



Anti-cancer action of a new recombinant lectin produced from *Acacia species*

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Abstract

The role of a new lectin protein from *Acacia species* found in Aseer region of Kingdom of Saudi Arabia, as anti-cancer agent on four cell lines viz. HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast cancer), HCT 116 (colon Cancer) and HEP-2 (Larynx cancer) was evaluated. *Acacia seyal del*, was collected from Aseer region of the Kingdom of Saudi Arabia. The plant tissue was subjected to RNA extraction, the extracted RNA was used for lectin gene amplification using specific primers. Cloning, subcloning of the *Acacia* 400bp gene was carried out and *in vitro* transcription, combined with protein purification was undertaken. The cytotoxicity of the recombinant lectin was performed on HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast cancer), HCT 116 (colon Cancer) and HEP-2 (Larynx cancer). Our study resulted in the observation of two amplicones with molecular sizes of 800 and 400bp. The 400bp amplicone was excised from the agrose gel, purified and sequenced. The sequence analysis revealed that the lectin gene isolated from *Acacia seyal del* showed similarity with *Lotus japonicus* nod factor binding lectin gene with an identity of 95%. The purified lectin exhibited a significant cytotoxic effect on MCF7cell. The IC₅₀ value for HepG-2 cells was 100 µg/ml and for MCF7 was 250 µg/ml interposed from linear regression curve. Thus we conclude that *Acacia* lectin has anti-cancer action at different concentrations especially at a high dose 100 µg/ml.

Keywords: Legume, *Acacia seyal*, lectin, anti-tumor, protein purification, cloning.

ABBREVIATIONS USED

AFAL: *Acacia farnesiana* lectin-like protein; Bel-7402: Type of human hepatoma cell line; bp: Base pairs; CTAB: Cetyl trimethylammonium bromide; cDNA: Complementary Deoxyribonucleic acid; Da: Daltons; DEPC: water: Diethylpyrocarbonate treated water; DNA: Deoxyribonucleic acid; dNTPs: Deoxy Nucleotide Triphosphates; dT: Deoxy thymine; *E-coli*: *Escherichia coli*; h : Hour; HepG-2: human liver carcinoma cell line; IC50: Inhibitory Concentration 50; IPTG: Isopropyl β-D-1-thiogalactopyranoside; LB medium: Lysogeny broth medium; MCF-7: Human breast cancer cell line isolated in 1970 at Michigan Cancer Foundation-7 ; min: Minutes; mM: millimoles; ng: nanogram; Ni-NTA resin matrix: nickel-nitrilotriacetic acid; °C: Degree centigrade; PCR: Polymerase Chain Reaction; pmol: Pico mole; RNA: Ribonucleic acid; rpm: Rounds per minute; SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis; sec:

Seconds; DIG System: Digoxigenin; TOPO TA Cloning: Topoisomerase Thymine Adenine cloning; µmol: Micromoles; µ/ml: Micromoles per millilitre; µL: Micromoles per liter.

INTRODUCTION

Acacia is an important plant genus that is commonly used in a variety of infections. It is widely distributed in Asia, Australia and America and efficacy has been demonstrated in the treatment of gonorrhoea, leucorrhoea, diarrhea, dysentery and wounds (Akinsulire et al., 2007). Lectins are a well-known family of cationic antibacterial peptides (AMPs) isolated from fungi, plants, insects, mussels, birds and various mammals (Seufi et al., 2011).

Lectins with different carbohydrate specificity have been isolated from forty nine different species, primarily from seeds. Lectins are the most abundant proteins (20-25% of total soluble proteins) in the park of several legume trees such as *Robinia pseudoscacia* and *Maackia amurensis*. Plant lectins have great potential as tools in the identification, purification, and stimulation of specific glycoconjugates. They also have been widely used to distinguish between cell types (Hamelryck et al., 1999; Chandra et al., 1999). Lectins are also present in microorganisms, plants and animals and have attracted great interest due to their varied physiological roles in cell agglutination (Khan et al., 2007), anti-tumour (Liu et al., 2009; Liu et al., 2010), immunomodulatory (Rubinstein et al., 2004), antifungal (Herre et al., 2004) and antiviral effects (Wong et al., 2005). Furthermore, lectin-mediated drugs have been acquired to target specific cells and some lectins with anti-proliferative properties were isolated and characterized from different parts of the plant like seeds (Lin and Ng, 2011), leaves (Park et al., 1997) and roots (Yan et al., 2009).

