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Original Article

Evaluation of Anticancer Activity of Boswellic Acid and Montelukast Sodium against Human Prostate Cancer Cell Line PC-3

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Abstract

Prostate cancer is a devastating disease for which current therapies are inadequate. Various lines of evidences have suggested the 5-lipoxygenase (5-LOX) pathway and the leukotriene receptor pathway are potential targets for prevention or treatment of Prostate cancer. Thus, search for new anti-cancer drugs targeting 5-LOX and leukotriene is very essential and important. The objective of the present study was to evaluate the *in vitro* anti- cancer effects of 5-LOX inhibitor-Boswellic acid and cysteinyl leukotrienes receptor antagonist-Montelukast sodium by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay and Sulphorodamine B assay (SRB) against human prostate cancer cell line PC-3. Cell viability was assayed by trypan blue dye exclusion assay. A study was carried out in 24, 48, and 72 hours. In addition, effect of combination of both the above drugs was also determined by the same assays. Boswellic acid and Montelukast sodium demonstrated a substantial anti-cancer effect against the PC-3 cell line. The cytotoxicity of both the drugs increased with increase in duration of drug treatment. A combination of both drugs showed significant reduction in cell viability but did not show any synergistic activity. Complete dose-response curves were generated and IC50 values were calculated for all the assays. IC_{50} Value for Boswellic acid was found to be 49.15- 45.80 µg/ml and 50.14-43.39 µg/ml by MTT assay and SRB assay, respectively at the same time IC_{50} value for Montelukast sodium was 49.27-46.77 µg/ml and 46.68-46.47 µg/ml by MTT assay and SRB assay respectively. In summary, Boswellic acid and Montelukast sodium are likely to be valuable for the treatment of prostate cancer, but further studies are required for their more extensive biological evaluations.

Key Words: Anti-cancer activity, Boswellic Acid, Montelukast Sodium, MTT assay, PC-3 cell line, SRB assay.

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1. Introduction

Prostate cancer is the most common form of malignancy and the second leading cause of cancer-related deaths in men living in the United States [1, 2]. Surgery, chemotherapy and radiation therapy have been used to control localized prostate cancer, but the management of metastatic prostate cancer still remains a major problem for which no satisfactory measure is available. Thus, screening and characterization of novel agents, that are specific and critical for prostate cancer cells, are of utmost significance to develop effective strategies to control prostate cancer.

The Lipoxygenases (LOXs) are a class of enzymes that converts arachidonic, linoleic, and other polyunsaturated fatty acid to its biologically active metabolites involved in the inflammatory and immune responses. Both epidemiological studies and experiments have related consumption of high-fat diets to occurrence of clinically evident prostate cancer [3,4,5], indicating that dietary fatty acids and their metabolic products may play an important role in the promotion and/or progression phases of prostate cancer. It has been observed that 5-LOX is overexpressed in human prostate cancer tissue, its expression in normal prostate glands is undetectable [6]. Several independent studies have proven the correlation between the 5-LOX over expression and cancer cell viability, proliferation, cell migration, invasion through extracellular matrix destruction, metastasis, and activation of anti-apoptotic signaling cascades [7]. Moreover Hassan et al., 2006 found that 5-LOX activity is required to stimulate prostate cancer cell growth by epidermal growth factor (EGF) and other cancer cell proliferating factors produced in the body. When 5-LOX levels were reduced, the cancer cell stimulatory effect of EGF and other growth factors were diminished [8]. Furthermore, inhibition of 5-LOX results in inhibition of cell proliferation and tumor angiogenesis whereas it induces rapid and massive apoptosis in both androgen-dependent and androgen-independent human prostate cancer cell lines [9, 10, 11]. Thus, critical role of 5-LOX in the development and progression of prostate cancer cells leads to searching and characterization of novel drugs that can target 5-LOX pathway, are of utmost significance to control prostate cancer.

5-LOX metabolizes arachidonic acid by different pathways to LTC4, LTD4, and LTE4 which are collectively known as cysteinyl leukotrienes (CysLT)) [12]. CysLT signal through two G-protein-coupled receptors, CysLT1R and CysLT2R, which differ in ligand specificities and distribution [13]. Recently, it has been shown that CysLT1R is highly expressed in a variety

human urological cancer cell lines (e.g., renal cell carcinoma, bladder cancer, prostate cancer, and testicular cancer) and LTD4-induced CysLT1R signalling results in cell proliferation, survival, and migration through distinct signalling pathways. It was also reported that CysLT1R antagonist treatments inhibit tumour growth by inducing apoptosis *in vitro* [14]. Moreover, Nazoki *et al*. 2013, has reported that Cysteinyl leukotriene receptor antagonists such as pranlukast inhibit tumor Metastasis [15]. So with this background, considerable efforts are being expended to produce 5-LOX as well as leukotriene antagonists, because the 5-LOX products, leukotrienes (LTB4, LTC4, LTD4, and LTE4) have been implicated as mediator of prostate cancer.

