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EFFECTS OF LECTIN EXTRACT OF THE SUDANESE ANASAZI BEANS (*Phaseolous vulgaris*) ON NATIVE LEUKEMIC CELLS

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ABSTRACT

Lectin was extracted from locally collected Anasazi beans (*Phaseolous vulgaris*), purified, precipitated by Ammonium sulphate and dialyzed against NaCl (0,145 M). Protein of the extracted lectin was estimated by the Lowery method.(1951). As sources of leukemic cells, freshly aspirated peripheral blood samples were collected from three previously diagnosed leukemic patients in Wad Medani Hospital. Two of the patients had acute lymphoblastic leukemia (ALL) and the third one had acute myelioblastic leukemia (AML). Density-gradient centrifugation was used for isolation of blast cells over 80%. Isolated lymphocytes were treated with Anazasi lectin at a concentration of 0.9 mg/ml. Vencristine was used as positive control at a concentration of 0.5 /ml. Then, cell count for viable cells was done to determine anti-proliferation activity of lectin using Trypan Blue Exclusion Assay, after incubation periods of 3, 24 and 96 hrs of leukemic cells culturing. In this study, methods used for separation and culturing of leukemic cells were found efficient with mortality rate of less than 0.01% after 96 hrs for the leukemic cells used as control. Anasazi lectin exhibited In-vitro selective cytotoxic effect against leukemic cells isolated from relapsed patients with (AML) and (ALL). Difference among treatments and the control were found to be highly significant (P=). Lectin gave comparable results with vencristine at 3hrs, 24 hrs and 96 hrs of in-vitro preservation. Regarding effects of lectin and vencristine on leukemic cells results showed highly significant differences (p=) among blood samples (patients) with lectin gave 70.1, 92.85 and 91.25 for patient 1, patient 2 and patient 3, respectively; whereas, vencristine gave mortality rates of 70.8, 97.1 and 97.2 for patient 1, patient 2 and patient 3, respectively. Results also showed high progress in mortality of leukemic cells with time for lectin and vencristine. Results pointed lectin of Anasazi bean as an alternative safe anti-leukemic product.

KEYWORDS : Lectin, *Agrobacterium tumefaciens*, Leukemia, Vencristine, Relapsed patients, Myelioblastic, Lymphoblastic

Lectins are defined as protein or glycoprotein that exhibited specific binding affinity for carbohydrates moiety of glycol-conjugates (Van Pammeet et al., 2003). Lectin has biological properties including anti-tumor, anti-fungal anti-human immune-deficiency virus (H and V) and mitogenic (Abuleav et al., 1997). Several plant lectins showed anticancer activity. Practically, lectins were used to distinguish between normal and cancer cells (Sharon et al., 1993). Several lectin studies observed the high affinity between interaction of lectins with normal cells and cancer cells (Kuwahara et al., 2002). Evidence have shown that Lectins have selective binding with cancer cells such as concanvalin (A) lectin and wheat germ agglutinin "WGA" (Nagata, 2000). Selective binding of lectins to specific carbohydrates on the outer-cellular membrane surface of tumor cell was considered as a diagnostic tool in addition to therapeutic use (Konska et al., 2008).

Acute leukemia (AL) is most common form in children. It is divided into Acute lymphoblastic leukemia (ALL) which the abnormal proliferation is in progenitor cells and Acute myeloid leukemia (AML) which involves the mycloid lingoes. The distinction between the (ALL) and (AML) leukemia is based on morphological, cytochemical and immunological. ALL is form of cancer of white blood cells characterized by excess lymphoblast and common observed in childhood at 2-5 years of age and another peak in old age. Acute myeloid leukaemia (AML) is a type of cancer that affects the blood and bone marrow. AML is characterized by an overproduction of immature white blood cells called myeloblasts or leukaemic blasts. These cells crowd the bone marrow, preventing it from making normal blood cells. They can also spill out into the blood stream and circulate around the body. Due to their immaturity, they are unable to function properly to prevent or fight infection. Inadequate numbers of red cells and platelets being made by the marrow cause anaemia, and easy bleeding and/or bruising (Zalcberg et al., 1995). The overall prognosis for patients with acute AL has improved

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over the last decades, with an overall survival of approximately 45-60% for adults (Faderl et al., 2010) and 80% for children (Robinson el al., 2008). Relapse of drug resistant leukemia remains a significant problem despite cure rates in children are high. Recurrent leukemia leads to death in 50-95% of cases depending on the site of recurrent (Gaynon, et al., 2000). Moreover, survivors often suffer from secondary neoplasms and chronic or late-occuring health problems (Cheng et al., 2003).

