

59P# Berberine Chloride inhibits TNBC cell metastasis by downregulating expression of matrix metalloproteinases

S. Rajoriya¹, M. Kataria², P. Nandhkumar³, M. Saini² and A. Kumar²

¹Nanaji Deshmukh Vet. Sci. Univ., Jabalpur, India, ²Indian Veterinary Research Institute, India, ³Tamilnadu Veterinary Department, India

Background

The triple negative breast cancer phenotype are characterized by an aggressive clinical history with poor disease-free and overall survival and show a high rate of metastasis. Matrix metalloproteinases are family of zinc dependentendoproteinases degrade the extracellular matrix, have been identified as prognostic markers and therapeutictargets for breast cancer patients. The effects of Berberine Chloride (BCI) on geneexpression and enzymatic activity of matrix metalloproteinase (MMP), cell invasion and cell migration were investigated MDA MB- 231 cell line.

Methods

1. The effects of BCI on The gene expression of MMP in cancer cells were examined by real time PCR:

MDA MB- 231 cells were cultured with different concentrations (0, 1, 5,10 and 15 µM) of BCI for 48 hrs. Total RNA from cells were extracted using Trizol reagent as per manufacturer protocol. Complementary DNA (cDNA) was synthesized from extracted RNA, using RevertAid First Strand cDNA Synthesis Kit. cDNA was used for amplification of the MMP1, MMP3, MMP 7, MMP 9, MMP11 and GAPDH using specific primers. Method described by Pfaffl (2001) was used for calculation of relative fold changes in gene expression

2. The effect of BCI on enzymatic activity of MMPs in TNBC cells: Gelatin and Casein zymography was done to evaluate enzymatic activity of MMPs with 0, 1, 5, 10 and 15 µM BCI.

3. The effects of BCI on MDA- MB 231 Cancer cell migration was done by wound healing assay:

MDA-MB-231 cells were seeded, cultured and treated with 0, 1, 5, 10 and 15 µM BCI. Wound is made and photographed at 0, 6, 24, and 48 h periods. Results are expressed as wound area percentage, remaining wound area uncovered by the cells relative to zero hr time point

4. The effects of BCI on MDA- MB 231 Cancer cell invasion was performed by Transwell chamber assay

Cancer cells were seeded on collagen based matrigel chamber and treated with1, 5,10 and 15 µM BCI. After 48hrs cells invaded through matrigel quantified by colorimetric method.

The present study is designed to identify the antimetastatic effect of Berberine Chloride in human breast carcinoma cells with following objectives:

- To study the effect of Berberine Chloride on different MMPs at transcriptional level in TNBC cell line model.
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- To evaluate effect of Berberine Chloride on cell migration of TNBC cell line model.
- To evaluate effect of Berberine Chloride on cell invasion of TNBC cell line model .

RESULTS

Fig. 1. Effect of BCI on gene expression of MMPs in TNBC cell line Table 1. Effect of BCI on gene expression of MMPs in TNBC cell line

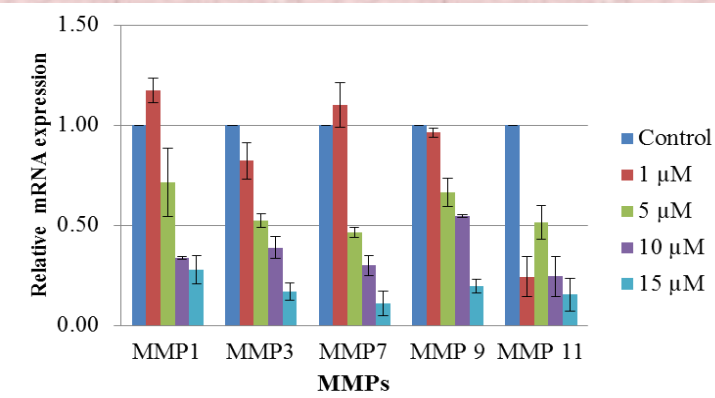


Table 2. Effect of BCI on enzymatic activity of MMPs in TNBC cell line

Dose	MMP1	MMP3	MMP7	MMP9	MMP11
Control	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00	100.00 ^a ±0.00
1µM	94.22 ^a ±1.14	92.51 ^a ±2.98	94.50 ^a ±2.48	97.79 ^a ±0.45	42.12 ^a ±6.45
5µM	79.18 ^a b±1.45	58.18 ^b ±0.32	48.25 ^b ±1.19	71.40 ^b ±1.06	52.17 ^b ±7.62
10µM	53.63 ^{ab} ±3.22	38.57 ^c ±1.44	32.50 ^c ±2.07	57.56 ^c ±2.00	39.78 ^b ±8.67
15µM	26.36 ^b ±3.97	18.78 ^c ±1.47	15.23 ^d ±1.23	26.72 ^d ±4.56	22.73 ^b ±3.23