The active principle of a gum resin from *Boswellia serrate Roxb*., Boswellic acid is 5-LOX inhibitor and has been traditionally used in treatments for various inflammatory diseases including arthritis and chronic colitis [16, 17]. Boswellic acid has also been shown to exert antitumor effects in human cell lines established from brain tumours [18], colorectal cancer [19] and leukaemia [20]. More recently, it is reported that Boswellic acid inhibits the growth of orthotopic tumours in mice with colorectal cancer [21] and pancreatic cancer [22]. Montelukast sodium, CysLTR antagonist, has been used in the treatment of bronchial asthma and allergic rhinitis.

In the light of above reports the present investigation was undertaken to study the *in vitro* cytotoxic activity and cell viability of 5-LOX antagonists-Boswellic acid and cysteinyl leukotriene receptor antagonist-Montelukast sodium as well as their combination against human prostate cancer cell line PC-3.

2. Materials and Methods

2.1. Cell Culture

Human prostate cancer cell lines PC-3 used in this study were procured from National Centre for Cell Science, Pune. The PC-3 cell line was maintained in control condition, i.e. Ham's F 12 K medium supplemented with L-Glutamine, at 37°C temperature and 5% CO_2 level in a CO2 incubator at section mutagenicity, Department of toxicology, at Jai Research Foundation, Vapi.

2.2. Qualitative Analysis of Boswellic Acid by HPTLC

Qualitative analysis of Boswellic acid was done by using HPTLC. The test compound was compared to standard *Boswellia serrate Roxb* by using MERCK/HPTLC silica gel 60 F254 aluminum sheets as stationary phase and hexane: ethyl acetate (7:3 v/v) as mobile phase. The derivitization was carried out with 10% sulfuric acid in methanol.

2.3. Trypan Blue Dye Assay

Preliminary cell viability test was performed using the trypan blue assay. In brief, the trypsinized cells from culture flask were seeded in 96-well plates at a density of $5x10^4$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 24 hours incubation, plates were inoculated with 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/ml concentration of Boswellic acid or 3.90, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 µg/ml of Montelukast sodium solubilized in DMSO (100%) and incubated at 37°C and 5% CO_2 atmosphere for 24 hours. In a separate experiment, cells were incubated with a mixture of Boswellic acid and Montelukast sodium at 1:25 ratio in DMSO at 37°C and 5% CO_2 atmosphere for 24 hours. Then the plates were subjected to trypsinization and 100 µl of cell suspension from each well was mixed with 100 µl of 0.8% trypan blue dye solution and kept aside for two minutes. Prepared suspension was transferred to the edge of haemocytometer and total 100 cells were counted and categorized into dead cell and viable (colorless) cell. The experiment was also carried out with the same drug concentrations for 48 hours and 72 hours treatment period in triplicate.

% cell viability = [total viable cells (unstained) / total cells (stained plus unstained)] x 100

2.4. MTT Assay

In vitro cytotoxic effect of Boswellic acid and Montelukast sodium was evaluated by using MTT assay, as described earlier [23, 24]. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.2 x 10^5 cells/ml using Ham's F-12 K medium containing 10% FBS. The trypsinized cells were seeded in 96-well plate at a density of $1.2x10^5$ cells/well in Ham's F-12 K medium containing 10% FBS and cultured at 37°C in 5% CO_2 to adhere. After 24 hours, the cells were incubated with 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5 and 25 µg/ml concentrations of Boswellic acid or 0.125, 0.250, 0.5, 1, 2, 4, 8 and 16 µg/ml concentrations of Montelukast sodium or a mixture of Boswellic acid and Montelukast sodium at different concentration (Table 1) solubilized in DMSO (100%) at 37°C and 5% CO_2 atmosphere for 24 hours. Culture medium and solvent were used as controls. After 24 hours incubation the cells were washed twice with phosphate buffered saline (PBS) and MTT (0.5 mg/ml PBS) was added to each well and incubated for 4 hours at 37°C. The supernatant growth medium was removed from the wells and replaced with 200 µl of DMSO to solubilize the colored formazan product. After 30 mins incubation, the absorbance (OD) of the culture plate was read at a wavelength of 540 nm with reference wavelength of 630 nm on an ELISA reader. The percentage cell viability was calculated by the formula

% Viability = Corrected OD of sample /Control OD ×100

% Inhibition = 100 - % viability.

