4. IN VITRO EFFECTS OF JACALIN ON CANCER CELLS BELONGING TO DIFFERENT LINEAGES

4.1 INTRODUCTION

The effects of lectins on cell proliferation is mainly attributed to the ability of lectins to identify and non-covalently bind to specific carbohydrates on the normal and transformed cell surface [245]. Upon binding, the lectins may modify the expression of molecules that are crucial for proliferation of cells and other functions. It also remains to be unveiled if the lectins directly bind to the target cell surface molecules or if it may possess the ability to bind to any other protein partner, thereby facilitating its passage into the cells. Though lectins are primarily known to bind to carbohydrates, it is to be ascertained if they bind with other proteins. For instance, the interaction of jacalin with deglycosylated CD4 present on the T-cells is mainly mediated via protein-protein interaction [246]. In the current study, the in vitro effects of jacalin on cells of different lineage were explored.

The TFD-binding lectins possess the ability to specifically bind to the TF-antigen. They have acquired substantial attention due to their promising diagnostic and/or therapeutic value. The binding of these lectins on the surface of the cancer cells were found to regulate a number of signaling pathways resulting in mitogenic, antiproliferative, autophagic or apoptotic effects on cancer cells, *in vitro* and *in vivo* [247]. Interestingly, the TFD-binding lectins exhibit marked and varied effects on proliferation of different types of cells. Though they bind to the same sugar, different lectins are known to exhibit adverse effects on the same cell; while, the same lectin exhibits contrasting effects on different cells [248]. For instance, while PNA inhibits the growth of breast cancer cell lines 734 B and ZR-75-1, the same lectin was found to act as a mitogen of HT29, T84 and Caco2 colon cancer cells [249,250]. Likewise, while a TFD-specific lectin isolated from *Sclerotium rolfsii* strongly induced apoptosis of human colon cancer HT29 cells, PNA that exhibits the same specificity was found to induce proliferation of those cells [152,251]. These

distinct effects make it obscure as to what are all the different factors that influence the activity of theses lectins on growth of different cancer cells. There are various reports that extensively report the mitogenic effects of PNA on various cells. It has been shown to act as a mitogen of proliferation of rectal epithelia [252]. Further, PNA also stimulates proliferation of colorectal cancer cells as well as normal colonic epithelium [251,253]. Also, PNA increased proliferation of cells isolated from the colonic mucosae of patients who suffer from inflammatory bowel disease [254]. In another study, PNA that appeared in the systemic circulation post consumption of peanuts, mimicked the action of galectin-3, an endogenous animal lectin, in promoting cancer cell metastatic spread [255]. In a similar study, a lectin isolated from potato was shown to induce the proliferation of T47D breast cancer cell line [256]. There are various reports of lectin-induced effects on cancer cell growth and survival. However, while most studies lay emphasis on mechanism underlying the inhibitory effects of lectins including jacalin, the mitogenic facets of lectins are yet to be explored in detail.

The antiproliferative effects of jacalin has been extensively studied. While jacalin induced dose-dependent, non-cytotoxic inhibition of growth of colon cancer cells HT29, it was found to inhibit the proliferation of A431 human epidermoid cancer cells by causing cell death [248,257]. In a more recent study, jacalin was found to inhibit formation of preneoplastic lesions *in vivo*, in a mouse model of colon carcinogenesis [258]. However, while jacalin exhibits anti-proliferative effects on certain cancer cells, it can also induce proliferation of primary cells [259]. Such contrary effects of jacalin on cell growth may be attributed to discrepancies in binding to different cells. Other than a previous study that had reported that jacalin induced differentiation of K562 cells along the monocyte lineage by triggering intracellular events [260], as per our knowledge no other study makes a direct indication of the proliferative effects of jacalin on cancer cells, thereby highlighting the importance of shedding more light on the proliferative effects of jacalin. In the present study, while jacalin was hindered the normal proliferation of all the other cancer cells, it was

shown to stimulate the proliferation of K562 chronic myelogenous leukemia (CML) cells.

Chronic myelogenous leukemia (CML)

CML is a hematopoietic stem cell malignancy in which there is abnormal proliferation of cells, regulated by BCR-ABL [261]; increased resistance to apoptosis [262-264] and decreased attachment to stroma cells and extracellular matrix [265]. The disease progresses through 3 phases, namely the initial chronic phase, the accelerated phase and the terminal phase that can also be known as blast crisis [266]. A prominent aspect is the absence of symptoms for at least a few years. Meanwhile, the leukemia starts to spread gradually. Imatinib mesylate, a small molecule kinase inhibitor that targets BCR-ABL, is the only drug that is being successfully used in the treatment of CML.

4.2. MATERIALS AND METHODS

4.2.1 Materials

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Chloroform, isopropyl alcohol and ethanol were purchased from SRL. Roswell Park Memorial Institute (RPMI) 1640 medium and Dulbecco's Modified Eagle's Medium (DMEM) powder were obtained commercially from Himedia and were prepared as per the protocol of the manufacturer. Trypsin-EDTA and fetal bovine serum (FBS) were purchased from Sigma-Aldrich Co. TRIzol was purchased from Invitrogen. cDNA reverse transcription kit was purchased from Applied Biosystems. SYBR green master mix was purchased from Himedia (MBT074).

4.2.2 Cell lines and Cell culture

K562 erythroleukemia cells were obtained from the National Centre For Cell Science (NCCS), Pune and were cultured in RPMI 1640 medium, HeLa cervical cancer cells, A549 non-small lung carcinoma cell, human colorectal adenocarcinoma cell HT29 and human embryonic kidney 293 (HEK 293) cells were cultured in DMEM. All the cells were cultured in medium containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin, streptomycin) and maintained at 37°C in a humified atmosphere containing 5% $CO_{2.}$.

4.2.3 MTT assay

The MTT tetrazolium assay implicates the reduction of MTT resulting in the formation of formazan crystals [267]. Since jacalin has the ability to bind to glycoconjugates present on the blood cells, 1% FBS was used in all the experiments. Initially, the proliferative behavior of K562 cells in RPMI 1640 medium that contained different concentrations of FBS was analyzed. K562 cells suspended in the appropriate medium, supplemented with decreasing concentrations of FBS (10% - 0%) and 1% antibiotics were seeded at a density of 3 x 10^3 cells/well into 96-well tissue culture plates. Thereafter, 20 µl of MTT (5 mg ml^{-1} in PBS) was added to each well and incubated at 37°C for 4 h in dark. The formazan crystals that were formed after 4 h were solubilized using

100 µl of DMSO and the microtiter plate was left for 15 mins at RT. Absorbance at 570 nm was measured using a multimode plate reader (Model Victor X3, Perkin Elmer). The absorbance of control cells were regarded as 100% cell growth.

To determine the effects of jacalin on cell proliferation, the respective cells suspended in the appropriate medium, containing 1% FBS and 1% antibiotics were seeded into 96-well tissue culture plates at a density of 3 x 10^3 cells/well. The seeded cells were cultured in the presence of different concentrations (500 to 3.123 µg ml^{-1}) of jacalin, and incubated at 37°C for 24 h, after which the cell viability was estimated by MTT assay. The absorbance given by control cells were regarded as 100% cell growth and the viability of the treated cells were calculated as a fraction of the control cells.

4.2.4 Light microscopic observation

K562 cells cultured in RPMI 1640 containing 1% FBS and 1% antibiotics were seeded at a density of 1 x 10^6 cells in each well of a 6-well plate. After the addition of 100 µg ml^{-1} jacalin to the cells, the images of the cells were obtained at different time points using the Zeiss Axioskop Fluorescence Phase Contrast Microscope. The Images of cells that were untreated were used for comparison**.** The varying degrees of K562 cell aggregation was determined at different time points (5 mins – 1 h) and were scored as follows: no aggregation (-), 1-5 cells/aggregates (1+), 6-10 cells/aggregate (2+),10-15 cells/aggregate (3+) and 15+ cells/aggregate (4+).

