Investigation of TF binding lectins from dietary sources and SRL on proliferation and cell cycle progression in human colon HT29 and SW620 cells.

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Abstract

TF antigen binding lectins from dietary source PNA, ACA, ABL, JAC and SRL from *Sclerotium rolfsii* have been reported to induce diverse effects on cancer cell proliferation by different mechanisms. This study aimed to compare effects of these lectins on growth and cell cycle progression in colon cancer HT29 and SW620 cells. As reported SRL, ABL and JAC inhibited whilst PNA and ACA increased cell proliferation. ABL and JAC treated HT29 cells showed increased cell population in G0/G1 phase. PNA, ACA, ABL and JAC increased SW620 cell population in S and decreased in G2/M phase. In contrast SRL and JAC increased hypodiploid population in both the cells. PNA and ACA reduced whereas SRL and

ABL diminished cell cyclin D1 expression. SRL, PNA and ACA also reduced cellular cyclin D3 level while SRL, ABL and JAC reduced cyclin E levels. ABL decreased CDK5 levels while SRL and ACA completely abolished CDK5 expression. All the lectins completely abolished cyclin D2 expression. These results not only confirms growth regulatory effects of TF-binding lectins but also indicates different effects of these lectins on cell growth is associated with regulation on expression of cell cycle associated proteins in G1-S phase and on cell cycle progression.

Keywords:, TF binding dietary lectins, SRL, Colon cancer cells, Cell cycle, Proliferation, Inhibition, Apoptosis.

Abbreviations: Thomsen Friedenreich antigen (TF), PNA (Peanut agglutinin), ACA (*Amaranthus caudatus* agglutinin), SRL (*Sclerotium rolfsii* lectin), ABL (*Agaricus bisporus* lectin) and JAC (Jacalin).

Introduction

Protein glycosylation is one of the important post-translational modifications (PTM) and half of the human proteins are estimated to be glycosylated [1]. Glycosylation plays very important role in protein folding, stability and in cell signalling. Changes of protein glycosylation occur in various pathophysiological conditions. In cancer, alteration of protein glycosylation, such as the increased appearance of Galactoseβ1-3-Nacetyl-galactosamine α-O-Ser/Thr (TF antigen) [1, 2] is common and is correlated with cancer malignancy and progression, which are sites for TF binding lectins. Hence consuming foods such as peanuts, mushrooms, jackfruit and others that are rich in TF specific lectin content is of concern, as these lectins are known to resist digestion, survive gut passage and bind to gastrointestinal

cells or enter the circulation intact, maintaining full biological activity [5]. Although glycosylation changes are often secondary to hyperplastic or malignant changes in the epithelium, they allow altered epithelial cell interactions with the environment. For example in the gut, it results in altered interaction of the gastrointestinal epithelium with carbohydrate binding proteins (lectins) in the food stuff, an effect that has been proposed to lead to the intestinal epithelium hyper-proliferation [3, 4].

Lectins are known bind to specific carbohydrate structures in reversible manner. The carbohydrate binding specificity of lectins has been exploited to identify carbohydrate changes on cell surfaces in cancer cells. There are many studies showing that lectin-glycan interaction on the cell surface can affect cell behaviours through activation of a variety of signalling events [6-8]. One of the most common lectin effects are on growth of the cells either stimulatory or inhibitory [9]. For example, the presence of the TF binding lectin from peanuts (PNA) is mitogenic to both the colon carcinoma cells [3] and for normal human colonic explants [4]. TF-antigen binding *Amaranthus caudatus* agglutinin (ACA) also stimulates cell proliferation of colon cancer cells [10]. On the other hands, TF binding lectins from the edible mushroom *Agaricus bisporous* (ABL) and jackfruit *Artocarpus integrifolia* (JAC) have shown to inhibit cell proliferation of several colon and breast cancer cells in a non-cytotoxic manner [10,11]. The ABL-mediated cell growth inhibition is associated with ABL internalization [17] and subsequent blockade of NLS-dependent nuclear protein import [18]. The growth inhibitory effect of JAC is associated with tyrosine phosphorylation of the putative human HLA-DR-associated protein I (PHAPI, also known as the tumor suppressor pp32) that causes release of PP2A protein phosphatase from association with PHAPI, allowing increased phosphatase activity of PP2A[19]. Recent studies from our laboratory have shown that another TF antigen binding lectin from *Sclerotium rolfsii* (SRL), isolated

from sclerotial bodies of *Sclerotium rolfsii* fungus, shows to inhibit cell proliferation by inducing cellular apoptosis in different cancer cells including HT29 human colon cancer cells [12-14,15]. It has been suggested that the diverse effects of these TF-binding lectins on cell proliferation are attributed to the subtle differences in the fine sugar specificity of these TF-binding lectins [10].