Table 1. Different concentrations of Boswellic acid, Montelukast sodium and their mixture used in the study for MTT and SRB assay.

Group	Boswellic acid (µg/ml)	Montelukast sodium (µg/ml)	Mixture	
			Boswellic acid (µg/ml)	Montelukast sodium (µg/ml)
1	25	16	6.25	16
2	12.5	8	3.125	8
3	6.25	4	1.56	4
4	3.125	2	0.78	2
5	1.56	1	0.39	1
6	0.78	0.5	0.195	0.5
7	0.39	0.250	0.098	0.250
8	0.195	0.125	0.049	0.125

Further experiment was carried out with same drug concentrations of both drugs and their mixture for 48 hours and 72 hours treatment period in triplicate. Mean of three absorbance values were calculated for each concentration and blank reading was subtracted from each reading. The graph was plotted for absorbance on Y-axis versus concentration of the drug on X-axis.

2.5. SRB Assay

SRB assay was carried out as described by Skehan *et al*., 1990, using Sulphorhodamine B dye [25]. The Sulphorhodamine B (SRB) assay is used for cell density determination, based on the measurement of cellular protein content. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.2 x 10^5 cells/ml using Ham's F-12 K medium containing 10% FBS. The trypsinized cells were seeded in 96-well plate at a density of 1.2x10^5 cells/well in Ham's F-12 K medium containing 10% FBS and cultured at 37°C in 5% CO_2 to adhere. After 24 hours, the cells were incubated with 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, and 25 µg/ml concentrations of Boswellic acid or 0.125, 0.250, 0.5, 1, 2, 4, 8 and 16 µg/ml concentrations of Montelukast sodium or a mixture of Boswellic acid and Montelukast sodium at different concentration (Table 1) solubilized in DMSO (100%) at 37°C and 5% CO_2 atmosphere for 24 hours. Culture medium and solvent were used as controls. After 24 hours, the plate was removed from the incubator and cell line was fixed in situ with 50 µl cold 10% TCA and incubated for 60 mins at 4°C. After completion of incubation period, plate was washed five times with tap water to remove TCA growth medium, low molecular metabolites, serum protein, etc. For washing, the wells of tissue culture plates were filled with distilled water and the liquid in the wells was discarded by sharply flicking plate over the sink and air dried. The air dried plates were stained with 200 µl of SRB dye 0.4% in 1% acetic acid and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing five times with 1% acetic acid. The

plates were then air-dried and bound stain was subsequently solubilized with 10 mM trizma HCl. The absorbance was read on an automated plate reader at wavelength 515nm.

The percentage growth inhibition was calculated using following formula:

% Viability = Corrected OD of sample /Control OD ×100

% Inhibition = 100 - %viability.

Further experiment was carried out with same drug concentrations of both drugs and their mixture for 48 hours and 72 hours treatment period in triplicate. Mean of three absorbance values were calculated for each concentration and blank reading was subtracted from each reading. The graph was plotted for absorbance on Y-axis versus concentration of the drug on X-axis.

3. Results and Discussion

Prostate cancer is a devastating disease for which current therapies are inadequate. Several researchers have identified the 5-LOX and Cysteinyl leukotrienes receptor pathway as potential targets for prevention or treatment of prostate cancer. Studies show conclusively that 5-LOX directly stimulates prostate cancer cell proliferation via several well-defined mechanisms [26]. It has also been reported that raised 5-LOX level stimulate the activity of EGF and VEGF that are used by the prostate cancer cell to proliferate and to induce the angiogenesis [9, 27]. Moreover, Ghosh and Myers, 1998 reported that selective 5-LOX inhibitors suppress cell growth of PC-3 cellline [28]. Inhibitors of 5-LOX and 5-LOX-activating protein (FLAP) can also induce apoptosis in PC-3 and LNCaP cell lines [29]. Arachidonic acid is metabolized to various leukotrienes by 5-LOX via different pathways. LTC4, LTD4, and LTE4 are collectively known as cysteinyl leukotrienes (CysLT). CysLT signal through two G-protein-coupled receptors, CysLT1 and CysLT2, which differ in ligand specificities and distribution [13]. Recently it has been shown that CysLT1R is highly expressed in a variety human urological cancer cell lines (e.g., renal cell carcinoma, bladder cancer, prostate cancer, and testicular cancer) and CysLT1R antagonist treatment have been shown to inhibit tumour growth by inducing apoptosis in the same cell lines. CysLT1R antagonist also is shown to inhibit tumour metastasis [14]. Moreover, it has been shown that LTD4-induced CysLT1R signalling results in cell proliferation, survival, and migration through distinct signalling pathways. In our study, we used the PC-3 cell line to check cytotoxic potential of 5-LOX inhibitor - Boswellic acid, CysLT1R antagonist - Montelukast sodium and their mixture.