4.2.5 Assessment of effects of jacalin-treated conditioned media (CM) on the viability of K562 cells

2x10^4 cells were seeded in each well of a 12 well plate and 100 µg/ml jacalin was added to the cells. After 3, 6 and 24 h, the CM were collected by pelleting the cells. The CM were filtered and stored at -80°C until use. Fresh K562 cells were seeded in a 12 well plate and cultured for 36 h using the 3, 6 and 24 h CM. The CM were mixed with equal quantities of fresh media, with

and without 300 µM galactose. The effects of CM on the viability percentage of fresh K562 cells were analyzed by MTT tetrazolium assay.

4.2.6 Quantification of mRNA levels of cytokines in jacalin-treated cells

(i) mRNA isolation

Cell lysis

$1x10^6$ cells were seeded in each well of a 6 well plate and 100 µg/ml jacalin was added to the cells. After 3 and 6 h of incubation, TRIzol reagent was added and the cells were homogenized by pipetting and were added into a fresh micro centrifuge tube. The cells were further lysed by vortexing the tubes after which the tubes were incubated at RT for 5 mins.

Phase separation

200 µl of chloroform was added to the lysed cells. The contents were mixed well by inverting the tubes and were incubated at RT for 5 mins. The cells were then subjected to centrifugation at 20,817g for 10 mins at 4°C. The top aqueous layer was collected in a fresh tube.

RNA precipitation

An equal volume of absolute isopropyl alcohol was added and the tubes were kept at -80°C. After 2 h, the contents of the tubes were subjected to centrifugation at 20,817g for 10 mins at 4°C. The supernatant was discarded.

RNA wash

The pellet was washed with 70% ethyl alcohol by spinning the tubes at 20,817g for 10 mins at 4°C. After the supernatant was discarded, the pellet was air-dried for 5 mins.

Redissolving RNA

The RNA pellet that was dissolved in diethylpyrocarbonate (DEPC) water was stored at -80°C.

(ii) **Reverse transcription**

5 µg of total RNA was converted to cDNA. Random primers were used for the synthesis of cDNA. The high capacity cDNA reverse transcription kit, obtained from Applied Biosystems, CA was used. Table 4.1 shows the preparation of 10 µl of 2X reverse transcription master mix from the kit components. Equal volumes of cDNA master mix was added to 10 µl of mRNA and the cDNA was synthesized as follows: 25°C for 10 mins, 37°C for 120 mins, 85°C for 5 mins.

Table 4.1 Preparation of 2X reverse transcription mix from the kit components

Components	Volume/reaction (µl)
10X RT buffer	2.0
25X dNTP MIX (100 mM)	0.8
10X RT random primers	2.0
Multiscribe reverse transcriptase	1.0
Neclease-free water	4.2

(iii) Sybr green Real time polymerase chain reaction (RT-PCR)

The mRNA expression of cytokines including interleukin-1β (IL-1β), interleukin -6 (IL-6), interferon-γ (IFN-γ), interleukin-10 (IL-10) and interleukin-4 (IL-4) were quantified by Quantitative real time (qRT-PCR), using Hi-Sybr Master mix (MBT074). The reaction mix was prepared by mixing together 1X Sybr green Universal PCR master mix, 500 nM of forward and reverse primers, 1 µl of cDNA. The following universal cycling conditions were used: 40 cycles of 95°C for 2 mins, annealing and extension at 55°C for 45 secs followed by melt curve analysis. Table 4.2 shows the list of primer sequences used. All the samples were run in triplicate and the threshold cycle (Ct) values were recorded in the real time PCR machine (Model CFX96, BioRad. Appropriate

no template control (NTC) were used. β-actin served as the endogenous control to enable normalization prior to comparison.

Table 4.2 List of primers used for quantification of mRNA expression of cytokines

Gene	Primer sequences (5'-3')	
	Forward primer	**Reverse primer**
Actin	GGACTTCGAGCAAGAGATGG	AGGAAGGAAGGCTGGAAGAG
IL-1β	ACAGATGAAGTGCTCCTTCCA	GTCGGAGATTCGTAGCTGGAT
IL-6	CCACCGGGAACGAAAGAGAA	TCTTCTCCTGGGGGTACTGG
IFN-γ	GGCAGCATCGCTTTAAACTC	GGAGGTGGGGGCTTTTATTA
IL-4	ACAAGTGCGATATCACCTTAC	CAACGTACTCTGGTTGGCT
IL-10	GCCTAACATGCTTCGAGATC	CTCATGGCTTTGTAGATGCC

4.2.7 Assessment of recovery of cells after jacalin treatment

$2x10^4$ cells were seeded per well of a 12 well plate, after which 100 µg/ml jacalin were added to the cells. After 1, 2, 3 and 6 h, the cells were pelleted and the supernatant was discarded. To allow the cells to recover post treatment of jacalin, the cells were cultured in RPMI 1640 containing 10% FBS and 1% antibiotics. The cells without jacalin were then incubated for 36 h. The percentage of cell viability was assessed by MTT tetrazolium assay. Cells grown for 36 h in RPMI 1640 conatining 10% FBS and 1% antibiotics were considered as control cells and the viability of the treated cells were calculated accordingly.

To assess the effects of jacalin on aggregation of the cells post recovery, 100 µg/ml jacalin was added to the cells. After 3 h and 6 h, the cells were pelleted and were cultured in fresh RPMI 1640. The images of cells were obtained after 36 h.

4.2.8 Quantification of mRNA levels of cytokines in jacalin-treated K562 cells, after recovery

$1x10^6$ cells were seeded per well of a 6 well plate after which 100 µg/ml jacalin were added to the cells. After 3 and 6 h, the cells were collected by centrifugation and were reseeded in RPMI 1640 containing 10% FBS and 1x antibiotic and incubated for 12 h at 37°C in a humified atmosphere containing 5% CO_2. The mRNA expression of cytokines were quantified by qRT-PCR.

4.2.9 Real time data analysis

The relative mRNA levels of the cytokines were denoted in terms of fold change ($2^{-\Delta\Delta Ct}$). The cells that were grown in RPMI 1640 containing 1% serum, without jacalin were regarded as the control cells. The fold change of expression was calculated with respect to the control cells. The relative abundance of the genes were normalized according to the expression level of the internal control gene, β actin.

Fold change = $2^{-\Delta\Delta Ct}$

Where, $\Delta\Delta Ct = (C_{t,\,Target} - C_{t,\,Actin})_{treated} - (C_{t,\,Target} - C_{t,\,Actin})_{untreated}$

treated – cells treated with jacalin for 3 h or 6 h

untreated –that were allowed to grow without jacalin

[268].

4.3 RESULTS AND DISCUSSION

4.3.1 Effects of different concentrations of FBS on the proliferation of K562 cells

K652 cells are routinely cultured in 10% FBS. However, it was observed that jacalin had no influence on K562 cell proliferation in the presence of increased concentration of FBS. As the surface of FBS contains specific sugars that can bind to lectins, the effects of lectins have been ascertained to be reduced or even nullified in the presence of 10% FBS [269,270]. It is possible that upon binding, these proteins neutralize the effects of lectins. Hence, cells cultured in medium that was supplemented with 1% FBS was used.

The effects of various concentrations of FBS on K562 cell proliferation was analyzed as it was deemed significant to find out that particular concentration of FBS which will neither neutralize the effects of lectins, nor will have a negative impact on cell proliferation;. As shown in figure 4.1, the K562 cell proliferation was found to decline with decreasing concentrations of FBS. In the presence of 1% FBS, only 70–75% of cell growth was observed as compared to those cells which were grown in RPMI 1640, containing 10% FBS. However, as the control cells were also allowed to grow using the same serum concentration, it can be corroborated that the observed results are not because of the difference in serum concentration.