Sun et al., (1992) found that the total proteins obviously inhibited ovarian cancer cell lines but showed no toxicity to human umbilical cord blood hematopoietic progenitor's *in vitro* (Sun et al., 1992; Zhu et al., 1999). Fu et al., (2007) also found that the 30% $(\text{NH}_4)_2\text{SO}_4$ deposition part of total proteins from *Pinellia ternata* rhizome could significantly inhibit human hepatocellular carcinoma cell line Bel-7402 growth and induce its apoptosis (Fu and Li, 2007). Santi-Gadelha et al. (2008) succeeded to isolate lectin like protein from *Acacia farnesiana* and they found that the isolated lectin protein sequence showed that AFAL has 68% and 63% sequence similarity with lectins of *Phaseolus vulgaris* and *Dolichos biflorus*, respectively (Santi-Gadelha et al., 2008).

Legume lectins are important to the pharmaceuticals but they are produced in low amounts in the plant seeds. Moreover, the genes controlling these proteins are conditionally active, i.e., they work under specific circumstances and not in regular manner. Looking into these limitations, we aimed this work to produce a recombinant lectin by extracting RNA from the tissues taken from *Accacia seyal del* and evaluate its use in the treatment of cancer.

MATERIALS AND METHODS

Plant Collection

Acacia seyal Del species was collected from different areas in Aseer region of Saudi Arabia. The collected young leaves and buds were washed with tap water, dried and then kept in aluminum foils and stored at -80°C until use. The *Acacia* tissues were used as a genetic pool, the tissue was ground as one sample.

RNA isolation protocol

Total RNA was extracted from the plant tissues using Qiagene RNA extraction kit (Qiagene comp, Germany). There are two species of *Acacia* found in Aseer, so genetic pool from these two species was made. 0.1 gm of the frozen tissues was minced in liquid nitrogen using mortar and pestle in the presence of buffer (100 mM Tris-HCl, 0.2% lysozyme and 0.1% glucose). The extraction process was performed according to the manufacturer procedure and the extracted RNA was re-suspended in 100 μL of DEPC water. As acacia contains high polysaccharides content in the tissues, we used CETAB method to diminish the polysaccharide from the extract and then the extract was completed using Qiagene for RNA extraction kit (QIAGene, Germany).

Reverse transcription of RNA

Reverse transcription reactions were performed using oligo (dT) primer. Each 25 μL reaction mixture containing 2.5 μL (5x buffer with MgCl_2), 2.5 μL (2.5 mM) dNTPs, 1 μL (10 pmol) primer, 2.5 μL of plant extracted RNA and 0.2 μL reverse transcriptase (MLVR, Fermentaz Company). PCR amplification was performed in a thermal cycler (Eppendorf) programmed at 95°C for 5 min, 42°C for 1 hr, 72°C for 10 min (Enzyme inactivation) and stored at 4°C (Chen et al. 2001).

Lectin DNA amplification using specific PCR

The synthesized cDNA of the plant was subjected to PCR amplification using the specific primers of lectin gene according to Maltas et al., (2011). PCR amplification was carried out in a total volume of 25 μL . The reaction conditions are showed as follows. 2 μL of (100 ng cDNA), 0.2 μL of 10 μmol each primer (forward 5'-TCAACGAAAACGAGTCTGGTG-3' and reverse 5'-GGTGGAGGCATCATAGGTAAT-3'), 2.5 μL 10x PCR Buffer, 2.5 μL of (2.5 mM) dNTPs, 0.3 μL of 1U Taq DNA polymerase (Qiagene, Germany). The program was initiated for 5 min of denaturation, followed by 10 cycles of amplification with denaturation for 15 sec at 94°C , first annealing for 20 sec at 55°C and an extension at 72°C for 15 sec and followed by 40 cycles of amplification with denaturation for 1.5 min, second annealing for 1.5 min at 53°C , an extension at 72°C for 1 min and a final extension at 72°C for 5 min on Eppendorf thermal cycler.

DNA sequencing and accession number

The PCR product (400 bp) was excised from the agarose gel using agarose DNA extraction kit, (Qiagene, Germany) and then the purified DNA was subjected to DNA sequencing (MACROGEN, Korea) using the designed forward primer in the sequence reaction. Sequence analysis using CLUSTAL W program were

performed. The nucleotide sequences were analyzed with the BLAST database (<http://www.ncbi.nlm.nih.gov>) and the DNA sequence was submitted into the GenBank under accession numbers (JQ964107).

Cloning and subcloning lectin gene

Cloning of amplified PCR products was done by T/A based cloning protocol by using TOPO TA Cloning[®] (with pCR[®] 2.1-TOPO[®] Cloning vector) and (a TOP 10 *E-coli* strain) (Invitrogen[™], USA). The recombinant bacteria were examined using Blue/White colony analysis. The well characterized clone were subjected to digestion using BamH1 restriction enzyme to release the gene from the pCR[®] 2.1-TOPO[®] vector, meanwhile the released fragment was purified by EzWay[™] Gel Extraction kit (Kombiotech comp, Korea) and ligated to the linearized prokaryotic expression pPROEX HT (life technologies, USA). The recombinant clones were examined by PCR for the extracted DNA plasmid and the recombinant clone was selected. The selected recombinant clones were grown on the LB medium containing ampicillin as antibiotic (100 µg/ml). For gene induction IPTG was added to the bacterial culture after two hours from inoculation time. The culture was grown in incubator shaker over night at 37 °C with shaking at 200 rpm and the cloning was done according to the protocols outlined by Life Technologies (Invitrogen Company, USA). For that reason the study aimed to design another primers to amplify the lectin full length gene and use it in this case with the 5 and 3 race RNA synthesis kit (SMARTer[™] RACE cDNA Amplification Kit, Clonetech Company, USA).