Qualitative analysis of Boswellic acid was done by HPTLC. Figure 1 illustrates the three pinkish color bands at an approximate R_f value of M1= 0.76, M2= 0.58, M3= 0.44 of the test reference solution of *Boswellia Serrata Roxb.* gum powder (S1). The major zone in the sample (T1) corresponds in color and position to that of

three markers of test reference solution (S1), confirming that the test compound is Boswellic acid (Figure 1).

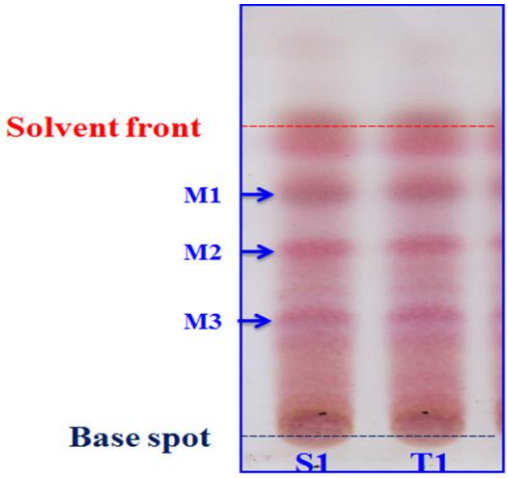

Figure 1. Qualitative analysis of Boswellic acid by HPTLC**.** Where, S1 = *Boswellia Serrata Roxb*. Gum Powder [Standard compound], T1 = Test compound, M1 = Marker compound 1, M2 = Marker compound 2, M3 = Marker compound

Effect of Boswellic acid, Montelukast sodium and their mixture on cell viability / proliferation was studied by tryptan blue dye exclusion assay, MTT assay and SRB assay. The quantification of cell viability and reduced proliferation was also done using a long-term incubation period i.e. for 48 hours and 72 hours treatment, which allows the determination of cells that remain viable and are capable of proliferating and those that remain viable but cannot proliferate and/or the detection of delayed programmed cell death.

Boswellic acid, the major constituents of a gum resin derived from the plant *Boswellia serrata*, have been traditionally used in treatments for various inflammatory diseases like arthritis and chronic colitis [16, 17]. Boswellic acid is known to be a non-redox and non-competitive inhibitor of 5-lipoxygenase. Boswellic acids have shown to exert antitumor effects in human cell lines established from

Table 2. Effect of various concentrations of Boswellic acid against PC-3 cell viability in trypan blue dye exclusion technique.

Concentration of Boswellic acid (µg/ml)	**% Cell Viability**		
	24 hrs	**48 hrs**	**72 hrs**
1.56	94.67	90	93.33
3.125	93	86	85.67
6.25	86	78.67	74
12.5	30.33	22.67	17.67
25	15.33	11.33	8.33
50	0	0	0
100	0	0	0
200	0	0	0

brain tumors, colorectal cancer and leukemia [18,19,20]. The anti-cancer effects of Boswellic acid may be due, in part, to its inhibitory effects on these intracellular signaling pathways. Bosewellic acid inhibited 100% cell growth in all wells treated with concentrations above 50µg/ml for 24 hours, 48 hours and 72 hours in tryptan blue dye assay, as shown in figure 2 and table 2.

From the results of trypan blue dye assay, 25 µg/ml of Boswellic acid and for combination, 6.25 µg/ml dose of Boswellic acid was selected as the highest concentration for further studies by MTT and SRB assay. From MTT and SRB assay data, dose response curve was constructed for % inhibition of cell viability obtained by Boswellic acid between the range of 0.15 – 25 µg/ml for a treatment period of 24 hours, 48

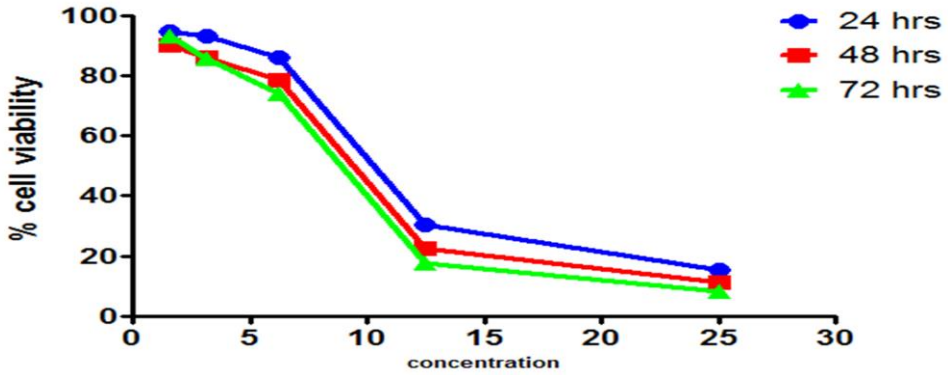

Figure 2. Effect of various concentrations of Boswellic acid against PC-3 cell viability in trypan blue dye exclusion technique.

