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APRICOXIB;

EVALUATION OF PRECLINICAL CYTOTOXIC EFFECTS OF APRICOXIB ON BREAST CANCEROUS CELL LINES: AN IN VITRO TRIAL

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ABSTRACT… Background: Breast cancer is most frequently diagnosed cancer globally but there is not any ideal economical and safer agent that not only decreases the progression but also resolve complexities associated with breast cancer such as inflammatory conditions. There was strong link between inflammation and cancer specially breast cancer. Thus by inhibiting the COX enzyme may inhibit the progression of cancer beside of its role in inflammatory conditions of breast. **Study Design:** Interventional In Vitro trial. **Setting:** Department of Pharmacology in alliance with PCMD. **Period:** The duration of study from April 2016 to February 2017. **Methodology:** For this purpose we used five cancerous lines MCF-7, MDA-MB-231, MCF-10, HT-29 and Hela cell lines. For demonstrating the cytotoxic effects of Apricoxib we used MTT assay (for all cell Lines) and Trypan blue dye exclusion assay (Primarily for MCF-7 cell lines). For calculation of minimum dose required for exert cytotoxic effects of Apricoxib and its selectivity towards cancerous cells of breast tissue we calculated its IC50 value and Selectivity Index (SI) by MTT assay. **Results:** Apricoxib significantly reduce the viability of MCF-7, MDA-MB-231, Hela, HT-29 as assessed by MTT assay in dose dependent manner (χ^2 (2) = 26.483, p<0.001), (χ^2 (2) = 26.49, p<0.001), (χ^2 (2) = 26.062, p<0.001) and (χ^2 (2) = 26.062, p<0.001) respectively. However Apricoxib had non-significant effects on % viability of MCF-10 cell line (χ^2 (2) = 4.167, p=0.654) as assessed by MTT assay. Furthermore Apricoxib had lowest IC50 value against MCF-7 cell line. **Conclusion:** This study demonstrated that beside of primarily anti-inflammatory effects Apricoxib have additional benefits in term of exerting the cytotoxic effects *(in vitro)* on cancerous cell lines as indicated by reducing the % viability and reducing the Absorbance value of test sample as compare to control. This opens the newer path for researcher to evaluate different aspects of Apricoxib in field of chemotherapy.

Key words: MTT assay, IC50, SI, MCF-7, MCF-10, MDA-MB-231, Apricoxib.

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INTRODUCTION

The commonest and leading cause of cancer related death in female is Breast cancer.[1] There are several risk factors of breast cancer but nowadays most debatable targeted risk factor is Cyclooxygenase enzyme (COX) especially COX-2.[2] There is clear evidence that breast cancerous cells had overexpressed with COX-2 enzyme receptor have worse prognosis because of its increase ability of metastasized with increase tumor growth.[3]

COX-2 derived Prostaglandins (PGs) specially PgE2 play a crucial role in tumor evolution by means of gratifying the nutritional necessities of tumorous cells by increases the angiogenesis by encouraging the synthesis of angiogenic growth factors particularly VEGF.[4]

Apart from that PGE2 can also promoting the tumor cells survival by inhibiting the apoptosis either by increases the expression of inhibitors of apoptosis[5] or by decreasing the expression of apoptosis promoting protein.[6]

PGE2 play an imperative part in development of breast cancer in postmenopausal women because it can cause non-ovarian production of estrogen by aromatase. This can cause increase breast tissue density which is the strongest risk factor for development of breast cancer in postmenopausal women.[7] Most importantly PGE2 can also promoting the cancerous cell proliferation by having effects on different

pathways such as EGFR and MAPK.[8]

Apricoxib is a newer COX-2 inhibitor that has proven anti-inflammatory and analgesics effects. Several trials showed that Apricoxib had additional benefit in terms of having cytotoxic effects on different cancerous cells.[9]

Thus keep viewing the above mentioned points the main objective of this trial to evaluate the anticancerous activity (*In Vitro*) of COX-2 inhibitors (Apricoxib) on breast cancerous cells.