Examination of gene function

Lectin purification using 6x Histidine affinity-tagged method:

Lectin purification was carried out by Ni-NTA resin matrix (QIAGEN Inc., USA). The induced bacterial cells was pelleted and re-suspended in 4 volumes of lysis buffer (50 mM Tris-HCl (PH 8.5 at 4 °C), 5 mM 2-mercaptoethanol, 1mM PMSF). The suspension was sonicated until 80% of the cells were lysed. The cell debris was removed by centrifugation; the supernatant was removed to a new tube (crude supernatant). Affinity purification was done according to the protocols outlined by Life Technologies, Invitrogen.

Solubilization and renaturation of lectin protein

The inclusion pellets were solubilized in 8M urea buffer (pH 8) as per the procedures developed by Marston *et al.* (1984). The urea mixture was incubated at 25 °C for 1 h before the insoluble molecules were removed by centrifugation. The urea solution was then diluted in a

high pH buffer (pH 10.7) for renaturation of lectin. After solubilization in 8 M urea, the inclusion body solution was diluted with phosphate buffer pH 10.7, the solution was incubated at 25 °C for 1 h, then pH adjusted to 8 and incubation was continued at 25 °C for 1 h. The solution was transferred to dialyze against buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM EDTA) at 4 °C overnight.

Hybridization using the 400bp as a probe

The small DNA fragment (400bp) was used as a probe to detect the lectin gene in the 800bp amplicones. The DIG System Nonradioactive and Highly Sensitive Detection of Nucleic Acids (Roche Applied Science, Germany) were used according to the manufacture procedures. The large fragment was purified from the agarose gel using agrose DNA extraction kit (QIAGEN, Germany) and the purified DNA was subjected to PCR amplification using the same conditions above. The PCR product was Cloned using T/A based cloning protocol by using TOPO TA Cloning[®] (with pCR[®] 2.1-TOPO[®] Cloning vector) and (a TOP 10 *E-coli* strain) (Invitrogen[™], USA). The recombinant plasmid was screened using white/blue method and the plasmid DNA examined with the same primers (lectin primers).

SDS-PAGE for the recombinant lectin

The purified lectin was separated on 12% polyacrylamide gel according to Sambrook *et al.* (2001). 10 µl of the purified protein was loaded and the gel was left for running at 80 Volt for two hours. Staining and destaining was performed and the gel was photographed.

Evaluation of the purified recombinant lectin as an anticancer agent

Four different human cell lines were used in this study to test the activity of the obtained recombinant lectins as anticancer agent. The cell lines used were HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast cancer), HCT 116 (colon Cancer) and HEP-2 (Larynx cancer). The cells were obtained and tested in Tumor Research Institute, Cairo University, Egypt.

Cell survival was determined using Sulpho-Rhodamine-B (SRB) method as previously described by (Skehan *et al.* 1990). The sulforhodamine B (SRB) assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation for large-scale drug-screening applications. Its principle is based on the ability of the protein dye sulforhodamine B to bind electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to and under mild basic conditions it can be extracted from cells and solubilized for measurement. Results of the SRB assay were linear with cell number and cellular protein measured at cellular densities ranging from 1 to 100% of

confluence. Its sensitivity is comparable with that of several fluorescence assays and superior to that of Lowry or Bradford. The signal-to-noise ratio is favorable and the resolution is 1000-2000 cells/well.

Cells were seeded in 96 well microtiter plates at a concentration of 1000-2000 cells/well (100 μ l/well). After 24 hrs, cells were incubated with various concentrations of drug (lectin) (0, 0.01, 0.1, 1, 10, 100 units), for 72 h. After 72 h treatment, the medium was discarded, cells fixed with 10% trichloroacetic acid 150 μ l/well for 1 h at 4 $^{\circ}$ C and finally wash with water 3 times (TCA reduce SRB protein binding). The wells were stained for 10-30 min at room temperature with 0.4% SRB dissolved in 1% acetic acid @ 70 μ l/well [kept in dark place]. Then the plates were washed with 1% acetic acid to remove unbound dye and air dried for 24 hours. The dye was solubilized with 150 μ l/well of 10 mM tris base (pH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 490 nm with an ELISA microplate reader. The IC₅₀ values were calculated using sigmoidal concentration– response curve fitting models (SigmaPlot software).

