Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.3810

EVALUATION OF NON-CYTOTOXIC DRUGS AS POTENTIAL CANDIDATES FOR BREAST CANCER

Mariam Davis[1], Sadia Rafique[2], Sidra Meer[3], Zunaira Afzal[4], Abdul Qader[5*], Anum Asghar[6], Muhammad Sajid Hamid Akash[5]

[1]Department of Pharmacology, Government College University, Faisalabad, Pakistan
[2]Department of Pharmacy, The University of Faisalabad, Faisalabad, Pakistan
[3]Department of Pharmacy, New Indus institute of medical sciences, Mianwali, Pakistan
[4]Department of Pharmacy, The University of Agriculture, Faisalabad, Pakistan
[5]Department of Pharmaceutical Chemistry, Government College University, Faisalabad, Pakistan
[5]Primary and Secondary Health Care Department Government of Punjab Pakistan
[6]Department of Pharmacy, Riphah International University, Faisalabad Campus, Faisalabad, Pakistan

***Corresponding Author:** Abdul Qader
*****Department of Pharmaceutical Chemistry, Government College University, Faisalabad, Pakistan
*****Primary and Secondary Health Care Department Government of Punjab Pakistan
Pharmacistqader316@gmail.com

Abstract
Suramin, a potent anti-parasitic agent has been used as anti-cancer drug during last years against a vast variety of cancers. This study highlight the use of suramin as an anti-cancer drug in treatment of breast cancer. The goal of the current investigation was to determine whether the breast cancer cell line MCF-7 exhibited increased expression of heparanase-mediated epithelial mesenchymal transition in vitro. Different cancer stem cell markers were examined using reverse transcription quantitative polymerase chain reaction with the following primers: Snail, Slug, E-cadherin, vimentin, NESTIN, NANOG, CXCR-4, OCT3/4, MDR, and MMP's. The effect of the test media on cancer cells was investigated using proliferation and viability assays, as well as gene expression and marker expression. Cell proliferation was identified by the treatment of MCF-7 cell line with suramin and using the test media (containing 200µM suramin) as an inhibitor of heparanase and epithelial-mesenchymal transition (EMT) in breast cancer cells. Suramin therapy decreased mRNA expression of EMT indicators, cancer stem cell markers, drug resistance proteins, matrix metalloproteinases, and heparanase in a time dependent manner. An inhibitory effect of suramin was also evaluatedin proliferation of cancer cells in MTT assay. In addition, suramin also exhibited a time-dependent inhibition of MCF-7 cell migration in the wound healing assay. As a result, the use of suramin as an anti-cancer agent provides the opportunity for novel drug design to address the issues of drug resistance, metastasis, and chemotherapeutic agent toxicity while improving therapeutic efficacy.

INTRODUCTION

Among global, the most prevalent cancer is the Breast Cancer that reports around 25% of all newly incident cancer cases in females (Buch et al., 2019). Breast cancer is a metastatic disease and the foremost source of cancer decease (Kitamura et al., 2015). Pakistan is known to have uppermost

frequency proportion of carcinoma of breast with at least 90,000 females suffering from breast cancer (Majeed et al., 2016). In a recent study on the nationwide prevalence of breast cancer, 90,000 instances were reported in Pakistan; however, the inability to diagnose these cases stems from a lack of appropriate indicators. Breast cancer contributes for 38.5% of all cancer cases in Pakistan (Amjad et al., 2018). Risk factors of breast cancer includes age, geographic distribution, age at menarche and menopause, age at initial gestation, family history, preceding benign breast ailment, radioactive rays, lifestyle, contraceptives, hormonal replacement treatment (Feng et al., 2018; Tariq et al., 2013). Chemotherapeutic drugs like anthracyclines, taxanes (Giordano et al., 2012), 5-fluorouracil (Deveci et al., 2018), cyclophosphamide (Hung et al., 2017), carboplatin (Sharma et al., 2017) have been tremendously used in the treatment of breast cancer. However these chemotherapy medications also induce damage to healthy cells, which has a negative impact on the patients which includes the following nausea, vomiting, diarrhoea, alopecia, hypersensitivity reactions, renal and hepatic toxicity, cognitive dysfunction, anaemia, gastrointestinal toxicity, neutropenia, fatigue, sexual dysfunction, cardiac toxicity thereby compromising the quality of life (Remesh, 2012). Chemoresistance is the key disadvantage in tumour cell insensitivity to treatment and is the leading cause of chemotherapy failure in breast cancer (Ji et al., 2019). There are different mechanism involved in chemoresistance including oncogenes, epithelial mesenchymal transition and its markers, tumour suppressor genes (p53), autophagy, cancer stem cells and its markers, drug transporters (Zheng et al., 2017).