METHODOLOGY

This interventional *In Vitro* trial conducted in department of Pharmacology BMSI, JPMC in collaboration with PCMD. For evaluating the cytotoxic effects of Apricoxib we used primarily four cancerous cell lines designated MCF-7, MDA-MB-231 (breast cancer cell lines), Hela (cervical cancer cell line) and HT-29 (Colorectal CA cell line). For demonstrated the dose dependent Anticancerous or cytotoxic activity of Apricoxib we used different dose ranges of Apricoxib (10μM - 150μM) and used MTT {3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide} assay (for all cell lines) and Trypan blue dye exclusion assay (used for MCF-10). From MTT assay we calculate IC50 value and % viability of studied cell lines.

MTT assay is most efficient assay for evaluation of in vitro anticancerous activity of drugs.[10] It is a calometric assay by which we calculated the % viability of cancerous cell lines by comparing the absorbance values of test (At), control (Ac) and Blank (Ab).[11] For this assay we incubate cultured cells with different dilutions of study drug for 48-72 hours and for each dose readings were repeat 4 times daily for at least 4 separate days as preclinical cellular culture based cytotoxicity assessment protocol described by Cumming et al., 2007.[12]

Trypan blue dye exclusion assay is another important assay by which we estimated the % viability thru calculating the Death, Viable and Total cell counts.[13]

For evaluating the selectivity of Apricoxib against cancerous cells we used MCF-10 cell line which representing the normal breast epithelial cell. Thus by comparing the IC50 value of Apricoxib against MCF-10 with IC50[14] value of breast cancerous cell lines we evaluated the selectivity index (SI)[15] of Apricoxib against breast cancer cells.

Statistical Analysis

For data analysis we used SPSS ver. 24.0 and to start with descriptive data have been executed and outcomes were provided as Mean, SD, minimum and maximum for every variable (together with Ac, At, Ab,% viability, Fa, IC50 and cellular counts as assessed through trypan blue dye exclusion assay. Kruskul-Wallis test was executed to appraise the mean distinction of variables among dosage subordinate impacts of study drug. A p-value of 0.05 or much less changed into considered as statistically huge and distinctly sizable at 0.01 or much less.

RESULTS

Comparison of dose dependent consequences of Apricoxib on cell viability of MCF-7 cell line as assessed via MTT assay shown statistically significant effects ($\chi 2$ (2)= 26.483, $p<0.001$) with viability % become 46.07 ± 0.6 with maximum dose from 99.74 ± 0.1 for dose zero with mean percent decrease turned into approximately -53.811 and mean IC50 value of 34.8 ± 2.5. As demonstrated in Table-I and Figure-1.

Further for MDA-MB-231 cell line contrast of dose dependent outcomes of Apricoxib on % viability discovered statistically good sized variations (χ^2 (2)= 26.49, $p<0.001$) with mean viability % turned into 47.11 ± 0.6 with 6th dose from 99.76 ± 0.1 for dose 0 with mean percent decrease change about -52.772 with IC50 value of 60.3 ± 5.6. As depicted in Table-II and Figure-2.

However for MCF-10 (representative of ordinary epithelial of Breast tissue) assessment of dose dependent effects of Apricoxib discovered statistically non-significant results on cell viability ($p=0.654$) with imply viability % became 99.759 ± 0.089 with dose 6 from 99.801 ± 0.075 and

average percent lower was approximately 0.0582. As proven in Table-III and Figure-3. Cytotoxic effects of Apricoxib was father supported by using that Apricoxib statistically appreciably reducing the % Viability of HT-29 cell line ($p<0.001$) with percentage decrease was approximately -43.885. As shown in Table-IV and Figure-4.

In contrast cytotoxic consequences of Apricoxib on Hela mobile line (representative of cervical carcinoma) although showed statistically significant effects on lowering the viability (χ^2 (2) = 26.062, $p<0.001$) but even though mean percentage lower was about -6.415. As depicted in Table-V and Figure-5.

Comparison of IC50 values among all studied cellular lines discovered statistically incredibly substantial variations ($p=0.001$), with lowest IC50 value of Apricoxib in opposition to MCF-7 cellular line. This cytotoxic impact of Apricoxib towards MCF-7 line changed into similarly supported by Trypan blue dye exclusion assay. As Apricoxib statistically drastically reducing the cellular viability assed through Trypan blue dye exclusion assay (χ^2 (2) = 19.636, p = 0.003). As depicted in Table-VI and Figure-6.