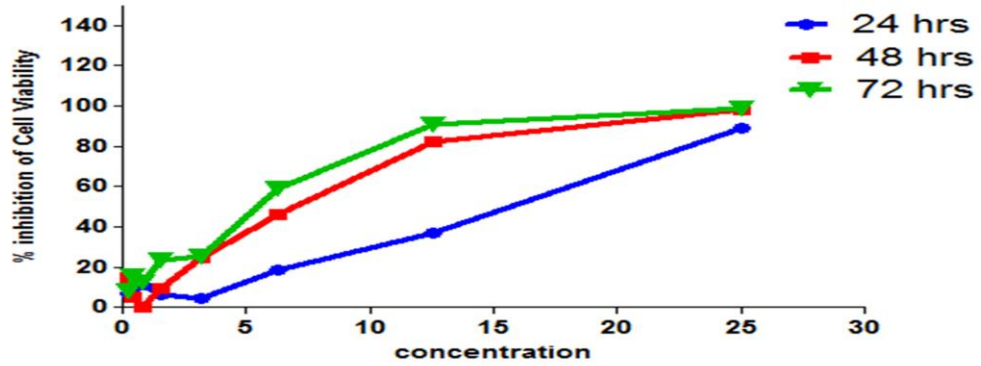

Figure 3. Cytotoxic effect of Boswellic acid against PC-3 cell line in MTT colorimetric assay.

hours and 72 hours (Figure 3,4). Calculation of IC_{50} and R^2 value was done using graphs generated from Microsoft excel 2007 edition. The susceptibility of cells to the drug exposure was characterized by IC_{50} values. Boswellic acid showed a significant cytotoxic effect against human prostate cancer cell line PC-3 above the 6.25µg/ml dose at 24, 48 and 72 hours with IC_{50} values of 49.15, 47.18 and 45.80µg/ml, respectively assayed by MTT assay (Table 3, Figure 3); Whereas with SRB assay, Boswellic acid demonstrated significant cytotoxic activity above the dose of 12.5µg/ml for all the treatment period. IC_{50} values obtained were 50.14, 43.08 and 43.39µg/ml at 24, 48 and 72 hours respectively (Table 4, Figure 4).

Cysteinyl leukotriene receptor antagonists –montelukast is a well-known inhibitor of slow-reacting substance of anaphylaxis (SRSA), a mixture ofLTC4, LTD4, and LTE4, and hence, useful for treating asthma and allergy. It is commercially available and currently in clinical use to treat asthmatic patients [15]. In addition, montelukast has been shown to induce the intrinsic apoptotic pathway, resulting in cleavage of caspases 3 and 9, and cell cycle arrest in neuroblastoma cell lines [31]. In tryptan blue assay, Montelukast sodium exhibited 0 % cell viability above 31.25µg/ml dose in PC-3 cell line at 24 hours, 48 hours and 72 hours (Table 5, Figure 5). Depending on the cell viability study results 16 µg/ml dose of Montelukast sodium was selected as highest concentration for the MTT and SRB assay. From MTT and SRB assay data, dose response curve was constructed for % inhibition of cell viability obtained by Boswellic acid between the range of 0.125 – 16µg/ml for a treatment period of 24 hours,

Table 3. Cytotoxic **e**ffect of various concentrations of Boswellic acid against PC-3 cell line in MTT colorimetric assay.

Concentration of Boswellic acid (µg/ml)	**24 hrs**			**48 hrs**			**72 hrs**		
	% inhibition	**IC_{50}**	**R^2**	**% inhibition**	**IC_{50}**	**R^2**	**% inhibition**	**IC_{50}**	**R^2**
0.195	6.88			14.99			8.3		
0.39	9.91			5.12			12.68		
0.78	11.53			0			15.88		
1.56	6.27	49.15	0.98	9.27	47.18	0.85	23.76	45.80	0.88
3.125	4.45			24.45			25.52		
6.25	18.42			46.06			58.98		
12.5	72.04			82.24			90.96		
25	88.86			98.22			98.54		

Table 4. Cytotoxic **e**ffect of various concentrations of Boswellic acid against PC-3 cell line in SRB assay.