The objective of this study was to examine effects of lectin from indigenous Anazasi bean on native leukemic cells (ALL and AML types) in comparison with vencristine as an anti-cancer drug.

MATERIALS AND METHODS

Experiments were conducted at the laboratory of the Sudanese Standards and Metrology Organization (SSMO) in Gezira State-Sudan in 2014-015. This study aimed at examining anticancer activity of Lectin extracts of the local Anazasi cultivar collected from the local market. Leukemic cells extraction, purification and preservation techniques were developed for the purpose of conducting this study. Such techniques were optimized during the course of this study for further applications in related studies.

Reagent

The leukemic drug Vincristine; antibiotics such as streptomycin and pencillin G were obtained from Wad Medani Hospital Pharmacy; RPMi, 1640 complete medium, histopaque 1077, trypan blue 0.4% were obtained from Sigma.

Plant Material Used

Anasazi beans (*Phaselous vulgaris*) were purchased from local market, which originated in North of Sudan (Shendi region).

Experimental Procedure

Five grams of Anasazi beans were ground to powder and mixed with physiological saline (0, 145 m) (1: 8 w/v) and kept overnight at 4^0C. The obtained homogenate was filtered through cheesecloth followed by filtering with Wattman number 1 filter paper. The supernatant solution was taken and subjected to centrifugation at 6000 rpm for 15 minutes at room temperature. The obtained supernatant protein on saline extract was fractionated with saturated ammonium sulphate at 30%, 60% and 80%, respectively. Then, the precipitated protein was collected by centrifugation at 6000 rpm for 10 minutes. The three protein pellets obtained at different concentration of ammonium sulfate were combined in one tube, dissolved with minimal volume of physiological saline and subjected to dialysis against physiological saline NaCl overnight with twice buffer changes. Protein was estimated by Lowery method (Lowery et al., 1951).

Patients Clinical Details

This study was conducted on three patients with confirmed diagnosis of acute leukemia (AL) with blast cells over 80%. All procedures were performed upon the approval of the Ethical Committee of Ministry of Health in Gezira State and Wad Medani Hospital (May 2015).

Cells Preparation

Three ml of freshly aspirated blood in heperinized tubes were collected from each patient. Leukocytes were isolated from blood samples by density gradient separation using histopaque 1077.

Cells Viability

Cells viability were determined by using trypan blue exclusion 0.4% mixed with pure cells suspension at a ratio of 1 : 1.

Cells Culture

Fresh isolated leukocytes were maintained in RPMi 1640 complete medium supplemented with 10% fetal bovine serum, 100 l/ml streptomycin and 100 u/ml penicillin G. The cells were cultured at 37^0C in incubator containing 5% CO_2.

Table 1 : Patients' Clinical Details

Samples	Source	Sex type	Old	Diagnosis
Sample 1	Patient 1	Female	4	ALL, newly diagnosed. She didn't receive any drugs
Sample 2	Patient 2	Female	70	AML cell blast over 80% relapsed
Sample 3	Patient 3	Male	7	ALL, cell blast over 80%