Doses (μM)	N = 28	Variables				
		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	4	3.8 ± 0.9	0.27 ± 0.013	0.27 ± 0.013	99.74 ± 0.1	0.003 ± 0.001
		(2.5 - 4.5)	(0.26 - 0.29)	(0.26 - 0.29)	(99.65 - 99.83)	(0.002 - 0.004)
22	4	3.9 ± 0.9	0.24 ± 0.012	0.27 ± 0.013	91.174 ± 1.064	0.087 ± 0.009
		(3 - 5)	(0.23 - 0.26)	(0.26 - 0.29)	(89.87 - 92.325)	(0.077 - 0.097)
25	4	3.7 ± 0.2	0.22 ± 0.012	0.27 ± 0.013	82.1 ± 1.1	0.18 ± 0.011
		(3.5 - 4)	(0.2 - 0.23)	(0.26 - 0.29)	(81 - 83.35)	(0.17 - 0.19)
30	4	3.1 ± 0.6	0.19 ± 0.008	0.27 ± 0.013	72.08 ± 0.4	0.28 ± 0.004
		(2.3 - 3.8)	(0.18 - 0.2)	(0.26 - 0.29)	(71.73 - 72.7)	(0.27 - 0.28)
35	4	2.9 ± 0.2	0.16 ± 0.012	0.27 ± 0.012	62.6 ± 1.6	0.37 ± 0.016
		(2.8 - 3.3)	(0.14 - 0.17)	(0.26 - 0.28)	(60.38 - 63.98)	(0.36 - 0.4)
40	4	2.7 ± 0.9	0.14 ± 0.013	0.27 ± 0.013	55.27 ± 1.2	0.45 ± 0.012
		(2 - 3.8)	(0.12 - 0.15)	(0.26 - 0.28)	(54.28 - 57)	(0.43 - 0.46)
45	4	3.7 ± 0.6	0.12 ± 0.005	0.26 ± 0.012	46.07 ± 0.6	0.54 ± 0.006
		(3.3 - 4.5)	(0.12 - 0.13)	(0.26 - 0.28)	(45.25 - 46.75)	(0.53 - 0.55)
p-value		0.115	< 0.001**	0.794	< 0.001**	< 0.001**

Table-I. Evaluation of dose dependent effects of Apricoxib on MCF-7 cell line assess by MTT assay

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in x10-3 '(Min - Max) in x10-3 **Significant at1%

Doses (μM)	N = 28	Variables				
		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	4	4.1 ± 0.5	0.341± 0.021	0.342 ± 0.021	99.76 ± 0.1	0.002±0.0007
		(3.5 - 4.8)	(0.31 - 0.36)	(0.31 - 0.36)	(99.65 - 99.83)	(0.002 - 0.004)
20	4	4.7 ± 0.7	0.31 ± 0.018	0.34 ± 0.022	91.83 ± 1.5	0.08 ± 0.015
		(3.8 - 5.3)	(0.28 - 0.33)	(0.31 - 0.36)	(89.85 - 93.15)	(0.07 - 0.1)
35	4	4.5 ± 0.5	0.28 ± 0.017	0.35 ± 0.011	82.99 ± 1.8	0.17 ± 0.018
		(3.8 - 5)	(0.26 - 0.3)	(0.33 - 0.36)	(80.95 - 85.15)	(0.15 - 0.19)
40	4	4.3 ± 0.7	0.25 ± 0.016	0.35 ± 0.011	73.41 ± 1.8	0.27 ± 0.018
		(3.8 - 5)	(0.23 - 0.27)	(0.33 - 0.36)	(71.48 - 75.9)	(0.24 - 0.29)
55	4	4.7 ± 0.6	0.22 ± 0.013	0.34 ± 0.012	64.35 ± 1.1	0.36 ± 0.011
		(4 - 5.3)	(0.2 - 0.23)	(0.33 - 0.36)	(62.98 - 65.7)	(0.34 - 0.37)
60	4	4.5 ± 1	0.19 ± 0.011	0.34 ± 0.011	55.79 ± 1	0.44 ± 0.01
		(3.3 - 5.5)	(0.18 - 0.2)	(0.33 - 0.35)	(54.53 - 56.63)	(0.43 - 0.45)
75	4	4.1 ± 0	0.16 ± 0.009	0.34 ± 0.011	47.11 ± 0.6	0.53 ± 0.006
		(2.8 - 5)	(0.14 - 0.16)	(0.33 - 0.35)	(46.55 - 47.93)	(0.52 - 0.53)
p-value		0.718	< 0.001**	0.944	< 0.001**	< 0.001**