Concentration of Boswellic acid (µg/ml)	24 hrs			48 hrs			72 hrs		
	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2
0.195	6.87			9.6			16.77		
0.39	2.5			2.6			14.35		
0.78	5			7.4			9.83		
1.56	6.25	50.14	0.88	25.6	43.08	0.83	30	43.39	0.87
3.125	0.31			29.67			41.2		
6.25	0.93			44.19			50.4		
12.5	20.67			64.6			69.83		
25	55			72.8			78.7		

Table 5. Effect of various concentrations of Montelukast sodium against PC-3 cell viability in trypan blue dye exclusion technique.

Concentration of Montelukast sodium (µg/ml)	% Cell Viability		
	24 hrs	48 hrs	72 hrs
3.9	89.33	87.67	69
7.81	45.67	40.33	37.33
15.62	17.68	16.33	12.67
31.25	0	0	0
62.5	0	0	0
125	0	0	0
250	0	0	0
500	0	0	0

48 hours and 72 hours (Figure 6,7). Calculation of IC_{50} and R^2 value was done using graphs generated from Microsoft excel 2007 edition. Montelukast sodium inhibited more than 70 % cell growth only at 16 µg/ml dose. It showed a moderate cytotoxic effect against human prostate cancer cell line PC-3 at doses of 4 and 8 µg/ml after 24 hours treatment with IC_{50} values of 49.27 and 46.68 µg/ml assayed by MTT assay (Table 6) and SRB assay (Table 7), respectively; but above dose of 4 µg/ml significant cytotoxicity at 48 and 72 hours treatment was noted. IC_{50} values were found to be 48.58 and 46.77 µg/ml for 48 hours and 72 hours respectively with MTT assay, whereas with SRB assay IC_{50} values obtained were 46.62 and 46.47µg/ml for 48 and 72 hours respectively (Table 6, 7).

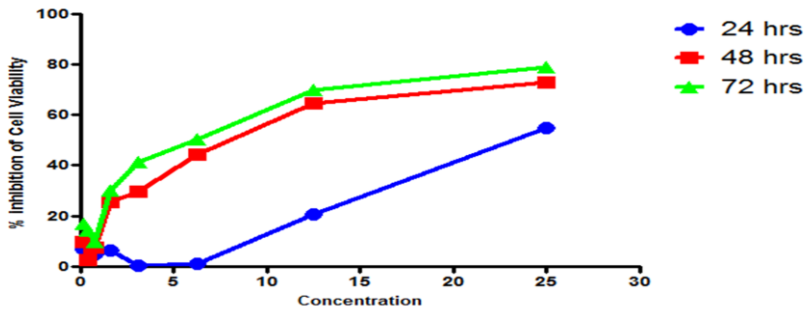

Figure 4. Cytotoxic effect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell line in MTT colorimetric assay**.**

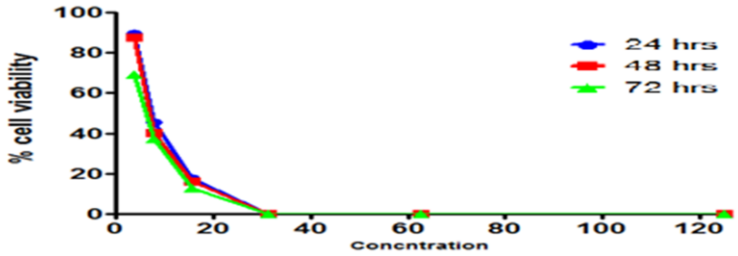

Figure 5. Effect of various concentrations of Montelukast sodium against PC-3 cell viability in trypan blue dye exclusion technique.

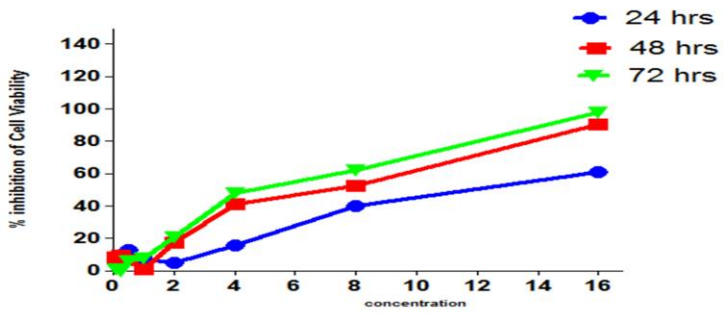

Figure 6. Cytotoxic effect of Montelukast sodium against PC-3 cell line in MTT colorimetric assay.