Molecular studies on two selected cell lines treated with the lectin and Doxorubicin for 72 hours:

The HepG-2 and MCF-7 were treated with the lectin with concentrations (99.2 and 208 μ g/l.) Also, HepG-2 and MCF-7 were treated with Doxorubicin (100 μ g/ml) drug under same conditions (previously described). Moreover, another cell was cultivated without any addition of any drug as control. The treated cells were incubated for 72 hours and samples were taken after 24, 48 and 72 hours post treatment. The cultured cells were harvested using centrifugation at 6000 rpm for 20 minutes. The harvested cells were washed with DEPC treated water and re-centrifuged and the cells were kept in liquid nitrogen until use.

Extraction of total RNA from treated cell lines

The treated cell lines were collected separately and subjected to RNA extraction. About 10⁶ cells were subjected to RNA extraction using the RNA extraction Mini Kit according to manufacturer's instructions (QIAGEN, Germany). The resultant RNA was dissolved in DEPC-treated water, quantitated spectrophotometrically and analyzed on 1.2% agarose gel.

The Quantitative Real Time-PCR

The extracted RNA from the treated and non-treated cell lines was used as template to examine the expression level of DNA cancer marker genes (P53, Bcl2, TNF- α) in the presence of housekeeping gene primers (GPDH). Moreover, some cytokines were also tested (Interlukins I,

12 and 14). The Real time reaction consists of 12.5 μ l of 2X Quantitech SYBR[®] Green RT Mix (Fermentaz, USA), 2 μ l of the extracted RNA (50ng/ μ l), 1 μ l of 25 pM/ μ l forward primer, 1 μ l of 25 pM/ μ l reverse primer (given above), 9.5 μ l of RNase free water for a total of 25 μ l. Samples were spun before loading in the rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95 $^{\circ}$ C for 10 min.; 40 cycles of 95 $^{\circ}$ C for 15 sec, annealing at 52 $^{\circ}$ C for 30 sec and extension at 72 $^{\circ}$ C for 30 sec. Data acquisition performed during the extension step. This reaction was performed using Rotor-Gene 6000 system (Qiagen, USA).

Data Analysis

Comparative quantitation analysis was done using Rotor-Gene-6000 Series Software based on the following equation.

$$\text{Ratio target} = \frac{\text{Fold change in target gene}}{\text{Fold change in reference}}$$

The data set of both samples and control of real-time PCR was analyzed with appropriate bioinformatics and statistical program for estimation of the relative expression of genes using Real Time PCR and the results normalized to GPDH gene (Reference gene). The data were statistically evaluated, interpreted and analyzed using Rotor-Gene-6000 version 1.7.

RESULTS

The results of PCR product revealed that an amplicone with molecular weight of 400bp was obtained. Different annealing temperatures were examined to diminish the 400bp from the PCR reaction but nothing happened. For that reason we expected that the 400bp amplicone may be another copy from lectin gene.

Sequence and sequence analysis

The sequence analysis using BLASTn revealed that the lectin gene isolated from *Acacia* showed similarity with *Lotus japonicus* nod factor binding lectin gene with identity of 95%. It is well known that, if the identity between two genes is not more than 97%, then the examined gene is a new gene. It could be concluded that the obtained gene is a new gene Figure 1.

Recombinant lectin purification from the transformed *E.coli*

The purified lectin separated on 12% polyacrylamide gel is shown in figure (2). Two proteins with molecular sizes of 17 and 15kDa were observed on the gel.

CCATGAACGATACGCTGCTAATCCTGAAGAAGCTGCAGAATCTCTGATTC
 CACTTCTAAAAGAAGCAGAAAATGTGGTTCCTGTGAGCCAGCAACCCAAC
 ACACCCGTTAAGCTTGGGGCAACTGCAGGTTTAAGGCTTTTGGAGGGGAA
 TGCTGCTGAAAATATATTGCAAGCGGTCGGGGATATGCTTTCAGCAACAG
 AAAAAGTGCCCTTGATGCAGTATCTATTCTTGATGGAACCCAAGAAGGTT
 CTTATCTTTGGGTGACAATTA ACTATCCCCTCTTGGGGAAAGTTGGGAAAAA
 GATTTACAAAGACAGTGGGAGTAGTTGATCTAGGAGGTGGGTTCAGTGCAA
 ATGACATATGCAGTCTCAAGGAACACAGCTAAAAAATGCTCCTAAG

Figure 1. The DNA nucleotide sequence for the amplified lectin gene from *Acacia seyal*.