Considering the presence of TF binding lectins in the dietary sources and their close resemblance in the structure and fine sugar specificity with SRL, the aim of this study was to compare effects of all these five TF-antigen binding lectins on cell cycle in human colon cancer HT29 and SW620 cells so as to gain further information of their diverse effects on cell proliferation and understanding their effect on cell cycle and expression of various cyclins.

Materials and Methods

Materials

Bovine serum albumin (BSA), protease inhibitor cocktail, nonidet P-40 (NP-40), ethylenediaminetetra-acetic acid (EDTA), 2-mercaptoethanol, Ribonuclease A, Triton X-100, trypan blue, Calcein-AM, glycine, DAPI, formaldehyde and propidium iodide were obtained from Sigma Chemical Co. (St. Louis, USA). PNA, ACA, ABL and JAC were purchased from Vector Laboratories, CA, USA. Acrylamide, bisacrylamide, Tris and sodium dodecyl sulphate (SDS) were obtained from GE Life Sciences (Pittsburgh, PA, USA). Dulbecco's Modified Eagle Medium (DMEM) and Fetal Bovine Serum (FBS) were from Gibco by Life technologies Pvt. Ltd. USA. PVDF membrane was procured from Millipore, USA. Antibodies to cyclin D1, D2 and D3, cyclin E, CDK5 and p21 were procured from Cell

Signalling technologies, Beverly, USA. Species-specific HRP-labelled secondary antibodies were procured from Bio- Rad, Hercules, USA. Chemiluminescence kit was procured from Bio-Rad Laboratories Inc. USA. All other chemical used were of analytical grade.

Cell culture

The human colon cancer HT29 cells were obtained from the European Cell Culture Collection via the Public Health Laboratory Service (Porton Down, Wiltshire, UK). SW620 cells were kind gift from Prof. Karunagaran D, from Department of Biotechnology, IIT Madras, India.The cells were cultured in DMEM supplemented with 10 % FBS, 100 units/ml penicillin, 100 µg/ml streptomycin (complete DMEM) at 37 °C in 5 % CO_2.

Purification of *Sclerotium rolfsii* lectin

SRL was purified from sclerotial bodies as reported before [20]. Briefly, SRL was extracted with 50 mM acetate buffer containing 100 mM NaCl (pH 4.3) and subjected to 30% methanol precipitation followed by ion exchange chromatography on CM cellulose matrix and finally purified on Superdex G-75 gel filtration column.

Assessment of Cell growth by Calcein-AM method

Briefly, sub-confluent HT29 and SW620 cells were seeded (4×10^4 cells/ml) in 96-well plates in DMEM with 10% FBS (complete medium) for 24 h. The cells were treated with SRL, ABL, JAC, PNA and ACA (each 25µg/ml) for 48h in serum-free DMEM with 0.5% BSA (incomplete medium) and maintained in humidified atmosphere (37°C, 5% CO_2)

for 48h. After 48h, cells were labelled with 10 µM Calcein AM at 37°C for 30 min in a CO_2 incubator, lysed with 100 µL of lysis buffer (50 mM Tris–HCl, pH 6.8, with 5% SDS and 2% mercaptoethanol). The fluorescent intensity was read using Tecan M200 pro microplate reader with an excitation wave length of 485 nm and an emission wave length of 535 nm.