Table-II. Comparison of dose dependent impacts of Apricoxib on viability of MDA-MB-231 evaluate by MTT assay

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in x10-3 '(Min - Max) in x10-3 **Significant at1%

Doses (μM)	N = 28	Variables				
		Ab'Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	4	4.3 ± 0.7	0.482 ± 0.009	0.485 ± 0.013	99.801 ± 0.075	0.0023± 0.0007
		(3.5 – 5.3)	(0.468 - 0.49)	(0.468- 0.498)	(99.637 - 99.785)	(0.0021 - 0.0036)
55	4	4.5 ± 0.7	0.484 ± 0.009	0.486 ± 0.0127	99.803 ± 0.064	0.002 ± 0.0006
		(3.8 – 5.3)	(0.4767- 0.488)	(0.47 - 0.499)	(99.742 – 99.894)	(0.0011 - 0.0026)
75	4	4.4 ± 0.3	0.478 ± 0.009	0.486 ± 0.014	99.735 ± 0.065	0.0026 ± 0.0006
		(4.0 – 4.8)	(0.464 - 0.486)	(0.469 - 0.501)	(99.674 – 99.793)	(0.0021 - 0.0033)
95	4	4.7 ± 0.2	0.477 ± 0.009	0.485 ± 0.014	99.80 ± 0.108	0.002 ± 0.001
		(4.5 – 5.0)	(0.463 - 0.485)	(0.468 - 0.501)	(99.684 – 99.947)	(0.0005 - 0.003)
115	4	4.3 ± 0.6	0.475 ± 0.009	0.483 ± 0.014	99.759 ± 0.073	0.0024± 0.0007
		(3.8 – 5.3)	(0.460 - 0.483)	(0.466 - 0.499)	(99.67 – 99.841)	(0.0016 - 0.0033)
135	4	4.1 ± 0.7	0.473 ± 0.009	0.482 ± 0.014	99.786 ± 0.043	0.021 ± 0.004
		(3.0 – 4.8)	(0.458 - 0.481)	(0.465 - 0.496)	(99.736 – 99.841)	(0.0016 - 0.0026)
150	4	4.2 ± 5.4	0.471 ± 0.009	0.481± 0.014	99.759 ± 0.089	0.0024 ± 0.0008
		(3.5 – 4.8)	(0.457 - 0.479)	(0.464 - 0.495)	(99.632 – 99.84)	(0.0016 - 0.0039)
p-value		0.895	0.232	0.961	0.654	0.654

Table-III. Comparison of effects on MCF-10 viability evaluate by MTT assay among the different doses of Apricoxib

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in x10-3 '(Min - Max) in x10-3 **Significant at1%

Doses (μM)	N = 28	Variables				
		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	4	4.3 ± 0.9	0.33 ± 0.027	0.33 ± 0.028	99.68 ± 0.1	0.003 ± 0.001
		(3 - 5)	(0.32 - 0.38)	(0.32 - 0.38)	(99.53 - 99.85)	(0.002 - 0.005)
10	4	3.9 ± 0	0.31 ± 0.039	0.33 ± 0.029	93.46 ± 1.1	0.07 ± 0.011
		(2.8 - 5)	(0.28 - 0.37)	(0.31 - 0.38)	(92.35 - 94.88)	(0.05 - 0.08)
12	4	4.3 ± 0.2	0.28 ± 0.039	0.33 ± 0.029	86.19 ± 2.2	0.14 ± 0.022
		(4 - 4.5)	(0.25 - 0.34)	(0.31 - 0.38)	(83.93 - 88.83)	(0.11 - 0.16)
15	4	3.9 ± 0.4	0.26 ± 0.039	0.33 ± 0.028	78.73 ± 2.7	0.21 ± 0.027
		(3.5 - 4.5)	(0.23 - 0.32)	(0.31 - 0.37)	(76.25 - 82.43)	(0.18 - 0.24)
20	4	4.3 ± 0.6	0.23 ± 0.041	0.33 ± 0.028	71.36 ± 4	0.29 ± 0.04
		(3.8 - 4.8)	(0.19 - 0.29)	(0.31 - 0.37)	(66.23 - 75.7)	(0.24 - 0.34)
22	4	4.1 ± 0.1	0.21 ± 0.043	0.33 ± 0.028	63.16 ± 4.4	0.37 ± 0.044
		(4 - 4.3)	(0.17 - 0.27)	(0.31 - 0.37)	(57.9 - 68.68)	(0.31 - 0.42)
25	4	3.8 ± 0.5	0.15 ± 0.011	0.33 ± 0.028	55.93 ± 6.4	0.44 ± 0.064
		(3.3 - 4.3)	(0.14 - 0.17)	(0.31 - 0.37)	(50.05 - 65.05)	(0.35 - 0.5)
p-value		0.784	0.002	0.777	< 0.001**	< 0.001**