In tryptan blue dye exclusion assay, treatment with both drugs together destroyed all cells above the concentration of 12.5 µg/ml of Boswellic acid and 31.25 µg/ml of Montelukast sodium as shown in figure 8. While at a lower doses percentage viability was significantly reduced in a dose dependent manner. However, there was no significant difference found in % inhibition of cell viability when compared between different treatment periods (Table 8).

Table 6. Cytotoxic **e**ffect of various concentrations of Montelukast sodium against PC-3 cell line in MTT colorimetric assay.

Concentration of Montelukast sodium (µg/ml)	24 hrs			48 hrs			72 hrs		
	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2
0.125	7.48			8.28			0.87		
0.25	5.66			9.66			0.14		
0.5	12.95			6.90			6.26		
1	7.28	49.27	0.78	0.98	48.58	0.9	8.01	46.77	0.96
2	5.12			17.61			20.55		
4	15.71			41.25			47.97		
8	49.79			52.76			62.32		
16	60.72			90.13			97.66		

Table 7. Cytotoxic **e**ffect of various concentrations of Montelukast sodium against PC-3 cell line in SRB assay.

Concentration of Montelukast sodium (µg/ml)	24 hrs			48 hrs			72 hrs		
	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2	% inhibition	IC_{50}	R^2
0.125	15			2.6			14.51		
0.25	13.43			0			13.38		
0.5	14.68			10.8			16.61		
1	0	46.68	0.64	19.6	46.62	0.77	16.61	46.47	0.91
2	3.12			44.8			21.93		
4	2.18			41.8			47.58		
8	26.56			54.2			65.8		
16	40			72.8			81.93		

The combination of both drugs showed more than 70 % inhibition at doses of 6.25 µg/ml of Boswellic acid and 16 µg/ml of Montelukast sodium in MTT assay (Table 9, Figure 9). Results indicate that the cytotoxic effect strengthens with an increase in the concentration of drug and with an increase in the duration of treatment. The combination of both drugs showed moderate % growth inhibition above doses 1.56 µg/ml of Boswellic acid and 4 µg/ml of Montelukast sodium for 24 hours treatment period in SRB assay; however, the % growth inhibition was found to be significant at 48 and 72 hours treatment period (Table 10, Figure 10).

Under the experimental conditions employed and within the limits of the data analyses applied, the MTT and SRB assays gave quite comparable results.

Cytotoxicity increased with an increase in the concentration and increase in duration of

Table 8. Effect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell viability in trypan blue dye exclusion technique.

Concentration of Boswellic acid+Montelukast sodium (µg/ml)	% Cell Viability		
	24 hrs	48 hrs	72 hrs
0.78+1.95	96	91	90
1.56+3.9	90.33	83.67	77
3.13+7.81	40	35	31.33
6.25+15.62	16.33	13.67	16.33
12.5+31.25	0	0	0
25+62.5	0	0	0
50+125	0	0	0
100+125	0	0	0

Table 9. Cytotoxic **e**ffect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell line in MTT colorimetric assay.

Concentration of Boswellic acid+Montelukast sodium (µg/ml)	% inhibition		
	24 hrs	48 hrs	72 hrs
0.049+0.13	90.08	3.15	1.6
0.098+0.25	80.16	1.77	0.29
0.195+0.5	15.78	3.74	0.58
0.39+1	15.78	1.57	0.14
0.78+2	15.99	2.16	22.59
1.56+4	34.16	41.63	53.2
3.125+8	39.67	63.11	69.67
6.25+16	82.38	96.44	96.35

Table 10. Cytotoxic **e**ffect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell line in SRB assay.

Concentration of Boswellic acid+Montelukast sodium (µg/ml)	% inhibition		
	24 hrs	48 hrs	72 hrs
0.049+0.13	8.43	3.8	5.16
0.098+0.25	6.25	5.6	6.29
0.195+0.5	11.87	4.4	2.09
0.39+1	12.18	11.4	8.22
0.78+2	15.62	16.8	17.25
1.56+4	13.12	30.4	32.25
3.125+8	21.56	45.8	59.67
6.25+16	44.68	74.4	74.35

treatment in both the assays.