Cell cycle analysis

Sub-confluent HT29 and SW620 cells were treated with or without SRL, ABL, JAC, PNA and ACA (25 µg/ml) for 24 and 48 h before harvested by gentle trypsinization. Cells were washed in PBS twice and fixed in 70% chilled ethanol for 30 min at 4 °C. Cells were then washed once in PBS and treated with 50 µl of Ribonuclease A (DNase-free 5 mg/ml in PBS,) for 10 min at room temperature and stained with 450 µl propidium iodide (50 µg/ml in PBS) for 2 h in the dark. DNA content of HT29 cells was analysed on FL-3 channel of flow cytometer (FC-500 Beckman Coulter) equipped with 488 nm argon laser at linear scale. Data was analysed using CXP analysis software for distribution of cells in different phases of the cell cycle. DNA content of SW620 cells was analysed on FL-2 channel of flow cytometer (BD Accuri C6) and the data was analysed using BD Accuri C6 Software.

Western blotting

Sub-confluent HT29 cells were treated with SRL, ABL, JAC, PNA and ACA (25 µg/ml) in serum free media at 37°C for 24 h. After treatment, the cells were lysed using RIPA lysis buffer (120 mM NaCl, 1.0% Triton X- 100, 20 mM Tris–HCl, pH 7.5, 10% glycerol, 2 mM EDTA, Protease inhibitor cocktail; Sigma Aldrich) and total protein was electrophoresed on 12% SDS–polyacrylamide gels and then blotted on to PVDF membranes. The membranes

were blocked with 3% BSA and were probed with primary antibodies (1:1000) to cyclin D1, cyclin D2, cyclin D3, cyclin E, CDK5 and Waf1/p21 for overnight at 4°C, followed by incubation of species-specific secondary antibodies (1:1000) for 1h. Finally the bands were visualized using chemiluminescence with Immun-Star™ HRP Luminol/Enhancer (BIO-RAD). β-actin was used as loading control. The bands were analysed using Image Studio Lite Ver 5.2 software.

Results

Effect of TF binding lectins on cell growth in HT29 and SW620 cells

As reported previously, the presence of each of the TF binding dietary lectins ABL, JAC, PNA, ACA and SRL, showed to affect growth of HT29 cells. PNA and ACA increased HT29 cell proliferation by 34.37 ± 3.85 and 6.90 ± 8.31% respectively whilst SRL, ABL and JAC caused growth inhibition by 39.19 ± 2.83, 15.86 ± 0.75 and 3.14 ± 3.37% respectively at 48h (Fig. 1.a). In SW620 cells, PNA increased cell proliferation by 17.7 ± 1.6 while the other four lectins, SRL, ABL ACA and JAC inhibited cell growth by 31.5 ± 1.36, 14.5 ± 2.35, 24.0 ± 0.20 and 19.8 ± 0.72% respectively at 48h (Fig1.b)

TF binding lectins show different effects on cell cycle

HT29 and SW620 cells treated with SRL, ABL, JAC, PNA and ACA were stained with propidium iodide (PI) and were subjected for cell cycle analysis by flow cytometry. After 24h treatment of HT29 cells, the proliferative lectins PNA and ACA did not show any significant effect on cell cycle compared to the untreated control cells. In contrast the anti-

proliferative ABL and JAC increased the cell population in G0/G1 phase and decreased cells in both S and G2/M-phase (Fig 2). Unlike PNA, ACA, ABL and JAC, SRL increased apoptotic/hypodiploid population and reduced the cell population in all the phases of cell cycle. After 48h PNA, ACA, ABL and JAC increased the cells in S phase, SRL, ABL and JAC decreased the cells in G2/M phase and SRL continued to increase the cells in hypodiploid phase (Table 1, Fig. 2). In SW620 cells after 24h treatment, PNA, ACA, ABL and JAC all showed to increase cell population in S phase, and all the five lectins decreased the cell population in G2/M phase. Both SRL and JAC increased the cells in hypodiploid phase. After 48h treatment, PNA ACA and ABL increased the cells in G2/M phase. The anti-proliferative lectins SRL and JAC decreased the cells in G2/M phase. SRL significantly increased hypodiploid population in SW620 cells compared to control or to other TF binding lectins, in consistent to its observed effect on apoptosis induction in HT 29 cells as reported earlier [11,19] (Table 2, Fig. 3). In SW620 cells, PNA and ACA slightly decreased the cell population in G0/G1, S and increased in G2/M-phase. Whereas SRL, ABL and JAC continued to increase the cells in hypodiploid phase and decreased the cells in S-phase.