Chemotherapy resistance is one of the features of breast cancer. Chemoresistance is characterised in major cancer cells by the over-expression of multidrug resistance molecules such as P-glycoprotein (P-gp), lung resistance protein (LRP), and multidrug resistance-associated protein (MRP) (Huh et al., 2006; Vtorushin et al., 2014). The epithelial-mesenchymal transition (EMT) is another source of chemoresistance (Sui et al., 2014). EMT plays avital role in development of tumor namely cancer metastasis (Hanahan and Weinberg, 2011). During EMT, cells exhibit reduced expression of epithelial cell markers such as E-cadherin while enhanced expression of mesenchymal cell markers (Snail, Slug, Vimentin, -SMA, fibronectin, collagen-I) and stimulated cell motility (Nieto et al., 2011; Thiery et al., 2009). In breast cancer, EMT was linked to heparanase hyper secretion (HPSE). Heparanase is a beta-endoglucuronidase and the mammalian enzyme. The role of heparanase in body is to cleave heparan sulfate (HS). When expression of heparanase is highly elevated it lead to pathological situations thereby, increasing cleavage of HS and degradation of ECM (Secchi et al., 2015). Heparanase is characterized by enzymatic and non-enzymatic activities. The cation-independent mannose 6-phosphate receptor (CD222), which is expressed on cell surfaces, is bound by enzymatically active HPSE and degrades extracellular matrix (Wood et al., 2008) with heparan sulfate, a side chain of heparin sulfate proteoglycans (HSPGs) (Nakajima et al., 1988). In non-enzymatic action Heparanase activates phosphorylation of Src, Akt, Stat This ultimately causes malignancy or modifies the function of substances like FGF-2 and TGF-β (Masola et al., 2014; Masola et al., 2012). Considering the toxicities of chemotherapeutic drugs and chemoresistance in breast cancer, there is a need to highlight the development of innovative drugs with more efficacy and lesser side effects, also including drugs from natural sources and non-cytotoxic drugs for the development of treatment of cancer. Among non-cytotoxic drugs, commonly used safest drugs with multi-model action are considered to be used for anti-proliferative effects in cancer.

Suramin, a polysulphonated naphthylurea, was initially prescribed for the treatment of parasitic infections, such as Gambian trypanosomiasis and Rhodesian (Steverding, 2010). Suramin has been used in the treatment of various malignancies such as (Nakajima et al., 1991), ovarian cancer (H. Li et al., 2015), lungs cancer (Mirza et al., 1997), liver cancer (Tayel et al., 2014), colorectal cancer (Falcone et al., 1995), gastric cancer (Shah et al., 2018), lungs cancer (Mirza et al., 1997), pancreatic cancer (Bhargava et al., 2007), cervical cancer (H. Li et al., 2015), renal cancer (Wade et at., 1992), esophageal cancer (Shin et al., 1997), bladder cancer (Gansler et al., 1992), neuroblastoma (Hensey et al., 1989) in time and dose dependent manner due to its anti-proliferative properties. With cytotoxic drugs or alone, suramin has been studied in clinical trials in different carcinoma (Eisenberg et al., 1993). In vitro, suramin is also shown to be a strong inhibitor of several nuclear enzymes, including

reverse transcriptase, DNA polymerase α, RNA polymerase, DNA topoisomerase II, and DNA primase. This inhibitory effect may be essential to suramin's cytotoxic action (H. Li et al., 2015).
Epithelial mesenchymal transition is correlated with elevated expression of heparanase in different cancers i.e. gastric adenocarcinoma (Shah et al., 2016), myelomas (Li et al., 2016) but correlation of heparanase and EMT in breast cancer is yet to be discovered. In this study, for the first time we evaluated role of heparanase in epithelial mesenchymal transition in breast cancer and its inhibition by treatment of suramin and metformin thereby decreasing cancer stemness leading to prohibited chemoresistance.