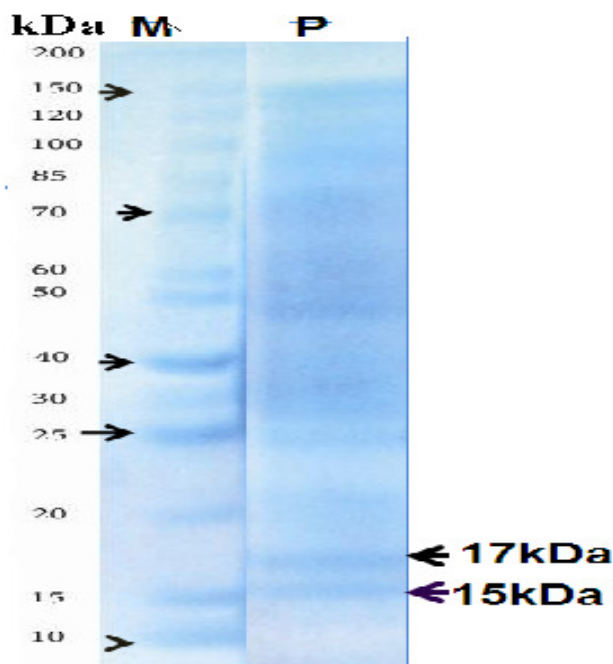


Figure 2. SDS-PAGE of recombinant lectin

The recombinant lectin as an anticancer agent

When the recombinant lectin was tested against the 4 different cell lines; HepG-2 (Hepatocellular carcinoma), MCF-7 (Breast cancer), HCT 116 (colon Cancer) and HEP-2 (Larynx cancer), whose results are presented in table (1) and figures (3, 4, 5 & 6) revealed that the lectin

was highly effective against liver cancer, breast cancer, colon cancer and lung cancer in respective manner. We assume that the lectin is more effective for the liver than the other three tested cancer cell lines. These results assure that the lectin could be used as anti-liver cancer followed by anti-colon cancer.

Table 1. IC₅₀ for the recombinant lectin as anticancer against various cell lines

Lectin treated cell lines → ↓ Parameter	HepG-2	MCF-7	HCT-116	HEP-2
IC ₅₀ (µg/ml)	99.1848	210.24	203.172	208.621
Residual fraction (resistant)	3.83	1.193	6.951	9.16

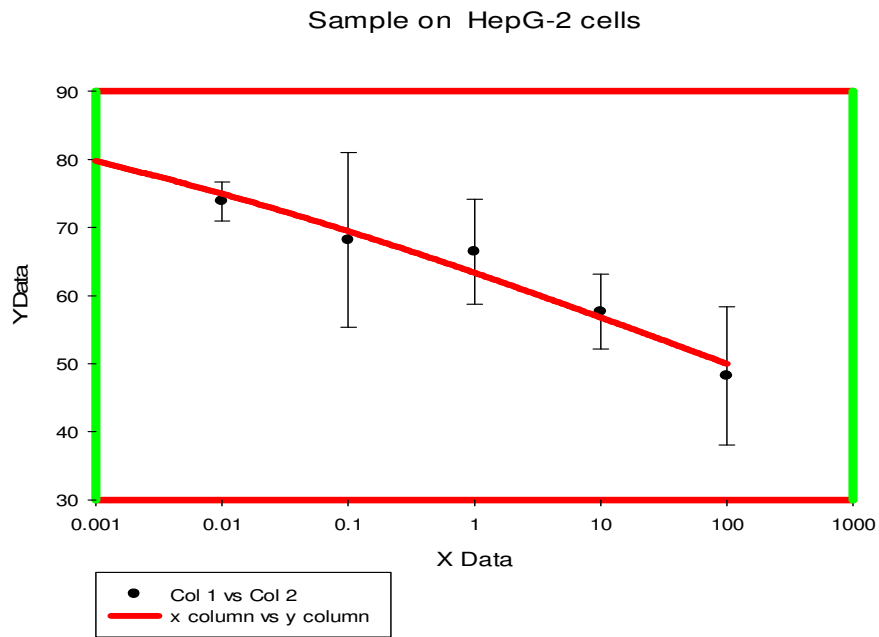


Figure 3. Effect of different lectin concentration on HepG-2 cells as anticancer agent