Table-IV. Assessment of cytotoxic effects of Apricoxib on HT-29 Cell line evaluate by MTT assay

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in x10-3 '(Min - Max) in x10-3 **Significant at1%

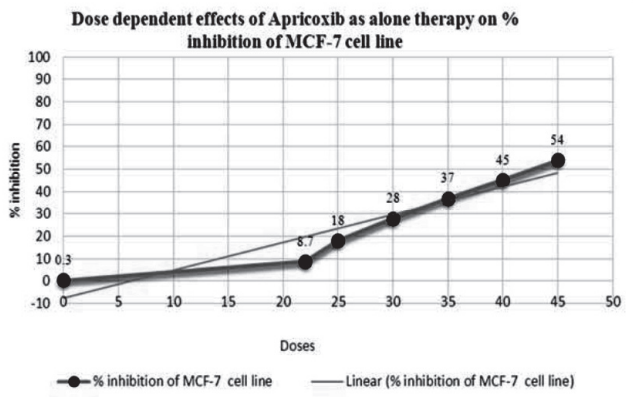

Figure-1. Evaluation of impacts of different doses of Apricoxib on MCF-7 cell line

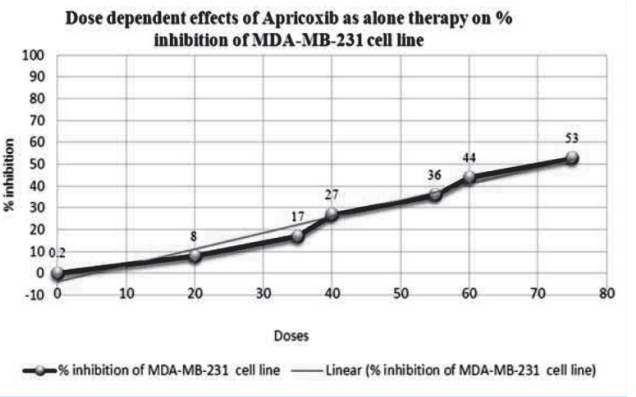

Figure-2. Comparison of Concentration dependent effects of Apricoxib on % inhibition of MDA-MB-231 cell line

Doses (μM)	N = 28	Variables				
		Ab' Mean ± SD	At Mean ± SD	Ac Mean ± SD	% Mean ± SD	Fa Mean ± SD
0	4	4 ± 0.2	0.4 ± 0.025	0.4 ± 0.025	99.768 ± 0.072	0.002 ± 0.001
		(3.8 - 4.3)	(0.36 - 0.41)	(0.36 - 0.41)	(99.675 - 99.85)	(0.002 - 0.003)
55	4	4.1 ± 0.3	0.39 ± 0.025	0.4 ± 0.015	98.718 ± 0.043	0.01 ± 0.003
		(3.8 - 4.5)	(0.36 - 0.41)	(0.38 - 0.41)	(98.675 - 98.775)	(0.01 - 0.01)
75	4	4.3 ± 0.6	0.39 ± 0.025	0.4 ± 0.015	97.568 ± 0.107	0.02 ± 0.001
		(3.5 - 5)	(0.35 - 0.41)	(0.38 - 0.41)	(97.5 - 97.725)	(0.02 - 0.03)
95	4	4.1 ± 0.3	0.39 ± 0.024	0.4 ± 0.014	96.112 ± 0.209	0.04 ± 0.002
		(3.8 - 4.5)	(0.35 - 0.4)	(0.38 - 0.41)	(95.975 - 96.425)	(0.04 - 0.04)
110	4	3.8 ± 0.5	0.38 ± 0.023	0.4 ± 0.014	95.181 ± 0.352	0.05 ± 0.004
		(3.3 - 4.5)	(0.35 - 0.4)	(0.38 - 0.41)	(94.825 - 95.575)	(0.04 - 0.05)
135	4	3.4 ± 0.3	0.38 ± 0.023	0.4 ± 0.014	94.006 ± 0.544	0.06 ± 0.005
		(3.3 - 3.8)	(0.34 - 0.4)	(0.38 - 0.41)	(93.48 - 94.75)	(0.05 - 0.07)
150	4	3.8 ± 0.7	0.37 ± 0.021	0.4 ± 0.014	93.475 ± 0.544	0.07 ± 0.006
		(3.3 - 4.8)	(0.34 - 0.39)	(0.38 - 0.41)	(93.475 - 94.75)	(0.06 - 0.07)
p-value		0.126	0.169	0.707	< 0.001**	< 0.001**