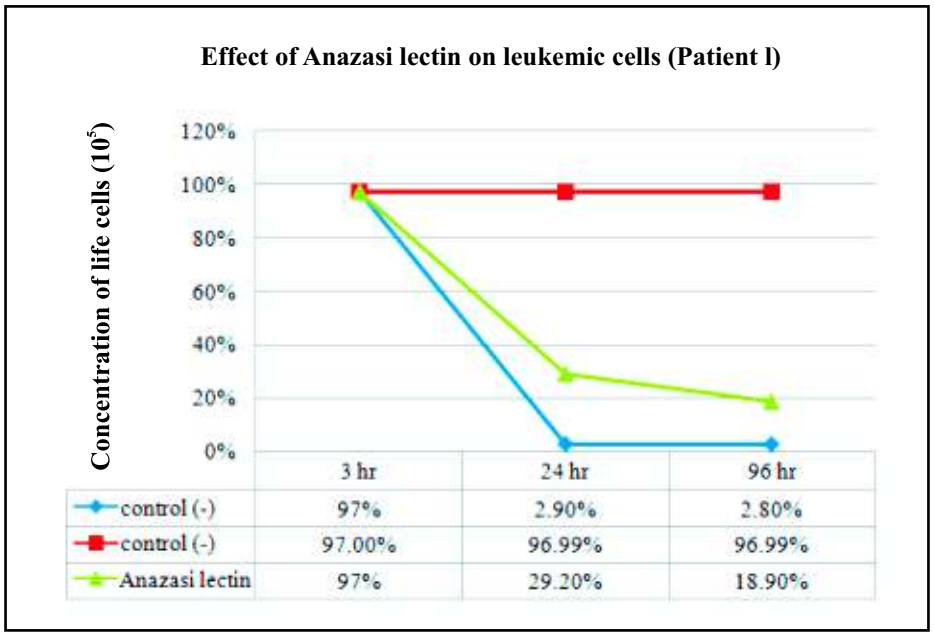

Figure 1 : Effect of Anazasi Lectin on Acute Lymphoblastic Leukemic Cells Sample Obtained from Patient 1

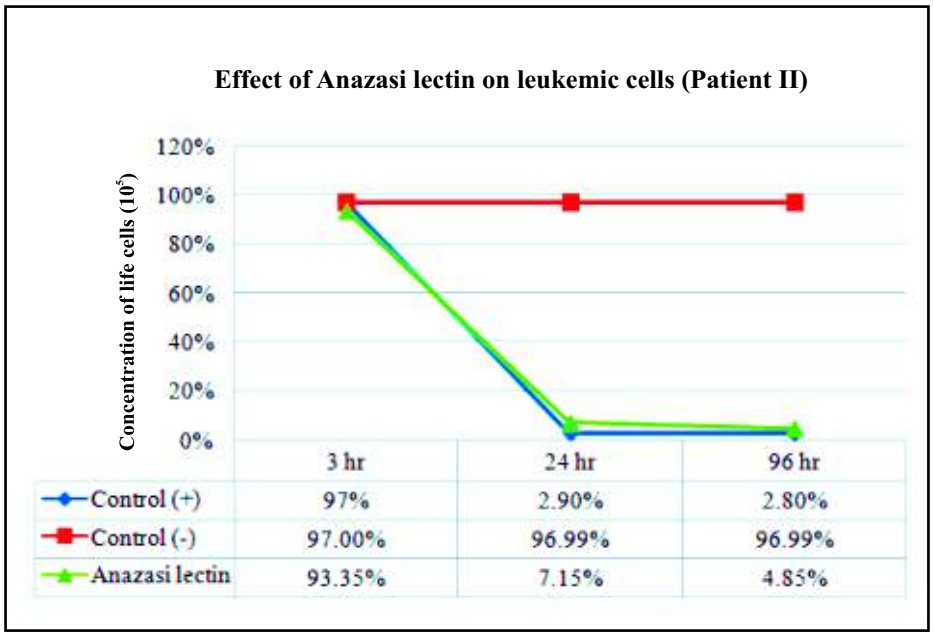

Figure 2 : Effect of Anazasi Lectin on Acute Myeloblastic Leukemic Cells Sample Obtained From Patient 2