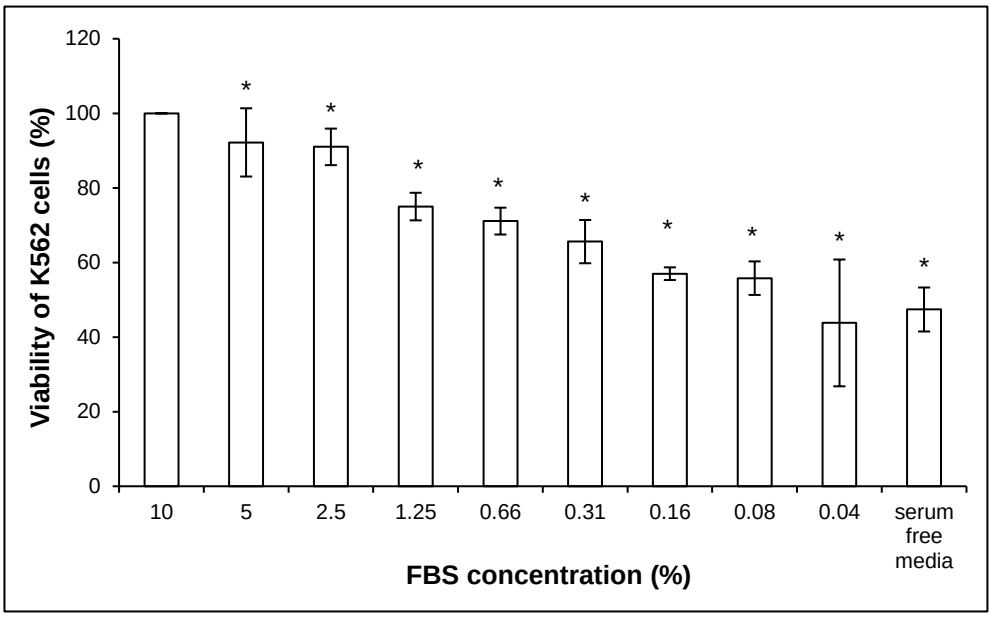


Figure 4.1 Effects of varying concentrations of FBS on K562 cell viability. K562 cells were allowed to grow in RPMI 1640 supplemented with decreasing concentrations of FBS (10%-0%) for 24 h and the rate of cell proliferation was assessed by MTT tetrazolium assay. Cells grown in 10% FBS were considered as 100% cell growth and the viability of the treated cells were calculated accordingly. Error bars indicate the standard deviations. *p≤0.05.

4.3.2 Differential effects of jacalin on the proliferation of cells of different lineage

Among the cancer cells analyzed, jacalin was found to exert dose-dependent inhibitory effects on the proliferation of A549, HeLa and HT29 cells [Figure 4.2a, b & c]. On the contrary, in case of K562 cells, jacalin was found to act as a mitogen. As shown in figure 4.2d, the cell viability percentage of K562 cells increased proportionally with increasing concentrations of jacalin. Overall, it may be suggested that jacalin may exhibit differential ways of binding to various cells, culminating in diverse effects on proliferation of cells. Further, to assess the effects of jacalin on non-tumorigenic cells, HEK 293 cells were treated with various concentrations of jacalin and the viability percentage of the cells was determined by MTT tetrazolium assay. While the viability of HEK 293 cells remained unaffected at jacalin concentrations up to 125 µg/ml, jacalin stimulated the proliferation of HEK 293 cells at higher concentrations (figure 4.2e). This is in accordance with a previous report where growth of normal cells have been shown to be stimulated by lectins [271].

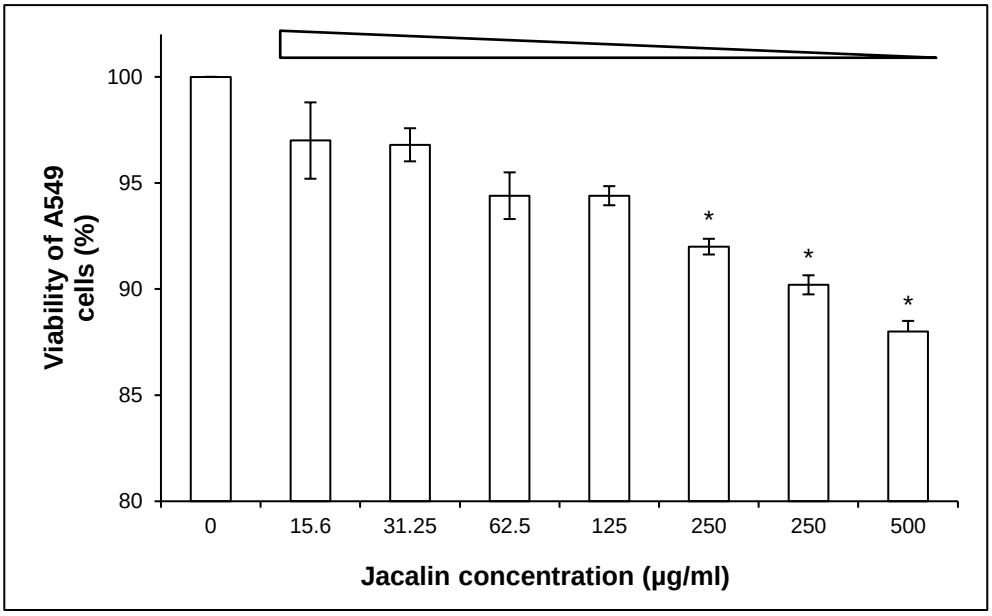


Figure 4.2a Effects of jacalin on A549 cell viability. A549 cells were cultured in various concentrations of jacalin (500 – 3.9 µg/ml) for 24 h and the viability percentage was assessed by MTT tetrazolium assay. Untreated cells grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of the treated cells were calculated accordingly. The values indicate the average of three wells. Error bars indicate the standard deviations. *p≤0.05.

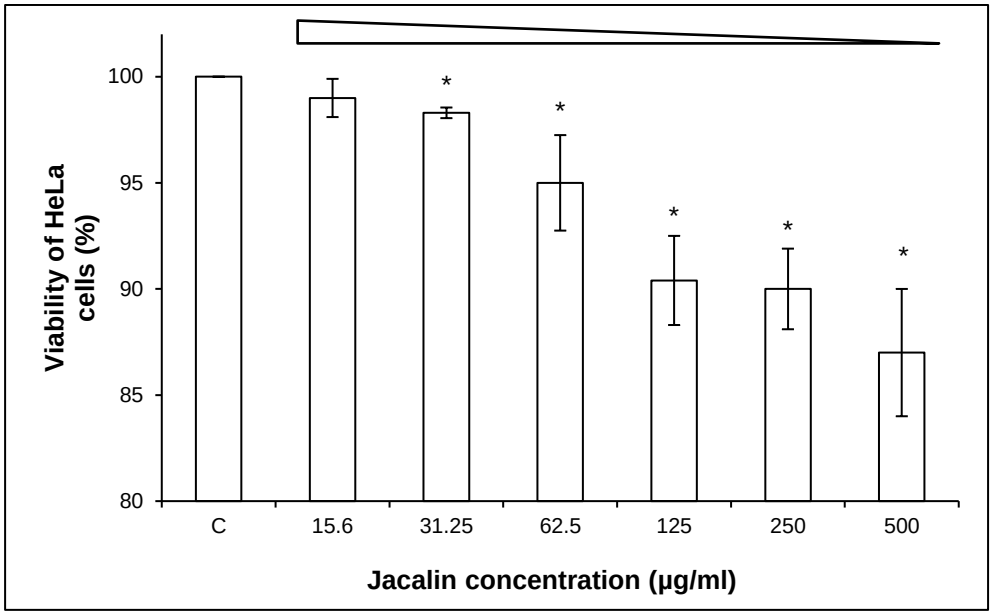


Figure 4.2b Effects of jacalin on HeLa cell viability. HeLa cells were cultured in various concentrations of jacalin (500 – 3.9 µg/ml) for 24 h and the viability percentage was assessed by MTT tetrazolium assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. The values indicate the average of three wells. Error bars indicate the standard deviations. *p≤0.05.