Effect of TF binding lectins on expression of cell cycle-related proteins in G1-S phase transition

Having observed the different effects on cell cycle of these TF-binding lectins, we then compared the expressions of cell cycle related proteins in G1-S phase transition (Fig. 4). Cyclin D1 expression was significantly diminished in response to SRL and ABL treatment (0.25 and 0.06 fold respectively), whereas PNA, JAC and ACA treatment slightly reduced cyclin D1 expression (0.26, 0.45 and 0.62 fold respectively). All the lectins completely diminished the expression of cyclin D2. Cyclin D3 expression was significantly reduced to

0.14 by SRL and to 0.67 and 0.48 by PNA and ACA respectively compared to lectin untreated control taken as 1. ABL and JAC showed no effect on cyclin D3 expression. Cyclin E expression was reduced in SRL and JAC treated cells to 0.36 and 0.49, respectively, but not by PNA, ACA and ABL. It is noted that reduction of cyclin E expression was more prominent in SRL treated cells (0.36) when compared to ABL or JAC treated cells (0.72 and 0.49 respectively). CDK5 expression was totally abolished by the treatment of SRL and ACA while it was only partially reduced by ABL treatment.

Discussion

Alteration of cell surface glycosylation allows altered interactions of the intestinal epithelium with TF-binding lectins from dietary (PNA, JAC and ACA), microbial (ABL and SRL) or endogenous human origins [23, 24]. All these TF-binding lectins have previously been shown to affect growth of human colon cancer cells in cell culture [25, 12]. The present study showed that the presence of each of these TF binding lectins affect growth of human colon cancer cells in culture and produced different effect on the expression of cell cycle-associated proteins.

PNA and ACA showed strong and mild proliferative effect, respectively, whereas ABL and SRL showed inhibitory effects on HT29 cell growth as reported before [4, 10]. JAC showed no significant growth inhibition at this concentration [10-12]. This lack of effect of JAC on cell proliferation may be related to the different concentrations of the lectins used or the different assay times or methods used. In earlier studies, thymidine incorporation assay

was used to assess effect of PNA, ACA, ABL and JAC on growth of HT29 cells, in the present study Calcien-AM assay was used.

Although all these lectins recognise TF antigen, these lectins are different in their sugar binding specificity toward addition on TF by other sugar residues. PNA binding to TF requires free hydroxyl groups at C-3 and C-6 of Gal but not at C-6 of GalNAc [26], whereas ACA binding requires free hydroxyl groups at C-4 of Gal and C-4 of GalNAc [27]. ABL binding to TF requires a free hydroxyl group at C-6 of GalNAc, but is not affected by substitution at C-3 or C-6 of Gal [26], whilst JAC also requires free hydroxyl group at C-6 of GalNAc, similar to ABL, but unlike ABL, also requires free hydroxyl group at C-4 of GalNAc [28]. SRL binding to TF requires a free axial hydroxyl groups at C4 of GalNAc similar to JAC and also C2 of Gal unlike other TF binding lectins [29]. Apart from these binding differences, these TF binding lectins also have distinct binding affinity towards TF anomers. SRL, ACA and JAC recognize only the "α anomer" of TFD (Galβ1- 3GalNAcα-Sp8; TFβα) whereas PNA and ABL recognizes only the "β anomer" (Galβ1-3GalNAcβ–Sp8; TFββ). Hence SRL resembles JAC and ACA more closely than PNA and ABL in binding to TF antigen [29].

The presence of lectins is known to influence cell cycle in culture. For example; *Agrocybe aegerita* lectin (AAL) which binds to *N*-acetylgalactoseamine, and *Legumi secchi* lectin, which has binding specificity to N-acetylglucosamine and mannose, decrease cells in the G2/M phase in cancer cells. AAL has also been shown to increase population in hypodiploid/apoptotic phase of cell cycle [30,31]. *Astragalus mongholicus* lectin showed to arrest HeLa cell cycle in S phase [32]. SRL has been shown to decrease cell population in all the phases of cell cycle and increase hypodiploid phase in human epithelial ovarian cancer PA1 cells [31]. In the present study, treatment of HT 29 cells with PNA and ACA for 24h did