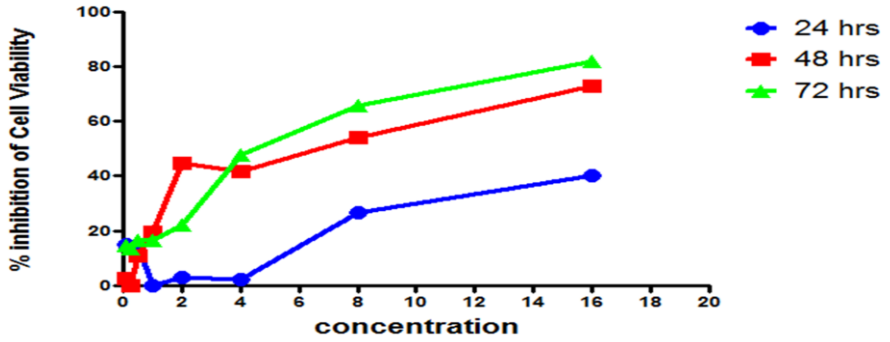

Figure 7. Cytotoxic effect of Montelukast sodium against PC-3 cell line in SRB colorimetric assay.

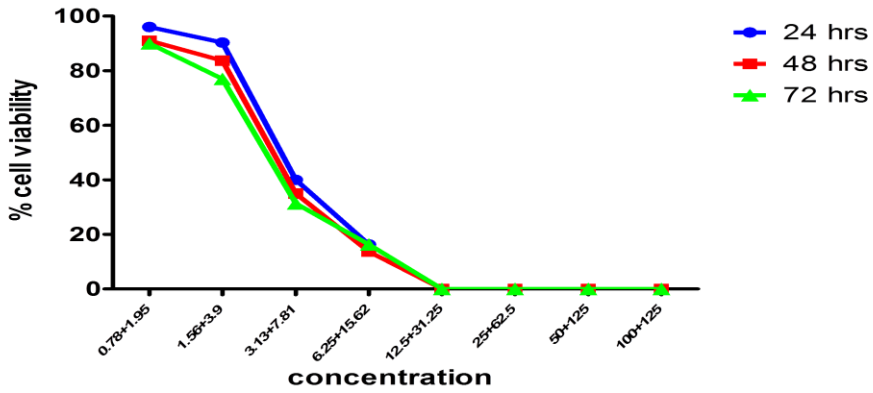

Figure 8. Effect of various concentrations of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell viability in trypan blue dye exclusion technique.

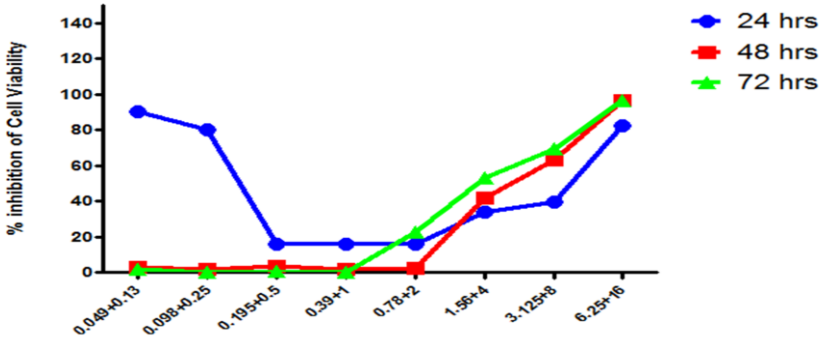

Figure 9. Cytotoxic effect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell line in MTT colorimetric assay**.**

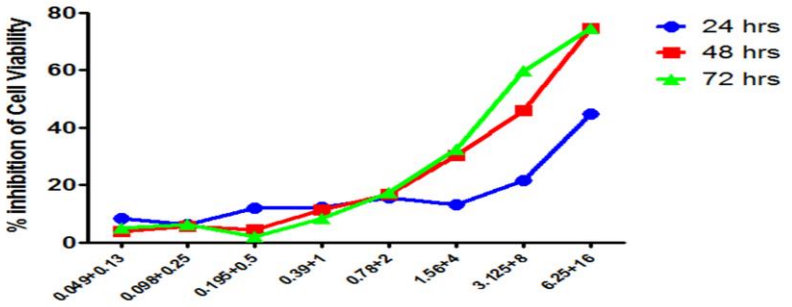

Figure 10. Cytotoxic effect of a mixture of Boswellic acid and Montelukast sodium against PC-3 cell line in SRB colorimetric assay

As previously discussed, the level of 5-LOX and its metabolites are seen to be raised in prostate cancer patient as compare to a healthy individual and they have direct impact on prostate cancer progression. In the present investigation both selected drugs are well known 5-LOX and leukotriene antagonist. So, decrease in cell growth might be due to antagonism of 5-LOX and its metabolites. But there was no synergism found when both drugs were used in combination.

4. Conclusion

To conclude, all these data suggest that Boswellic acid and Montelukast sodium have significant anti-cancer potential *in vitro* and can be developed as novel chemotherapeutic agent for treatment of prostate cancer.

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