MATERIALS AND METHODS

Materials and reagents

Different types of chemicals were used during this experimental work like Ethanol (Sigma Aldrich), Antibiotics (Thermo-Scientific), Iso-propanolol (Sigma Aldrich), Chloroform (Sigma Aldrich), MTT reagent (Invitrogen-Thermo), EDTA disodium salt dehydrate (VWR Chemicals), Trypsin (Life technology Thermo), DMEM (Life technology Thermo), Acridine orange (Santa Cruz Biotechnology, Inc.), Dimethyl Sulfoxide (DMSO) (Sigma-Aldrich), Foetal Bovine Serum (Invitrogen- Thermo), TRIzol reagent (Ambion), Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific), Primers, Maxima SYBR Green (Thermo Scientific), Agarose Gel (bio PLUS Fine Research Chemicals), Formalin (Sigma-Aldrich), Phosphate Buffer Saline, TRIS BASE (bio PLUS Fine Research Chemicals), Sterile water for injection.

Cell lines and drug

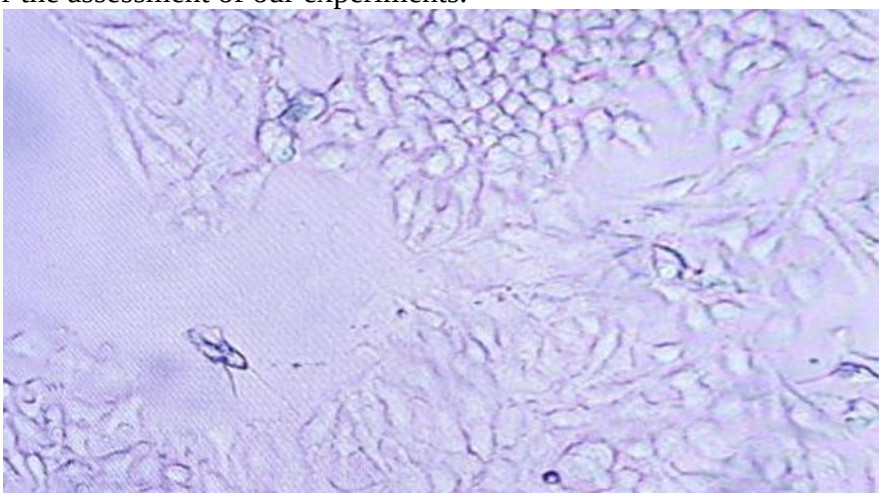
Human cancer cell line used were MCF-7 was obtained from the American Type Culture Collection (ATCC). Drug used in this study was suramin (Sigma Chemical Co, St. Louis, MO, USA).

Culture

Cells lines were grown in culture media. Culture media comprised of DMEM medium with 10% fatal bovine serum (FBS), antibiotics like penicillin, streptomycin and L-glutamine. Cell lines were continued at room temperature with humid atmosphere that contain 5% carbon dioxide gas. Cell were cultured in culture dish and permitted to breed up to nearly 75% union prior to the collection of the cultured cells for the assessment of our experiments.

Culture medium:

Cells lines were grown in culture media. Culture media comprised of DMEM medium with 10% fatal bovine serum (FBS), antibiotics like penicillin, streptomycin and L-glutamine. Cell lines were continued at room temperature with humid atmosphere that contain 5% carbon dioxide gas. Cell were cultured in culture dish and permitted to breed up to nearly 75% union prior to the collection of the cultured cells for the assessment of our experiments.



MCF-7 cells growing in culture medium

Cell viability assay

MCF-7 cell lines were cultivated at 37^0C and 5% CO_2 in culture media. Viability assay was performed by using MTT reagent (3-(4, 5-dimethylthazol-2-yl)-2, 5-diphenyl tetrazolium bromide at the dose of 5 mg/ml. The concentration of the solubilized formazan was analysed and read at 490 nm spectrophotometrically.

The cytotoxic effects of the suramin and metformin on two cancer cells were determined by MTT assay. Cells in each well were incubated with culture media containing suramin and metformin. Viability of the cells was assessed after the drug was incubated for 24 hours by adding 10 µL of MTT reagent solution to each well and allowing it incubate for 4 hours. After 4 hours of incubation, half of the culture media was removed then dimethyl sulfoxide (DMSO) was added to every well of the 96 well plate and wait for 15 minutes. The absorbance was read at a wavelength of 570nm in a plate reader. Some wells were left cell-free as blank controls for background absorption.

Migration Assay

Cancer cells were grown (80% confluency) in 12 well plate with or without drug treatment for the period of 24 or 48 hours. The ability of tumour cell was assessed with the help of scratch assay. Stripped zones were generated over dormant cell layers of cancer cells through scrabbling by a sterile pipette tip. Then layer were washed two times by phosphate buffer saline and incubated in media having no FBS, with or without drug. The cells were snapped at various intervals of time. The thicknesses of wound were measured at dissimilar spots to obtain a mean value, and invasions were noted in micrometer amongst dimension at point of scratch and different intervals.