Fig. 3. Effect of BCI on cell migration of MDA MB -231 cancer cell

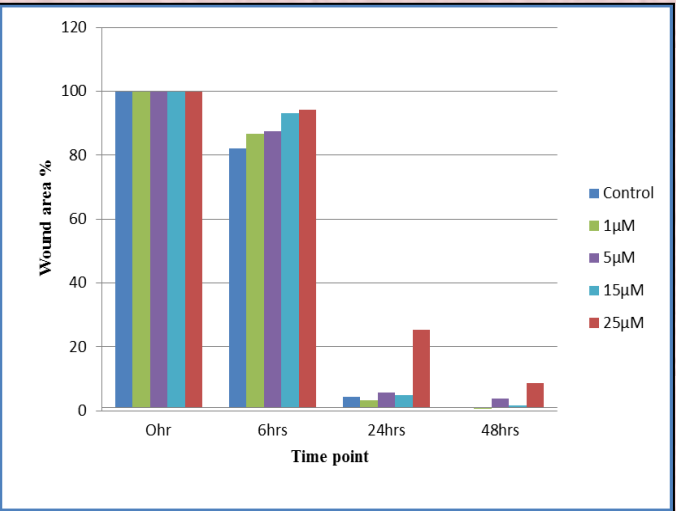
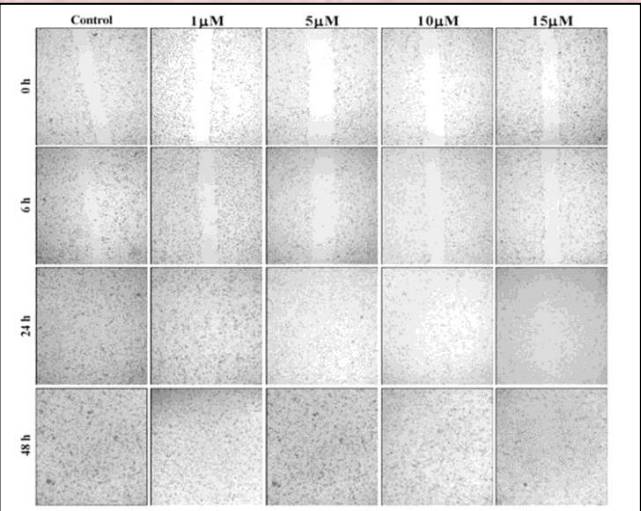


Fig 4. Effect of BCI on cell invasion of TNBC cell line

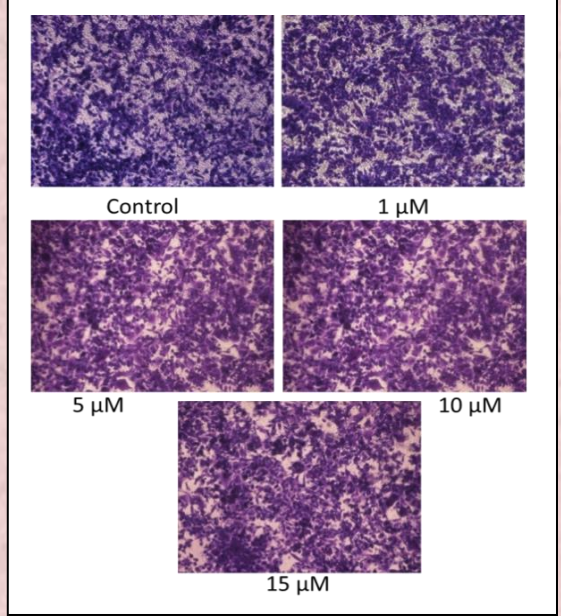
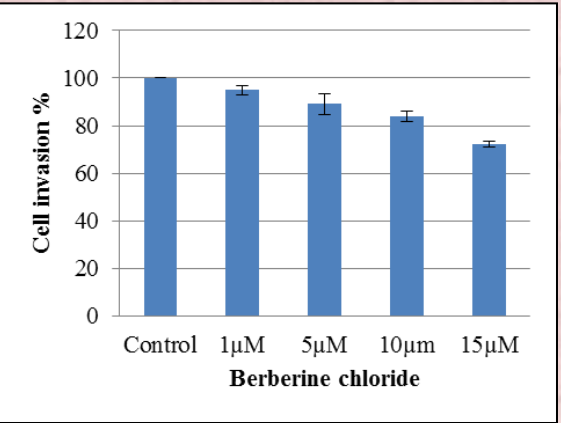
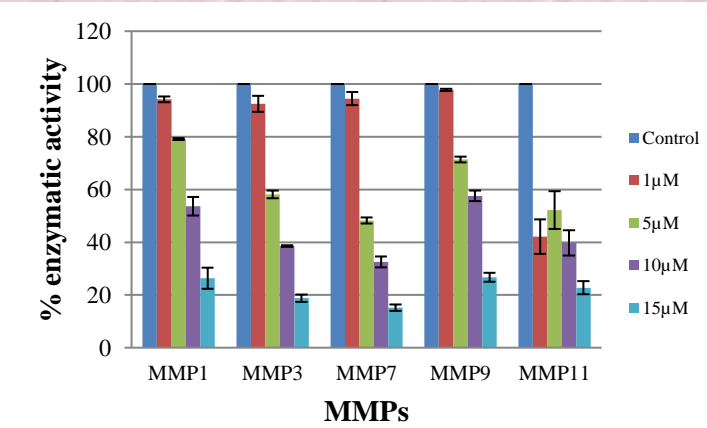


Fig. 2. Effect of BCI on Enzymatic activity of MMPs in TNBC cell line



Conclusions

In conclusion, Berberine Chloride reduced the mRNA expressions and enzymatic activity of MMP₁, MMP₃,MMP₇, MMP₉ and MMP₁₁ in MDA MB-231 cells. Berberine Chloride also inhibited the invasion and migration ofTNBC cells..

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