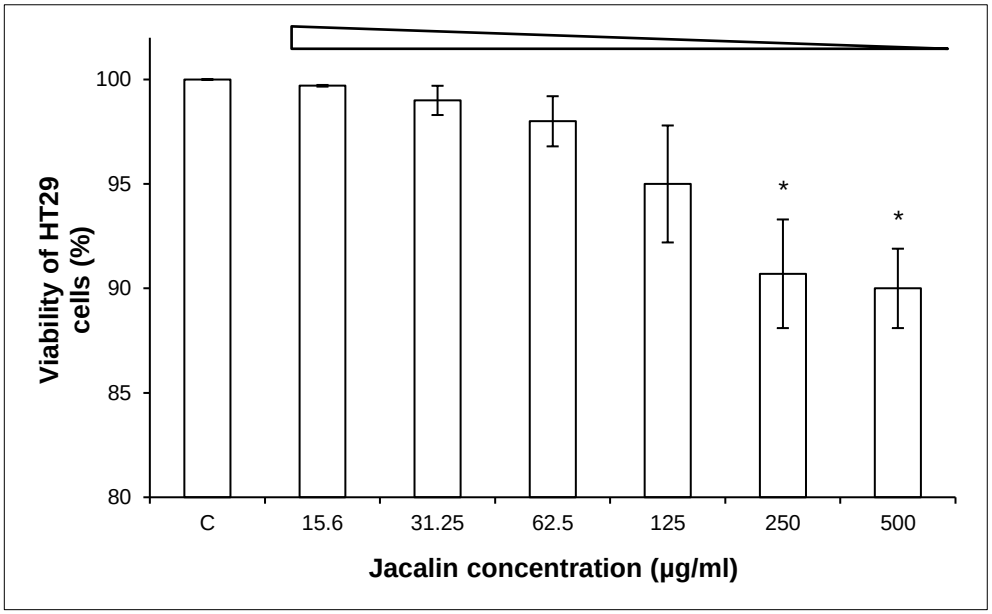


Figure 4.2c Effects of jacalin on HT29 cell viability. HT29 cells were cultured in various concentrations of jacalin (500 – 3.9 µg/ml) for 24 h and the viability percentage was assessed by MTT tetrazolium assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. The values indicate the average of three wells. Error bars indicate the standard deviations. *p≤0.05.

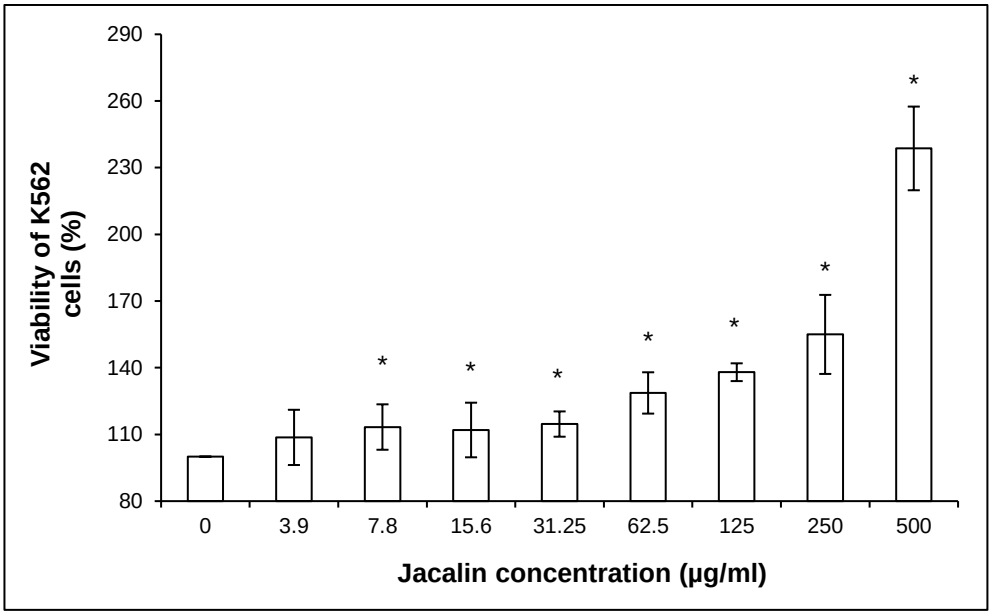


Figure 4.2d Effects of jacalin on K562 cell viability. K562 cells were cultured in various concentrations of jacalin (500 – 3.9 µg/ml) for 24 h and the viability percentage assessed by MTT tetrazolium assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. The values indicate the average of three wells. Error bars indicate the standard deviations. *p≤0.05.

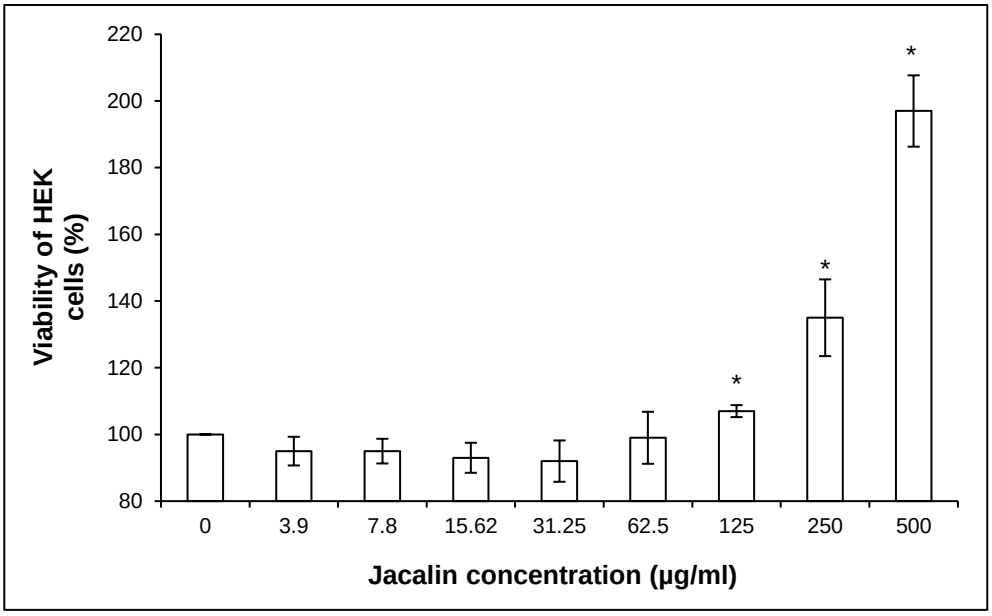


Figure 4.2e Effects of jacalin on viability of HEK cells. HEK cells were cultured in various concentrations of jacalin (500 – 3.9 µg/ml) for 24 h and the viability percentage assessed by MTT tetrazolium assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. The values indicate the average of three wells. Error bars indicate the standard deviations. *p≤0.05.

4.3.3 Effects of D-galactose on the viability of K562 cells

To prove that jacalin binds to the Gal/GalNac on the surface of K562 cells, the K562 cells were treated with jacalin and galactose, which is the specific jacalin binding sugar. However, to rule out the possibility that galactose might inhibit/stimulate K562 cell proliferation, the rate of proliferation of K562 cells in the presence of various concentrations of galactose (100 mM-1.5 mM) were analyzed. As shown in figure 4.3a, galactose at higher concentrations were shown to be toxic to the cells while concentrations of 300 µM and lower had no impact on K562 cell proliferation.

Thereafter, 100 µg/ml jacalin were added to the K562 cells, along with decreasing concentrations of galactose to find out the optimum concentration which will neutralize the effects of jacalin without being toxic to the cells 300 µM of galactose was found to completely nullify the mitogenic effect of 100 µg/ml of jacalin (figure 4.3b).

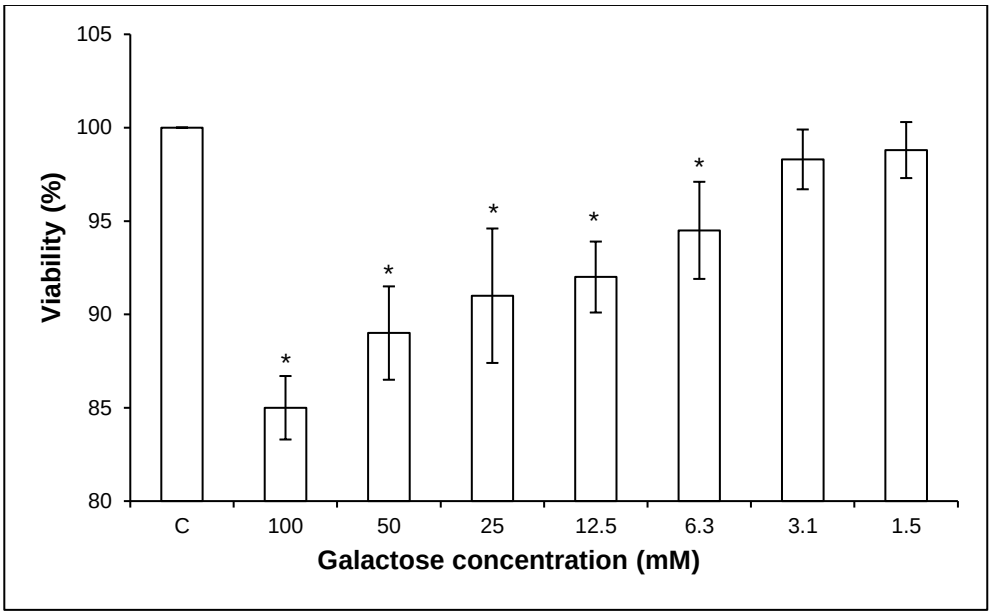


Figure 4.3a Effect of D-galactose on the K562 cell viability. K562 cells were cultured with varying concentrations of D-galactose (100 mM – 1.5 mM) and the viability percentage was determined by MTT tetrazolium assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. Error bars indicate the standard deviations. *p≤0.05.