not show any effect on cell cycle. In contrast, the anti-proliferative lectins ABL and JAC increased the cell population in G0/G1 phase and decreased cells in both S and G2/M-phase, suggesting ABL and JAC treatment arrested the cells at G0/G1 phase. SRL treatment of the cells showed to reduce cell population in all the phases of cell cycle thereby shifting the cells to apoptotic/hypodiploid phase in a time dependent manner. In SW620 cells, all the TF binding lectins except SRL increased the cell population in G0/G1 and S phase and all the lectins decreased the cells in G2/M phase. SRL and JAC also increased SW620 cells in hypodiploid phase. At 48h, PNA and ACA treatment showed to slightly increase the cell population in G0/G1 and S phase, in HT 29 cells, whereas ABL and JAC marginally decreased the cells in G2/M phase. In SW620 cells, PNA and ACA slightly decreased the cell population in G0/G1, S and increased in G2/M-phase. Taken together, these results suggest that ABL and JAC inhibit HT 29 cell growth by arresting the cells in G0/G1 phase whilst the proliferative effect of PNA and ACA is linked with decrease of cells in G0/G1 phase and increase in S phase. In consistent to its effect on apoptosis induction, SRL increased hypodiploid population and decreased cells in all the three phases G0/G1, S and G2/M of cell cycle. These results are in line with our earlier observation that several genes involved in cell cycle pathways were significantly affected by SRL suggesting that the initial signalling cascades lead to the disruption of cell cycle and induction of apoptosis [20].

Eukaryotic cell division is tightly regulated and the key fate decisions are made in the G1 phase of cell cycle i.e. whether the cell should proliferate or not. In cell cycle, there are a set of cell cycle regulatory proteins like, D-type cyclin proteins, cyclin D1, D2 and D3, cyclin-dependent kinases (CDK)- 2, -4 and-6 that control the progression of cells from G1 to S phase [33]. Cyclin D-CDK4/CDK6 complex is known to phosphorylate the retinoblastoma susceptibility gene product Rb in mid- to late G1 phase and triggers the onset of S phase by

inducing the release of E2F transcription factor which transcriptionally activates genes involved in the activation and maintenance of DNA synthesis [35].

In the present study, all the five TF binding lectins differentially regulated cyclins involved in G1 to S transition phase. PNA, ACA and JAC slightly reduced cyclin D1 expression whereas SRL and ABL totally diminished D1 expression which falls in line with our previous reports that SRL induces cellular apoptosis in HT 29 cells[12]. Cyclin D2 expression was completely abolished by the presence of each of the lectins compared to control. Surprisingly all the five lectins have shown to reduce the expression of Cyclin D2 including PNA and ACA contradicting their proliferative response. Cyclin D3 expression was significantly reduced by SRL and moderately reduced by PNA and ACA. In contrast cyclin D3 levels were not affected either by ABL or JAC. Overexpression of cyclins D1, D2 and D3 has been reported in a large number of tumours. Overexpression of cyclins D1 and/or D2, but not cyclin D3, is reported to correlate with colon carcinogenesis [35]. Inhibition of cyclin D1 expression has shown to inhibit growth and tumorigenicity of human colon cancer cells [37]. Hence down-regulation of cyclin D1 and D2 levels by SRL, ABL and JAC consequently arrests the cells at G0/G1 phase. In contrast to cyclin D1 and D2, cyclin D3 has been shown to play positive role in colon epithelial cell differentiation but not in colon carcinogenesis [38]. Proliferative lectins PNA and ACA may promote cell proliferation by decreasing cell cyclin D3 levels which is known to induce differentiation.

Cyclin E is another important G1 phase regulator which regulates normal cell proliferation and development. Cyclin E expression in HT29 cells was markedly reduced by the presence of SRL, ABL and JAC, but not by PNA and ACA. Cyclin E/CDK2 complex regulates progression of cells from the G1 to the S phase. Cyclin E deregulation plays a role in tumorigenesis [39] and its overexpression is correlated with carcinogenesis of many

cancers including colorectal cancer [40]. Cyclin E is required by S phase entry of cells in growth. The presence of SRL, ABL and JAC, but not PNA and ACA reduced cyclin E expression in HT29 cells; this is consistent with ABL and JAC effect on cell rest in G1-S phase.