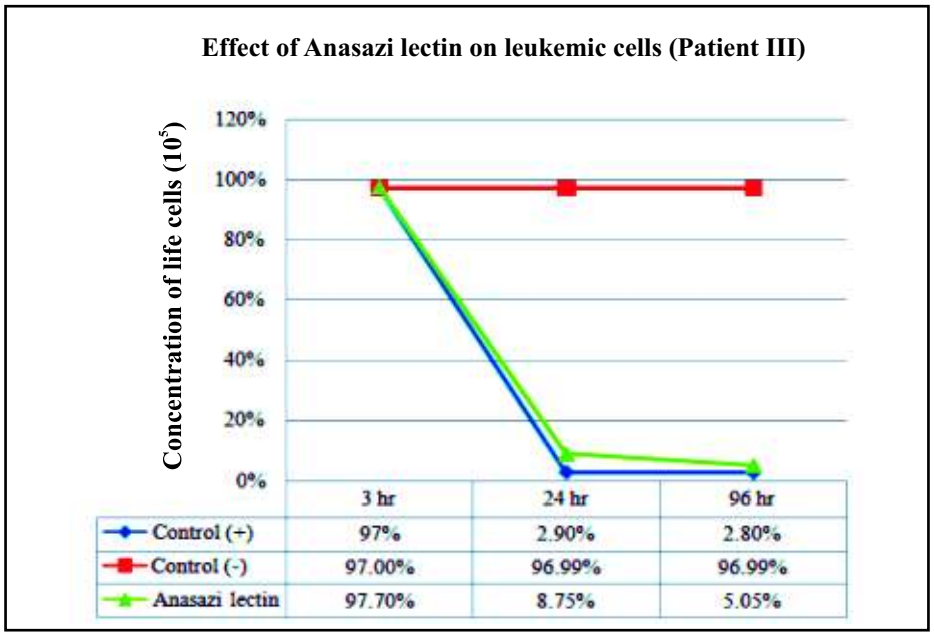

Figure 3 : Effect of Anazasi Lectin on Acute Lymphoblastic Leukemic Cells Sample Obtained from Patient 3

Assessment of Anti-Proliferation

Pure cells were suspended in RPMi 1640 medium and seeded in 24 well micro plates culture tubes at a density of 105 cell/well and immediately treated with lectin extract (100 /ml) and vincristine (0.5 /ml) as positive control in separate tubes. Untreated cells were used as negative control. At each time point of 3, 24 and 96 hrs, cell suspensions were mixed with trypan blue 0.4% at a ratio of 1:1. Then the viable cells were counted using hemacytometer champer.

Morphological Changes

Treated and untreated cells were photographed under inverted microscope after staining with trypan blue 0.4%.

RESULTS

The potato disc technique was developed at the University of Gezira-Sudan in 2010 and utilized to assessing anticancer activity of some natural products using the indigenous strain of *Agrobacterium tumefaciens* SDB 0012 (Yousif et al., 2011). The development of this technique opened a room for identification of more than 13 products having anti-tumor activity (Yousif et al., 2012). Among these lectin extracts from the Sudanese Anasazi (Phaseolus vulgaris) lectin (Abd Elmoniem, 2015).

Several studies practiced purification and characterization of extracted protein from anasazi beans such as Sharma et al., 2009. On the other hand, different methods for determining sensitivity of leukemic cells to drugs were developed, among these the popular In-*vitro* technique for assessment of anti-proliferative effect of natural extracts on human cancer cells (Fruebauf and Bosanquet 1993). It was the first time to use such technique in Sudan.

Results obtained in this study on leukemic cells used as negative control showed 0.01% cell death in the leukemic cells of the three patients after 96 hours of incubation (Table 2). Whereas, the anti-leukemic drug vencristine scored 94.2% cell death in the leukemic cells of the three patients, after 96 hrs of incubation. The anti-proliferative effect of the extracted lectin from Anasazi beans towards ALL (Sample 1 and Sample 3) and AML

Table 2 : Effect of Vencristine and Anasazi Bean Extract on the Leukemic Cells 96 hrs After Treatment

Treatment	Cell Death After 96 hrs (%)		
	Sample1	Sample 2	Sample 3
Control	0.01	0.01	0.01
Vencristine	97.20	97.20	97.20
Anasazi extract	81.10	95.15	94.95

(Sample 2) type of leukemia was significantly (P=0.001) reduced the percentage of cells death after 24 hours at 67.8% for Sample 1, 86.2% for Sample 2 and 88.95% for Sample 3. Percentage of cell death increased to 87.1%, 88,5% and 92.65% for Sample 1, Sample 2 and Sample 3, respectively, after 96 hrs of incubation; with no significant differences between ALL and AML type of leukemia for life cells (Fig 1, Fig 2 and Fig. 3). Anazasi lectin was found effective with no differences among samples collected from patients of different ages mentioned in Table 1. Results concluded Anazani lectin extract to be one of the effective products against both relapsed and drug resistant types of leukemia. Drug resistance is considered to be the major cause of chemotherapy failure (Mishra et al., 2005). An example of products used against relapsed and resistant cases of leukemia is concanvalin (A) lectin "Con A" (Faherina et al., 2011), which triggering an intrinsic mitochondrial pathway and increasing reactive oxygen species (ROS) in leukemic cells. Therefore, results obtained in this study announced Anazasi lectin to be one of the products that may act similarly as con A lectin.

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