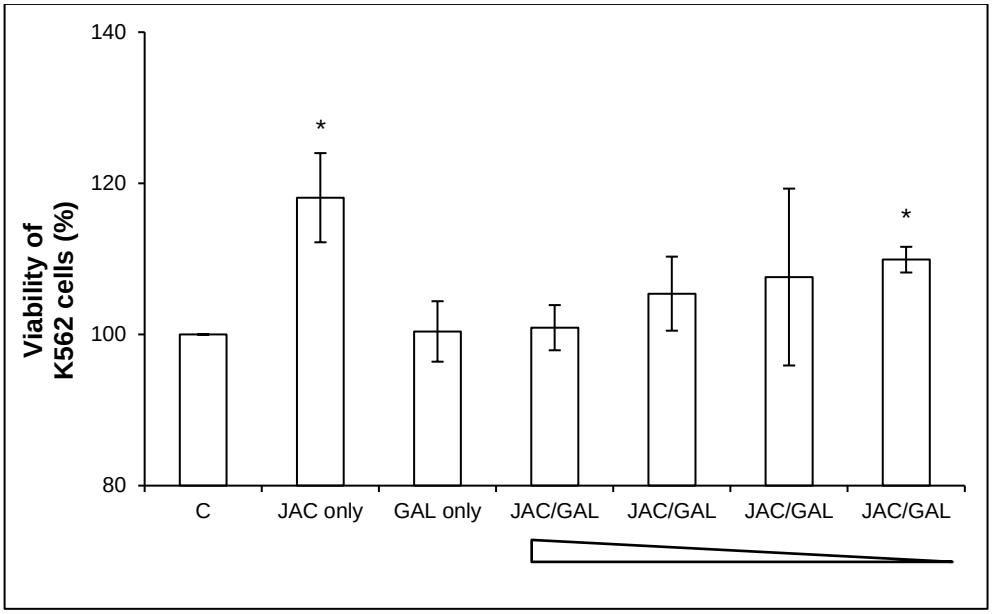


Figure 4.3b Effects of D-galactose on jacalin induced proliferation of K562 cells. 100 µg/ml jacalin was added to K562 cells that were preincubated with varying concentrations of D-galactose (300 µM – 37.5 µM) and the viability percentage was determined by MTT assay. Untreated cells that were grown in the presence of 1% FBS were regarded as 100% cell growth and the viability of treated cells were calculated accordingly. Cells allowed to grow in the presence of 100 µg/ml jacalin alone were referred to Jacalin control. Gal only denotes cells that were grown in the presence of 300 µM galactose alone. *p≤0.05.

4.3.4 Effects of jacalin on aggregation of K562 cells

Cancer cell aggregation is known to facilitate better survival of cells as compared to individual cells. Cells in aggregates easily metastasize to secondary sites and are capable of escaping anoikis, which facilitates existence of cancer cells in the lymphatic circulation [272,273].

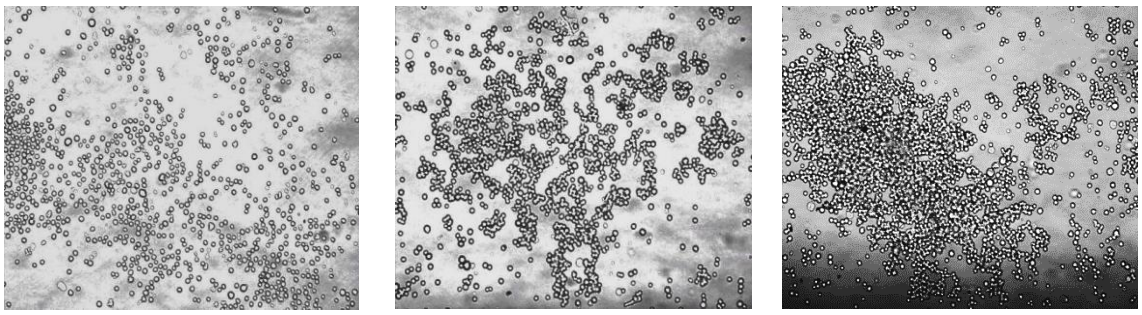
100 µg/ml jacalin was shown to have a remarkable effect on aggregation of K562 cells which is clearly visible even within 1 h of treatment (figure 4.5a). Nevertheless, when the cells were incubated with jacalin along with 300 µM of galactose, remarkable decrease in the amount of visible aggregates of K562 cells was observed (figure 4.4b). This is due to the ability of galactose to bind to jacalin, thereby preventing jacalin from binding to the β gal residues on the K562 cell surface. Table 4.3 shows the varying degrees of homotypic aggregation of K562 cells upon jacalin treatment. The formation of aggregates was monitored at regular intervals of time (5 mins – 60 mins) and the total number of cells in each aggregate was recorded.

Table 4.3 Varying degrees of homotypic aggregation of K562 cells upon jacalin treatment

Incubation time	Degrees of cell aggregation
5 mins	-
10 mins	1+
15 mins	1+
30 mins	2+
45 mins	2+
60 mins	3+

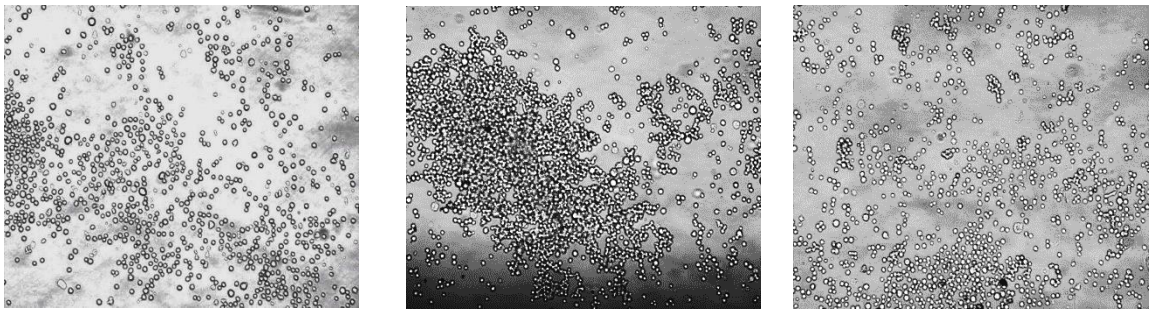
Degrees of cell aggregation: (-) - no aggregation,
(1+) - 1-5 cells/aggregate,
(2+) - 6-10 cells/aggregate,
(3+) - 10-15 cells/aggregate,
(4+) - 15+ cells/aggregate

(a)



Control **Jacalin (100µg/ml) 1h** **Jacalin (100µg/ml) 3h**

(b)



Control (untreated) **Jacalin (100µg/ml) 3h** **(jacalin+gal) 3h**

Figure 4.4 (a) Jacalin induces homotypic aggregation of K562 cells.
(b) Galactose inhibits jacalin induced aggregation of K562 cells.
Images of K562 cells that were cultured under different conditions were obtained using Zeiss Axioskop Fluorescence Phase Contrast Microscope.