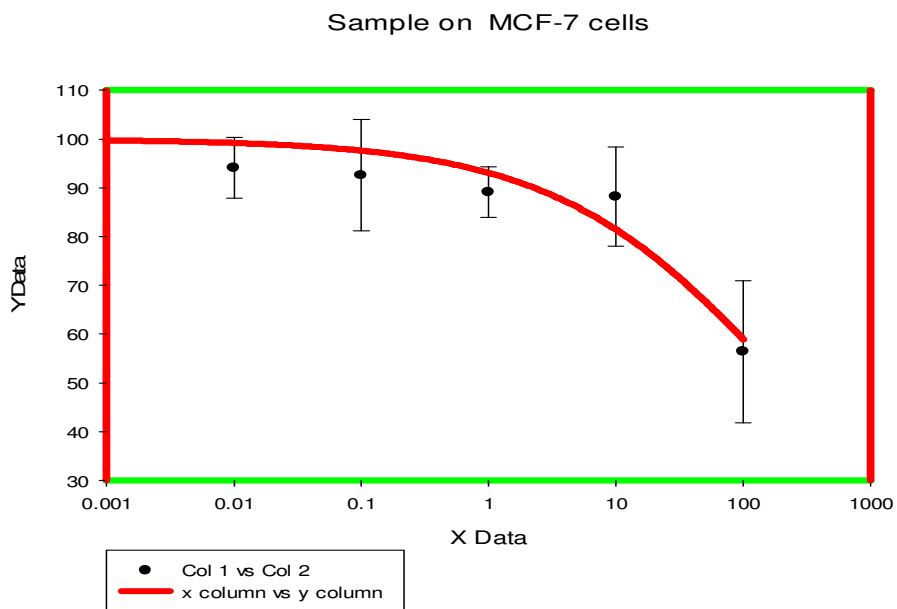


Figure 4. Effect of different lectin concentration on MFC-7 cells as anticancer agent

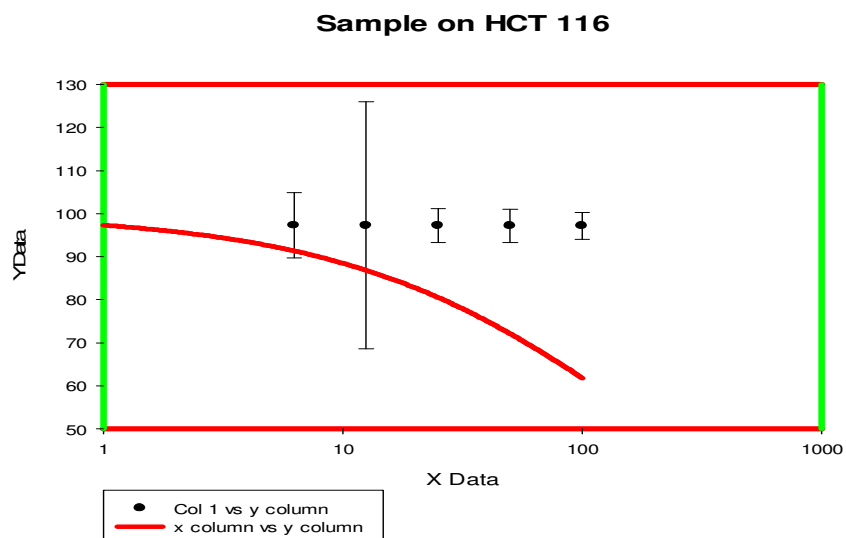


Figure 5. Effect of different lectin concentration on HCT-116 cells as anticancer agent

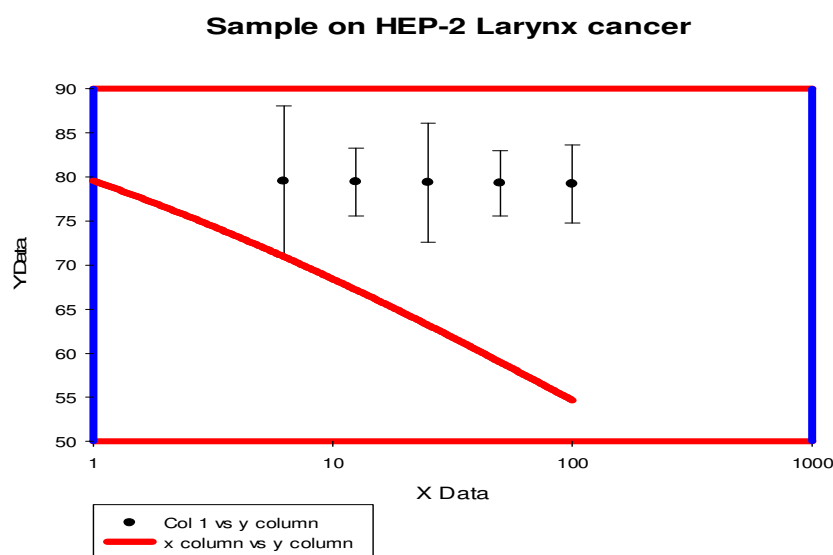


Figure 6. Effect of different lectin concentration on HEP-2 cells as anticancer agent

The recombinant lectin as anticancer drug against liver and lung cancer

Based on IC_{50} for the 4 examined cell lines, both liver (HepG-2) and lung cancer (Hep-2) were treated with the IC_{50} of each and incubated at the previous conditions for 48 hours. Samples were taken after 24 and 48 hours in respective manner. The results revealed that the samples treated with lectin were more effective as anti cancer agents.

