin Isolation. Proteins were extracted from *Datura innoxia* seed powder by phosphate buffer (15 mM) at pH 7.5 with 0.9% NaCl at 4 C with constant stirring for 20 h. The extract was separated by centrifugation at 300 rpm for 15 min. The extraction was repeated twice. The resulting extracts were combined. Protein was precipitated by ammonium sulfate (45%) overnight at 4 C. The precipitate was collected by centrifugation at 1200 rpm for 30 min. The precipitate was resuspended in phosphate buffer, dialyzed against distilled water, and lyophilized.

Hemagglutination activity of protein was determined using 2% human erythrocytes suspended in 96-well polystyrene planchettes by the standard method [14].

Total sugar content was determined spectrophotometrically with anthrone-H_2SO_4 reagent. A protein solution (1 ml) was treated with anthrone solution (3 ml, 0.1%) in conc. H_2SO_4, incubated for 10 min at 100 C, and cooled to room temperature. Absorption was measured at 620 nm. Total sugar content was determined from a calibration curve.

Proliferation Activity. A total of 80,000 KML cells (per ml) were placed in a tube with RPMI-1640 nutrient medium (3 ml) with 10% calf embryo serum, L-glutamine (200 mM), and antibiotics. Lectin was introduced simultaneously with the cells in doses of 100, 10, and 1 μg/ml. The mixture was cultivated for 24 h at 37 C in a CO_2-incubator. Then ^{3}H-thymidine (1 mCi) was introduced into the tube in 10 μCi doses over 30 min. The culture medium was poured off. The cells were washed three times with phosphate buffer at pH 6.8 and three times with 5% CCl_3CO_2H over 10 min, and three times with distilled water over 10 min. They were dried with alcohol, a mixture of alcohol and ether, and ether. Scintillant (toluene—POPOP—PPO, 5 ml) was added to this same tube and counted on a γ-counter.

Mitotic activity was determined by the literature method [15]. The mitotic index was calculated by determining the number of mitoses per 1000 KML cells (%). Colchicine was added at the mitosis peak at a dose of 0.3 μg/ml of medium per 1.5 h. Lectin was added at a dose of 10 μg/ml.

The protein content was determined by the Lowry method [16].

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