4.3.5 Effects of jacalin-treated K562 cells CM on the viability of fresh K562 cells

To decipher the events that may be responsible for increase in K562 cell proliferation, the effects of CM from jacalin-treated K562 cells on the growth of fresh K562 cells were analyzed. The main aim of the study was to determine the soluble factors in the jacalin-treated K562 CM (J CM) which promotes K562 cell proliferation. The cell secretome or CM consists of proteins such as cytokines and various soluble mediators which the cells require for their normal growth. The main function of theses soluble mediators is to synchronize the binding between that occurs between the cells and between the cell and the matrix [274].

CM were obtained from K562 cells that cultured with 100 µg/mL jacalin for 24, 6 and 3 h. Fresh K562 cells were then allowed to grow with the following CM; 3h J CM, 6h J CM, 24h J CM, along with fresh media in 1:1 ratio. However, the CM also have jacalin; therefore the effects of J CM on K562 cells were also analyzed after addition of 300 µM of galactose which will neutralize the effects of jacalin. K562 cells cultured in RPMI 1640 media was taken as control and the viability of cells cultured in CM were calculated accordingly. The cells cultured with all the three CM followed an almost similar growth trend (figure 4.5a). However, in case of cells cultured with 3h and 6h J CM along with galactose, no remarkable difference in proliferation was observed (figure 4.5b). Thus, the initial increase in proliferation of cells cultured in 3 h and 6 h CM may be attributed to the presence of biologically active jacalin in the CM. However, in case of K562 cells that were treated with 24h J CM and galactose, a remarkable increase in cell viability confirms the presence of certain soluble factors in the 24h J CM that are responsible for increased cell proliferation (figure 4.5b).

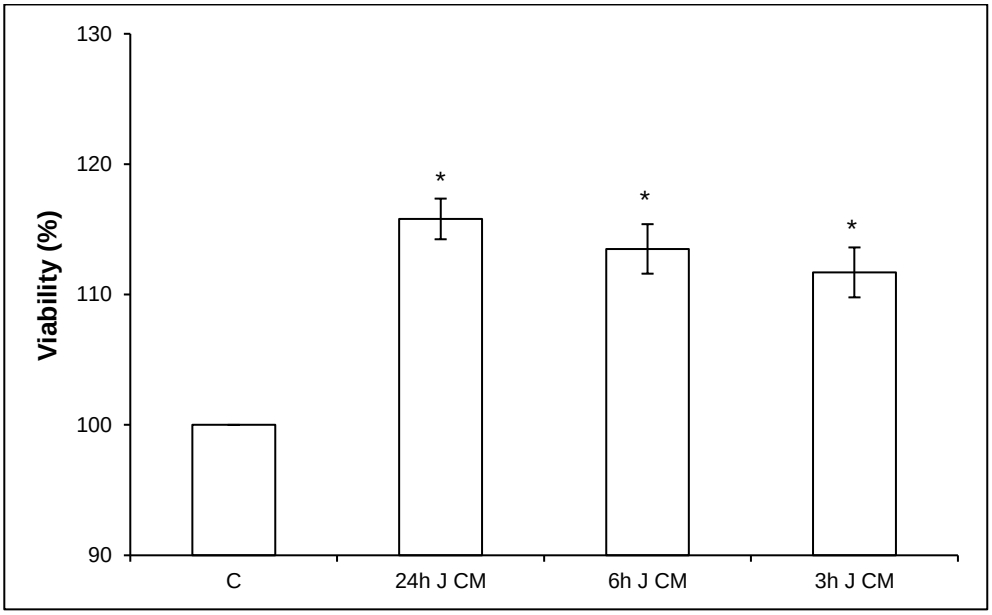


Figure 4.5a Effects of jacalin treated conditioned media on K562 cell proliferation. 100 µg/ml jacalin was added to the K562 cells. After 3, 6 and 24 h, the CM were collected. Fresh K562 cells were allowed to grow in the respective CM along with equal volumes of fresh RPMI medium for 36 h. The cell viability was determined by MTT assay. Untreated cells were regarded as 100% cell growth and the viability of the treated cells were calculated accordingly. *p≤0.05.

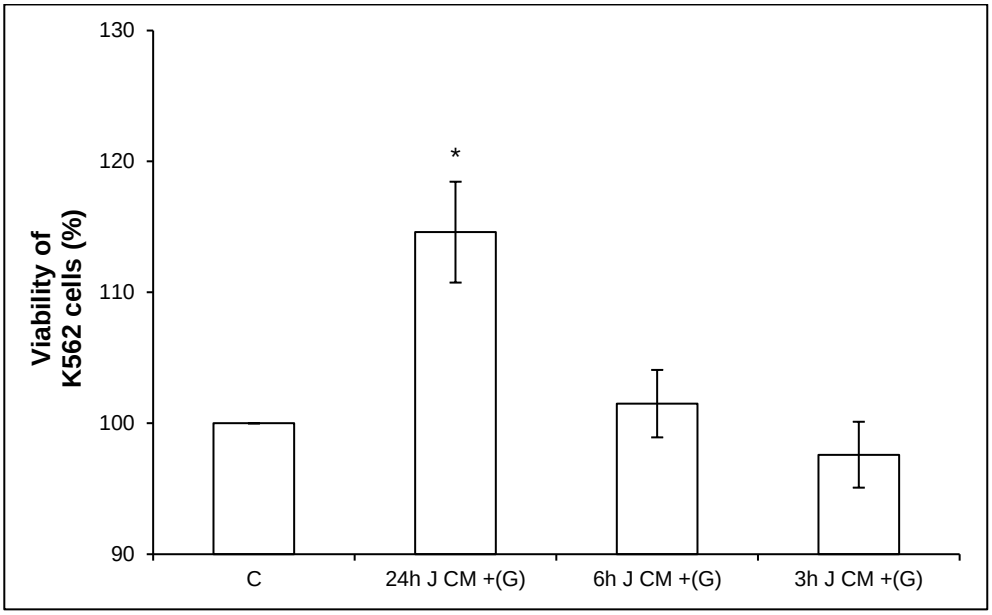


Figure 4.5b Effects of jacalin treated CM along with galactose on K562 proliferation cell. K562 cells were cultured in the presence of 100 µg/ml jacalin. After 3, 6 and 24 h, the CM were collected. Fresh K562 cells were cultured for 36 h in the respective CM along, in the presence of 300 µM galactose along with equal volumes of fresh RPMI medium. The cell viability was determined by MTT assay. Untreated cells were regarded as 100% cell growth and the viability of the treated cells were calculated accordingly. *p≤0.05.

4.3.6 Quantification of mRNA levels of cytokines in jacalin treated K562 cells

The pro-inflammatory and anti-inflammatory cytokines play a major role in progression of cancers [275]. Thereupon, the mRNA expression certain pro-inflammatory cytokines including IL-1β, IFN-gamma IL-6, and anti-inflammatory cytokines IL-10 and IL-4 were analyzed.

As shown in figure 4.6, a remarkable increase in mRNA expression of IL-1β and IL-6 were observed in the 3 h and 6 h jacalin treated K562 cells, as compared to the untreated control cells. IL-1 β is a pro-inflammatory cytokine that supports tumor invasiveness, metastasis and angiogenesis through the expression of cell adhesion molecules on malignant and endothelial cells, [276]. Further, IL-1 β can also activate NF-κB which in turn induces the expression of other tumor-promoting proteins including the MMPs, urokinase-type plasminogen activator and TNF-α [277]. Likewise, IL-6 is a multifaceted pro-inflammatory cytokine, the expression of which has been found to be altered in several cancers. Increased expression of IL-6 has been associated with tumorigenesis and metastasis of cancers including that of the prostate, colon and breast [278-280]. In esophageal carcinoma and myeloma cells, IL-6 was shown to inhibit apoptosis thus exerting a remarkable pro-survival function [281,282]. Overall, the elevated levels of IL-6 in cancer patients has been shown to be correlated with poor prognosis [283,284].

Among the other cytokines, the mRNA levels of IL-4 was found to be high in 6 h jacalin treated cells, as compared to the control cells. However, no remarkable change in mRNA levels of IL-10 and IFN-gamma was observed (figure 4.6). Though IL-4 primarily acts as an anti-inflammatory cytokine, there are reports to prove that IL-4 can also support the survival of cancer cells by activation of AKT pathway [285].