Real time PCR and cancer marker gene expression

The sample keys are the following:

1- Control MCF-7 breast cancer

- 2- Control HepG-2 liver cancer cell
 3- D MCF-7 24 breast cancer cells treated with Doxorubicin for 24 hours
 4- D MCF-7 48 breast cancer cells treated with Doxorubicin for 48 hours
 5- DHepG-2 24 liver cancer cells treated with Doxorubicin for 24 hours
 6- DHepG-2 48 liver cancer cells treated with Doxorubicin for 48 hours
 7- T MCF-7 24 breast cancer cells treated with lectin for 24 hours
 8- T MCF-7 48 breast cancer cells treated with lectin for 48 hours

Table 2. Real time results for the Real Time PCR of gene expression in treated and non- treated cancer cell lines.

Serial	Experiment	Ex/cont Ct	Hous/cont	(Ex/cont)/ Hous/cont %
P53				
No.1	Control BC	1.0	0.53	189%
No.2	Control HepG-2	1.0	0.47	189%
No.3	D MCF-7, 24	0.71		134%
No.4	D MCF-7, 48	0.72		136%
No.5	DHepG-2, 24	0.71		151%
No.6	DHepG-2, 48	0.71		151%
No.7	TMCF-7, 24	0.83		157%
No.8	T MCF-7, 48	0.67		126%
No.9	THepG-2, 24	0.71		151%
No.10	THepG-2, 48	0.59		126%
IL-4				
Serial	Experiment	Ex/cont Ct	Hous/cont	(Ex/cont)/ Hous/con %
No.1	Control BC	1.0	0.78	128%
No.2	Control HepG-2	1.0	0.69	145
No.3	D MCF-7, 24	0.80		103%
No.4	D MCF-7, 48	0.86		110%
No.5	DHepG-2, 24	0.77		112%
No.6	DHepG-2, 48	0.73		106%
No.7	TMCF-7, 24	0.73		94%
No.8	T MCF-7, 48	0.76		97%
No.9	THepG-2, 24	0.76		110%
No.10	THepG-2, 48	0.78		113%
TNF				
Serial	Experiment	Ex/cont Ct	Hous/cont	(Ex/cont)/ Hous/cont
No.1	Control BC	1.0	0.83	128
No.2	Control HepG-2	1.0	0.80	125
No.3	D MCF-7, 24	1.01		122
No.4	D MCF-7, 48	0.99		119
No.5	DHepG-2, 24	1.09		136
No.6	DHepG-2, 48	1.37		171
No.7	TMCF-7, 24	0.96		116
No.8	T MCF-7, 48	0.96		116
No.9	THepG-2, 24	1.0		125
No.10	THepG-2, 48	0.99		124
IL-12				
Serial	Experiment	Ex/cont Ct	Hous/cont	(Ex/cont)/ Hous/con %
No.1	Control BC	1.0	0.86	117%
No.2	Control BC	1.0	0.82	122%
No.3	Control HepG-2	1.11		129%
No.4	D MCF-7, 24	1.02		119%
No.5	D MCF-7, 48	1.18		144%
No.6	DHepG-2, 24	1.25		152%
No.7	DHepG-2, 48	1.04		121%
No.8	TMCF-7, 24	1.07		124%
No.9	T MCF-7, 48	1.13		138%
No.10	THepG-2, 24	1.15		134%

Table 2 continues

Bcl-2				
Serial	Experiment	Ex/cont Ct	Hous/cont	(Ex/cont)/ Hous/con %
No.1	Control BC	1.0	0.93	107%
No.2	Control HepG-2	1.0	0.96	104%
No.3	D MCF-7, 24	1.58		170%
No.4	D MCF-7, 48	1.88		202%
No.5	DHepG-2, 24	1.89		197%
No.6	DHepG-2, 48	1.72		179%
No.7	TMCF-7, 24	1.46		157%
No.8	T MCF-7, 48	1.48		160%
No.9	THepG-2, 24	1.41		147%
No.10	THepG-2, 48	1.44		150%

9- THepG-2 24 liver cancer cells treated with lectin for 24 hours

10- THepG-2 48 liver cancer cells treated with lectin for 48 hours

Housekeeping gene GPDH

Results listed in the Table (2) summarizes the results obtained by Real Time PCR for the five different tested genes on the treated and non-treated cell lines with the two used drugs. In case of the P53 the expression level of this gene was decreased in the cells treated with both lectin and Doxorubicin but the expression was lower in the lectin. This result assures that the lectin is more effective as anticancer agent when compared to Doxorubicin. In case of interleukin-4 (IL-4) the expression of this gene in the treated and non-treated is mostly the same. Thus a slight modification in the IL-4 expression was observed. In case of the tumor necrosis, the tumor necrosis factor (TNF) expression, the same trend was seen in the two types of the non-treated cells. The expression of the IL-12 was increased in the treated cells with two drugs and the expression was higher in case of hepatocarcinoma more than the breast cancer cell lines. In case of the Bcl-2, the expression of this gene was increased with cells treated doxorubicin compared with the non-treated ones.