The presence of ABL reduced CDK5 levels and SRL and ACA completely abolished in SRL and ACA- CDK5 expression. CDK5 is a proline-directed serine/threonine kinase and controls various cellular events, including cytoskeletal organization, cell adhesion, membrane trafficking, cell cycle exit and neuronal differentiation, and thereby regulates multiple aspects of brain development and function [41]. CDK5 Overexpression is shown to occur in metastatic tumors in comparison in primary colon tumors. Inhibition of CDK5 expression by gene knockdown or small molecule inhibitors reduced cell proliferation and migration, increased cell death and arrested cells in the G2/M transition *in vitro* and halted tumor progression and tumor weight *in vivo* [42]. A decreased level of CDK5 in HT29 cells by the presence of SRL and ABL is in consistent with the growth inhibitory effect of these two lectins supports in those cells. Antiproliferative lectins SRL and ABL have reduced the overall expression of Cyclin D1, D2, D3, Cyclin E and CDK5which fall in line with their ultimate response**.**

In conclusion, all the TF binding dietary lectins and SRL produce different effects on proliferation of human colon cancer cells and these effects are associated with different influences of the lectins on expression of cell cycle associated proteins or cyclins and cell cycle progression.

Acknowledgements

This work has been supported by funding from the British council under UKIERI, CPEPA and S. Belur acknowledges UGC for RGNJRF.

Conflict of interest

The authors declare no conflict of interest.

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Figure Legends

Fig. 1. Effect of TF binding lectins on cell proliferation

HT29 (a) or SW620 (b) cells were treatment with PNA, ACA, SRL, ABL and JAC (25µg/ml) in serum free DMEM for 48h before cell proliferation was analysed by Calcein-AM assay as described in the materials and methods section. (*p<0.05, **p<0.01, ***p<0.001)

Fig. 2. Effect of TF binding lectins on cell cycle progression in HT29 cells

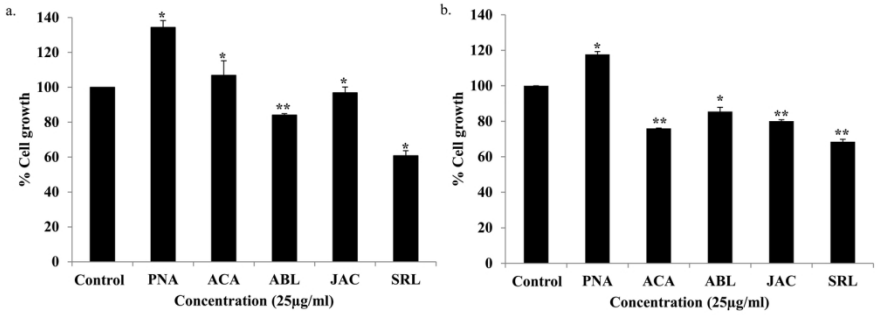
HT29 cells were incubated with PNA, ACA, SRL, ABL and JAC (25 µg/ml) for 24 (A) and 48h (B). Cells were stained with PI and then analysed on FL3 channel of flow cytometer. X-axis represents DNA content of cells and the Y-axis represents cell number. C, D, E and F represent cell population in hypodiploid/apoptotic, G0/G1, S and G2/M phases, respectively.

Fig. 3. Effect of TF binding lectins on cell cycle progression in SW620 cells

SW620 cells were incubated with PNA, ACA, SRL, ABL and JAC (25 µg/ml) for 24 (A) and 48h (B). Cells were stained with PI and then cells was analysed on FL-2 channel of flow cytometer. M1, M2, M3 and M4 represent cell population in hypodiploid/apoptotic, G0/G1, S and G2/M phases, respectively at 24 h and 48 h.

Fig. 4. Effect of TF binding lectins on expressions of cell cycle-associated proteins in G1-S phase

HT29 cells were treated with PNA, ACA, SRL, ABL and JAC (25 µg/ml) for 24 h and whole cell lysate was electrophoresed and transblotted on to PVDF membrane and probed with antibodies against cyclin D1, cyclin D2, cyclin D3, CDK5, cyclin E and p21. β-Actin was used as loading control. Data are representative of two similar experiments. The bands were analysed using Image Studio Lite Ver 5.2 software and change in expression is mentioned compared to control.