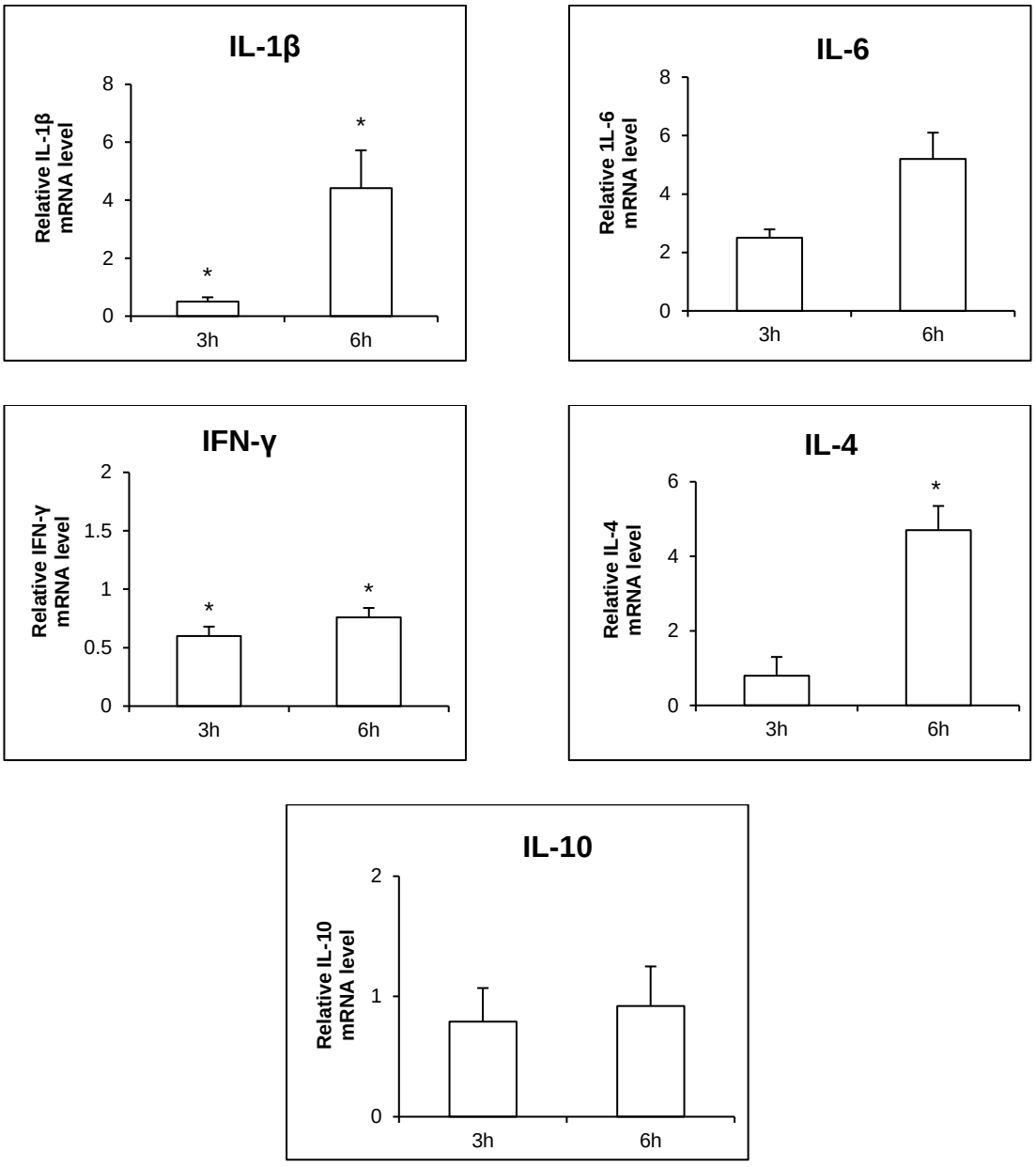


Figure 4.6 Relative mRNA levels of cytokines in jacalin-treated K562 cells. K562 cells were grown in the presence of 100 µg/ml jacalin. After 3 and 6 h of treatment, the mRNA expression of the cytokines IL-1, IL-4, IL-6, IFN-γ and IL-10 were analyzed with respect to the untreated control. Bar graph denotes the respective fold change in expression. β-actin serves as the housekeeping gene. *p≤0.05.

4.3.7 Assessment of recovery of cells after jacalin treatment

The K562 cells were initially subjected to jacalin treatment for 1, 2, 3 and 6 h. After the respective time points, the cells were subjected to centrifugation, collected separately and cultured in fresh RPMI 1640 containing 10% FBS and IX antibiotic for 36 h. After 36 h of incubation, viability percentage was found to be directly proportional to the time point of jacalin treatment. Moreover, the cells were not able to recover from jacalin treatment even after culturing them for 36 h, without jacalin.

As shown in figure 4.7a, the proliferative response of the cells were found to correspond to the initial time of exposure to jacalin as the vitality percentage of 6 h jacalin treated cells were observed to be high compared to cells that were treated for a 3, 2 and 1 h and then allowed to recover.

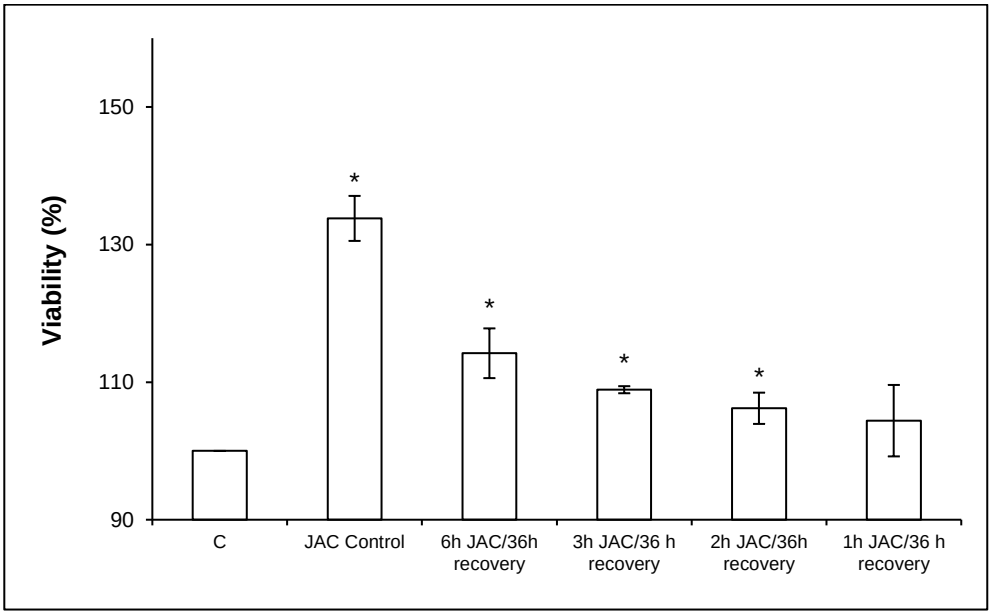
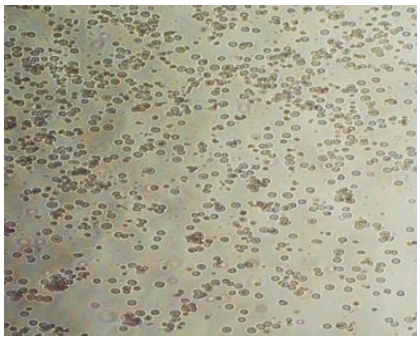
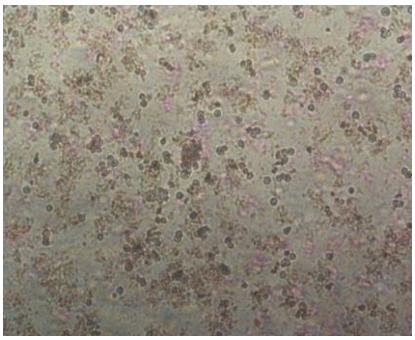


Figure 4.7a Viability of K562 cells, post removal of jacalin. K562 cells were grown in the presence of 100 µg/ml jacalin. After 1, 2, 3 and 6 h, the cells were collected by centrifugation and were allowed to grow for 36 h in fresh RPMI 1640, without jacalin. The viability percentage was determined by MTT tetrazolium assay. Untreated cells were regarded as 100% cell growth and the viability of the treated cells were calculated accordingly. Jacalin control denotes cells that were continuously under jacalin treatment. *p≤0.05.