DISCUSSION

Carbohydrate-binding proteins which agglutinate erythrocytes and precipitate glycoconjugates are very common in plant tissues. These phytohaemagglutinins or lectins may be simple proteins or glycoproteins and they exhibit considerable binding specificity towards carbohydrates. The nature and properties of plant lectins have been reviewed by Bog-Hansen (1981). Leguminous seeds are a rich source of lectins, many

of which possess homologous segments of amino acids and, hence, may have an evolutionary relationship (Hankins et al., 1979). Few examples of enzymes possessing lectin activity have been reported but cumannosidase from *Phaseolus vulgaris* seeds (Paus and Steen, 1978) and α -galactosidases obtained from several species of leguminous seeds have been shown to be lectins (Hankins et al., 1980). Lectin is very important for human health, so the studies aimed to purify and intensify the efficiency of new lectin specially derived from legumes. It is well known that several C-type lectins are present in serum in a soluble form (collectins, galectins, ficolins), where they function as agglutinins and opsonins, promoting phagocytosis by binding to microbial surface carbohydrates. Moreover, Membrane-bound C-type lectins are designed to capture pathogens for intracellular destruction, degradation and antigen loading of major histocompatibility complex molecules, whereas soluble lectins (such as the collectins) function by ligating and opsonizing microorganisms, or might have a role in antigen transport.

A recombinant lectin, in a soluble form within *E. coli* cells has been recovered and purified as shown in figure-2. The sequence analysis using BLASTn revealed that the lectin gene isolated from *Acacia* showed similarity with *Lotus japonicus* nod factor binding lectin gene with identity of 95%. Moreover, the obtained DNA sequence is completely new. When the sequence obtained for this new lectin was compared with the other lectins presented on the data base of the Gene Bank, it was observed that the *Acacia* lectin was a new outer group for all the other lectins. For this reason this gene was chosen to be cloned and overexpressed. The Recombinant lectin purification from the transformed *E.coli* revealed two proteins with molecular sizes 17 and 15kDa (Figure-2). The molecular weight is different from the other recorded before. These new recombinant lectin proteins encourage us to study their cytotoxicity on

cancer cell lines as anticancer agent. Other legume lectins also exhibit antiproliferative activity toward cancer cell lines (Wong and Ng, 2003). Similarly the recombinant lectin from acacia showed to be more effective as chemotherapeutic agent for cancer.

Aseer region, in the southern region of Kingdom of Saudi Arabia, is at an altitude of about 2000 feet from the sea level and has its own confidential flora. With regard to the type I C-type lectins, there is evidence that macrophage mannose receptor is associated with a signal transduction pathway leading to tumour necrosis factor (TNF) and interleukin-12 production (Garner et al., 1994). No such findings have yet been reported for the recombinant lectin from *Acacia species*. The expression of the investigated genes (table 2) was increased in the cells treated with the Doxorubicin. But the expression was mostly the same in the hepatocarcinoma cells but lower in the breast cancer cells treated with lectin. This means that the expression of the TNF was inhibited/suppressed in the breast cancer cells rather than the HepG-2. The expression of the IL-12 was increased in the treated cells with two drugs and the expression was higher in case of hepatocarcinoma more than the breast cancer cell lines. This mean that the immune system was induced when treated with the two examined drugs to resist the cancer effect. In case of the Bcl-2, the expression of this gene was increased with cells treated with doxorubicin compared with the non-treated ones. On the other hand this expression was lower in the cells treated with lectin. This means that the presence of lectin make more suppression for the Bcl-2 when compared with the doxorubicin. These results assure that the lectin is more effective than the Doxorubicin.

CONCLUSION

In fact, many studies affirm that any lectin can explore cellular surfaces, linking carbohydrates to glycoconjugates that are present on the cell. Therefore, they have been used to detect alterations in the composition of these carbohydrates. Lectins isolated from *Acacia* has variance in their identity from the other identified ones in genbank. These lectins have anticancer action on HepG-2 and MCF-7 cells. So this study introduces these new recombinant lectins (15 and 17kDa) in the treatment of hepatocarcinoma and breast cancer. The lectin is more effective than the widely used anticancer drug - doxorubicin treating different kinds of cancer as it is able to:

- Make more suppression for the Bcl-2 when compared with the doxorubicin.
- Decrease the expression of P53. The expression was lower in the case of lectin than Doxorubicin.
- IL-4 expression was the same with lectin and Doxorubicin.

- Tumor necrosis factor (TNF) expression is the same in the two types of treated cells.

- The expression of the IL-12 was increased in the treated cells with two drugs and the expression was higher in case of hepatocarcinoma more than the breast cancer cell lines.

ACKNOWLEDGMENT

The authors are indebted to the Deanship of Scientific Research, King Khalid University for financial support of this project (kku-med.-11/015).

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How to cite this article: Patel A, Hafez E, Elsaid F, Amanullah M (2014). Anti-cancer action of a new recombinant lectin produced from *Acacia* species. *J. Med. Med. Sci.* 5(1):1-11