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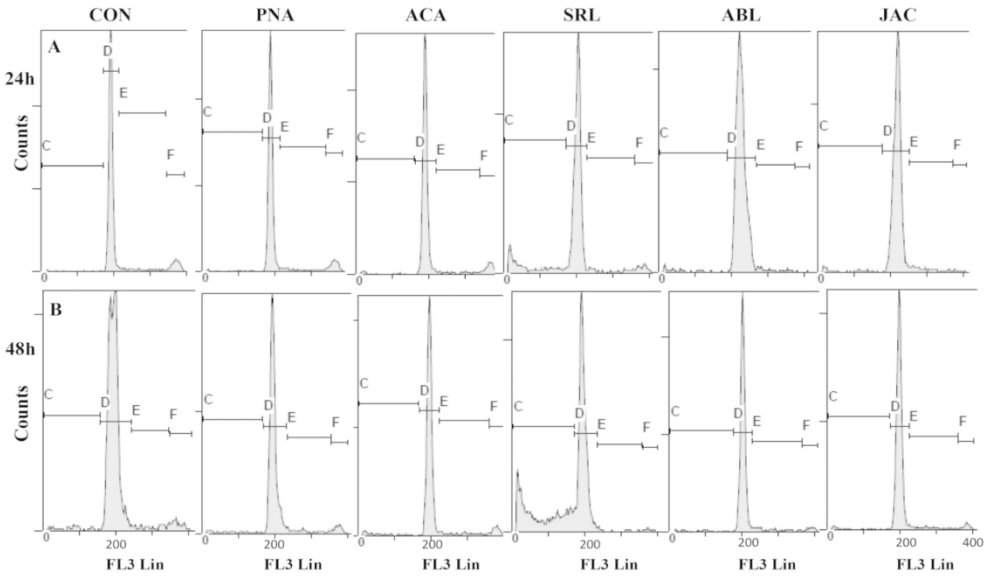

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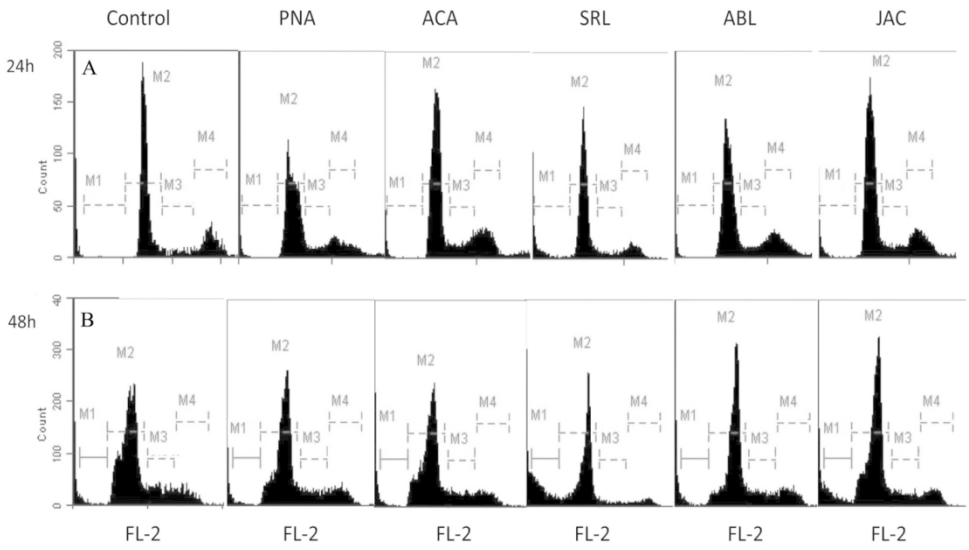