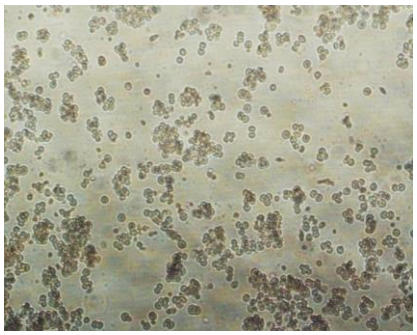
The ability of the cell to form homotypic aggregates also depends on the time point of exposure to jacalin as evident from images of cells that were initially treated with jacalin for 3 and 6 h and later allowed to recover. More significantly, the cells do not get separated from the aggregates even when they were reseeded under normal conditions, after initially being exposed to jacalin for specific time periods (figure 4.7b).



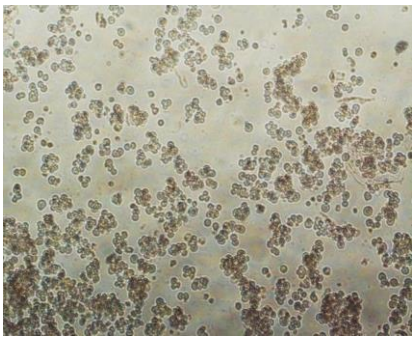
Control K562 cells



Jacalin control



3h Jacalin stimulated/ 36h recovery



6h Jacalin stimulated/ 36h recovery

Figure 4.7b Effects of jacalin on aggregation of K562 cells are sustained. K562 cells were allowed to grow in the presence of 100 µg/ml jacalin. After 3 h and 6 h, the cells were collected by centrifugation and were reseeded in fresh RPMI medium. The images of cells were taken after 36 h. Jacalin control are cells that were continuously grown under the influence of jacalin.

Besides, the mRNA expression of pro-inflammatory cytokines including IL-1β, IFN-gamma IL-6 and anti-inflammatory cytokines IL-10 and IL-4 were analyzed in cells that were grown for 12 h under normal conditions after having treated with jacalin for 3 h and 6 h. As shown in figure 4.7c, an increased in mRNA levels of IL-1β was observed in K562 cells that were initially stimulated with jacalin for 3h and 6h. Further, a slight increase in mRNA levels of IL-6 and IFN-γ were observed in cells that had initially been subjected to jacalin treatment for 6 h. IL-6 has been implicated as a pro-tumorigenic factor in various cancers including lungs, breast, ovarian, colon and has often been associated with poor prognosis [286]. Depending on the cancer microenvironment, IFN-γ can exhibit pro- or anti-tumorigenic effects [287]. On the other side, the relative mRNA expression IL-4 and IL-10 which are anti-inflammatory cytokines was found to be decreased. IL-10 is an immunoregulatory cytokine that exhibits antitumor and antimetastatic potential [288,289].

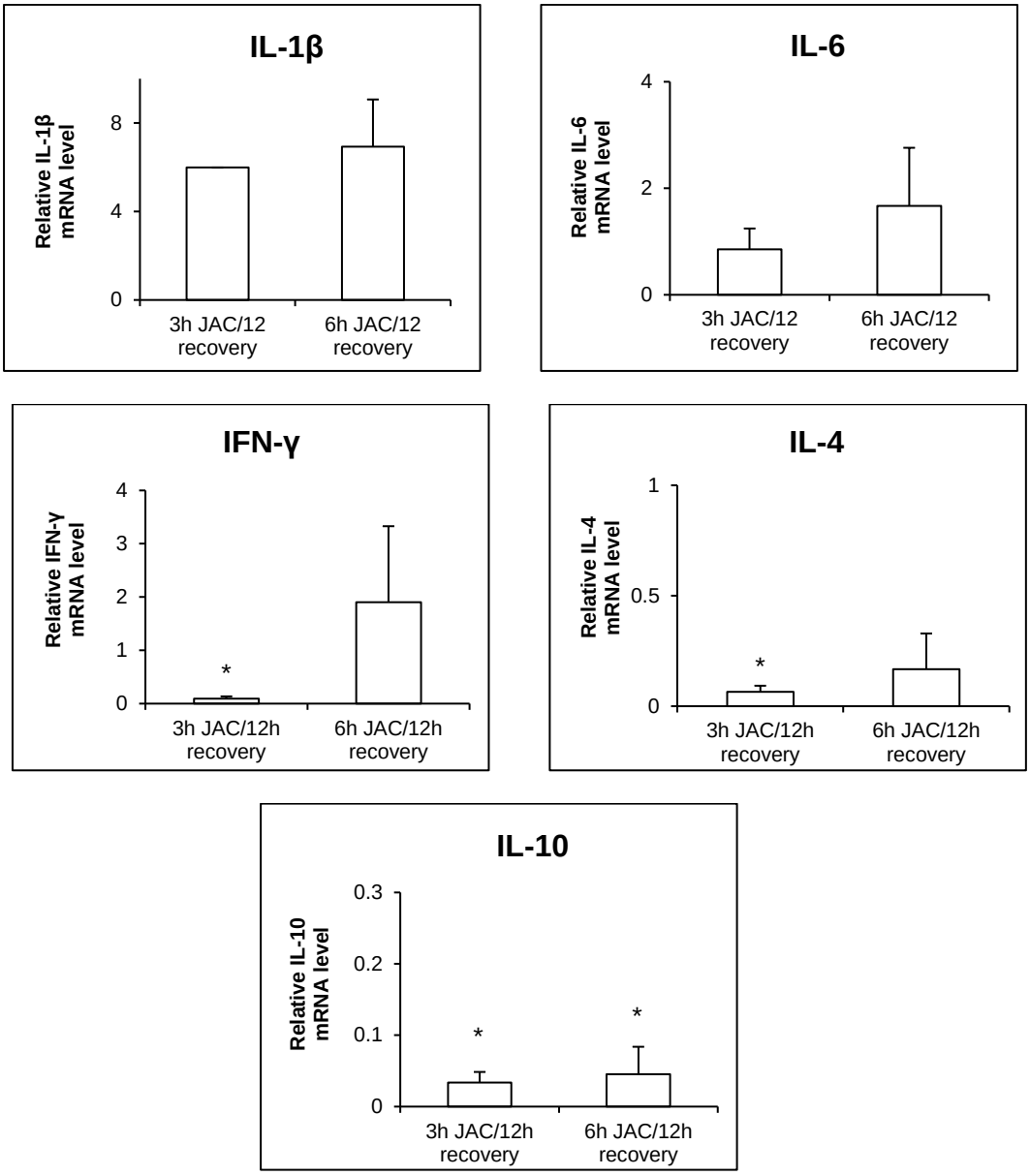


Figure 4.7c Relative mRNA levels of cytokines in K562 cells, after recovery from jacalin treatment. K562 cells were grown in the presence of 100 µg/ml jacalin. After 3 and 6 h, the cells were collected by centrifugation and allowed to grow in fresh RPMI medium for 12 h. The mRNA expression of the cytokines IL-1, IL-6, IL-4, IFN-gamma and IL-10 were analyzed with respect to the untreated control. Bar graph denotes the respective fold change in expression. β-actin serves as the housekeeping gene. *p≤0.05.

4.4 CONCLUSION

In summary, in this chapter, the outcome of jacalin treatment on cells of different lineages were assessed, with emphasis on the mitogenic facet of jacalin on K562 cells. While jacalin was found to be highly mitogenic to K562 erythroleukemia cells, the same lectin was found to inhibit the proliferation of other cancer cells studied. These observations suggest that jacalin might exhibit varying modes of binding to different cells, resulting in adverse outcome on cell proliferation. Further, the lack of previous reports highlighting the mitogenic facet of jacalin, further enhanced the significance of the particular observation that jacalin induced the proliferation of K562 cells. The sustained effect of jacalin on K562 cell proliferation may be attributed to secretion of soluble molecules. Further, an increase in mRNA levels of IL-1β and IL-6 provide more support to the notion that the jacalin-mediated effects occurs due to the activation of certain signaling pathway.