Table-V. Comparison of cytotoxic effects of Apricoxib on Hela cell line evaluate by MTT assay

N=4 samples per day for each dose for 4 days so N=16 but Data analysis were done after entering mean value for each dose for each day so N became 4 for each dose and Total N=28 for individual drug

'Mean ± SD in x10-3 '(Min - Max) in x10-3 **Significant at1%

Cell Lines N=5	IC50 Mean ± SD	P-value
MCF-7	34.8 ± 2.5	0.001**
	(32.3 - 38.2)	
MDA-MB -231	60.3 ± 5.6	
	(54.7 - 66.1)	
HT-29 human colorectal adenocarcinoma	24.3 ± 1.4	
	(22.3 - 25.4)	
Hela cell line	969.3 ± 22.4	
	(940.1 - 987.6)	
MCF-10	666.9 ± 21.8	
	(651.6 - 699.1)	

Table-VI. Comparison of IC50 values of Apricoxib between studied cell lines

Mean ± SD (Min - Max) **Significant at 1%

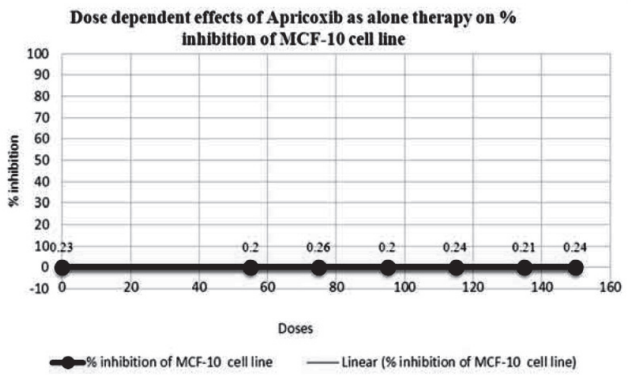

Figure-3. Comparison of Dose subordinate impacts of Apricoxib on % inhibition of MCF-10

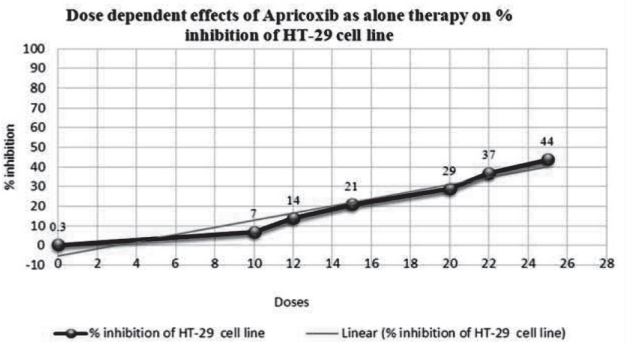

Figure-4. Comparison of Dose dependent effects of Apricoxib as single therapy on % inhibition of HT-29 cell line

DISCUSSION

Breast cancer is one of the commonest public health problem worldwide and ranked as second foremost cause of cancer related death and also had significant burden in terms of not only their treatment point of view but also in term of their diagnosis.[1] Thus researchers paid more attention in search of newer and economical approaches for both diagnostic and interventional point of view.[2]

Recently there are so many targeted therapeutic options for therapy of breast cancer out of which the most debatable option for both presumptive