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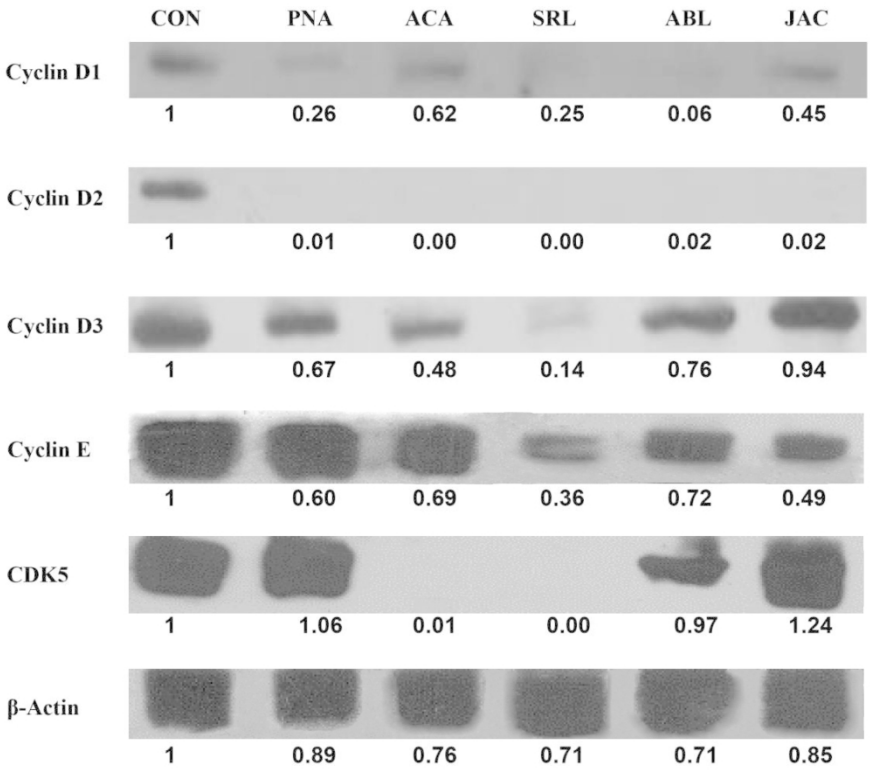

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HT29 cells were treated with PNA, ACA, SRL, ABL and JAC (25 µg/ml) for 24 h and whole cell lysate was electrophoresed and transblotted on to PVDF membrane and probed with antibodies against cyclin D1, cyclin D2, cyclin D3, CDK5, cyclin E and p21. β-Actin was used as loading control. Data are representative of two similar experiments. The bands were analysed using Image Studio Lite Ver 5.2 software and change in expression is mentioned compared to control.

218x185mm (300 x 300 DPI)

Table 1: Effect of TF binding lectins on different phases of cell cycle in HT29 cells at 24 and 48h.

				TF antigen binding lectins				
		Time point	Control	PNA	ACA	SRL	ABL	JAC
Percentage of cells in different phases	Hypodiploid	24h	3.67	5.11	5.38	19.87	7.71	8.70
		48h	7.55	9.40	8.83	38.79	7.75	7.14
	G0/G1	24h	76.60	76.03	77.05	66.15	84.55	80.02
		48h	79.71	75.47	74.21	54.98	77.61	79.00
	S	24h	10.10	9.49	8.33	8.45	6.62	8.94
		48h	7.03	9.38	10.67	4.24	10.34	9.36
	G2/M	24h	9.58	9.16	8.87	5.50	1.10	2.08
		48h	5.56	5.50	6.03	1.83	4.19	4.29

Table 2: Effect of TF binding lectins on different phases of cell cycle in SW620 cells at 24 and 48h.

				TF antigen binding lectins				
		Time point	Control	PNA	ACA	SRL	ABL	JAC
Percentage of cells in different phases	Hypodiploid	24h	2.52	2.02	2.15	11.13	1.62	4.86
		48h	2.96	2.62	2.80	24.07	3.97	9.31
	G0/G1	24h	67.58	68.54	65.64	67.60	67.26	66.60
		48h	69.62	69.17	70.79	61.48	67.81	68.70
	S	24h	8.93	11.86	12.58	7.88	11.08	9.87
		48h	15.74	14.18	14.01	6.29	13.74	10.99
	G2/M	24h	22.85	17.56	19.60	13.37	20.02	18.65
		48h	11.66	14.01	12.38	8.14	14.46	10.99