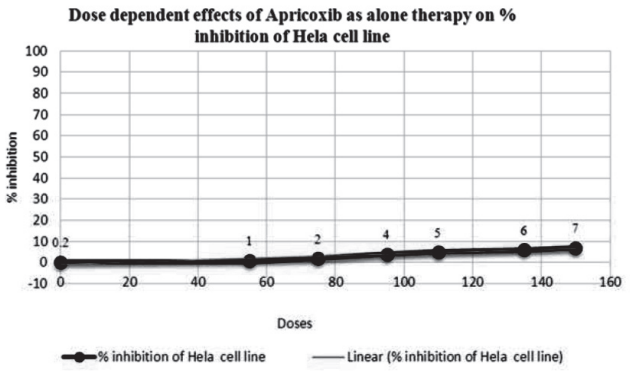

Figure-5. Comparison of cytotoxic effects of Apricoxib as alone therapy on % inhibition of Hela cell line

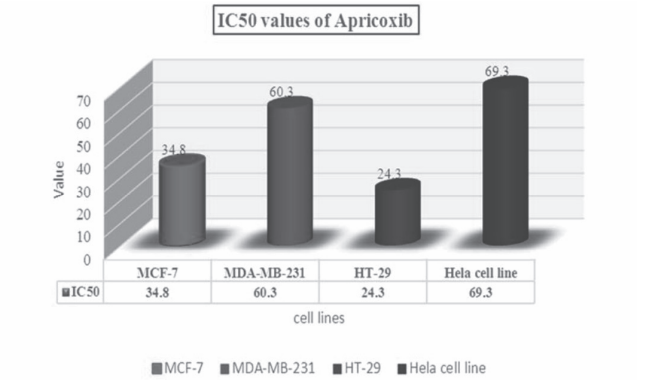

Figure-6. Comparison of IC50 values Apricoxib for all studied cell lines

treatment and preventive role of COX2 inhibitors in breast cancer.[3] Because increased COX2 enzyme expression reported over the most aggressive breast cancer cell line (MDA-MB-231).[4] As per Nerko et al. (2005) COX-2 expression was itself an important tumorigenic marker.[5] Previously several studies demonstrated the preventive and also cytotoxic effects on several cell lines especially on colonic cancer.[6]

Therefore in this trail we studied the preclinical cytotoxic effects of Apricoxib as cell cultured model primarily on breast tumor cell lines in dose subordinate manner. For this purpose we used MCF-7 and MDA-MB-231 cell lines. Apricoxib significantly reduces the viability of both cell lines in dose subordinate manner from 99.74 ± 0.1 to 46.07 ± 0.6 for MCF-7 and for MDA-MB-231 cell line from 99.76 ± 0.1 to 47.11 ± 0.6. These outcomes were matched with the study lead by Thill et al (2015).[7] As they reveal the cytotoxic activity of COX-2 inhibitors (Celecoxib) on breast tumor cells. They publicized that Celecoxib ominously decreasing the viability of studied cell lines (MCF-7 and MDA-MB-231) as both as alone and combination therapy with Calcitriol.

Another trial conducted by Robertson et al. (1998)[8] demonstrated the cytotoxic effects on non-selective COX inhibitor (Ibuprofen) on breast cancer model of rat. As Ibuprofen can cause significant regression of tumor size in 12 dimethylbenz[a]anthracene induced breast cancer animal model.

Another trial conducted by Abbul-Issa et al., (20001)[9] revealed that COX-2 inhibitor (Celecoxib) significantly had cytotoxic effects on breast tumor in dose dependent manner in animal model of breast cancer.

Cytotoxic effects of Apricoxib was further supported by its significant effects of HT-29 cell line as it statistically significantly reducing the cellular viability in dose dependent manner 99.68 ± 0.1 to 55.93 ± 6.4. This was in accordance with the study lead by Burrows (2011).[10]

CONCLUSION

This trial demonstrated that in the hunt of safer, economical and effective therapy against the most prevalent breast cancer we should not forget COX-2 inhibitors. Because COX-2 inhibitors has its role in not only prevention (as previously thought)[11] but also for treatment of breast cancer by having cytotoxicity against cancerous cells.

RECOMMENDATIONS

Its preventive and therapeutic effects can also prove by larger preclinical trial in cell cultured model by using its different concentration and also on animal model of breast cancer. Later on prove by small clinical trials.

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It always seems impossible until it's done.

– Nelson Mandela –

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Fatima Rizvi	Designed study, collect data and perfoemed statistical analysis.	
2	Syed Mahboob Alam	Review and proofreading of article.	
3	Farah Asad	Final editing of statistical analysis.	
4	Hina Shams	Help out in editing.	