RNA isolation, RT and real-time PCR

Total RNA was isolated from a cell line using the TRIzol reagent according to the manufacturer's instructions. RNA samples were reverse transcribed to cDNA in a 20µL volume using the Qiagen QuantiTect reverse transcription kit. The heat cycling included real-time PCR under the following conditions: 5 minutes at 95° C, then 40 cycles (denaturation for 15 seconds at 95° C, annealing for 20 seconds at 60° C, and extension for 20 seconds at 72° C). Supplementary Table 1 lists the primer sequences and PCR product size for the reference and target genes. Using Mesa Blue qPCR Master Mix Plus for SYBR assay on the Mastercycler Realplex2 (Eppendorf), real-time PCR was used to determine the mRNA expression levels of various markers, with ß-actin serving as an internal reference. Using realplex software and the comparative threshold cycle (CT) approach, relative quantitation was computed. To compute ΔCT, or the difference in CT for target and internal reference (ß-actin) genes, the mean CT of three measurements was taken. To determine ΔΔCT, the difference between the ΔCT of each sample and the ΔCT of the control experiment (MCF-7) was computed. The mRNA fold increase was computed using $2^{-\Delta\Delta CT}$.

PCR products of cell lines and tissue samples after real-time PCR were electrophoresed by E-Gel Precast Agarose Electrophoresis System.

RESULTS

Figure 1

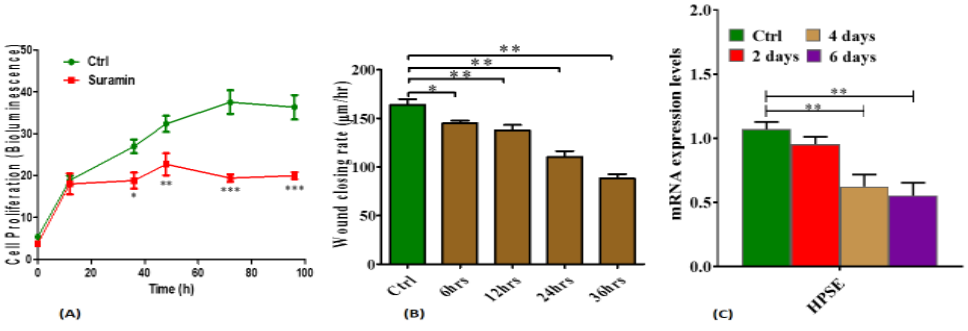


Figure 2

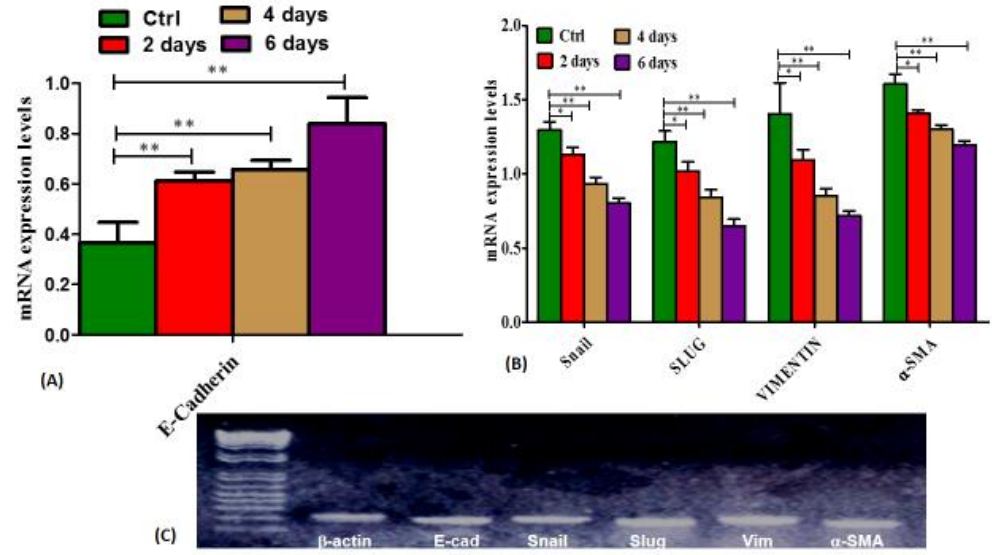


Figure 3

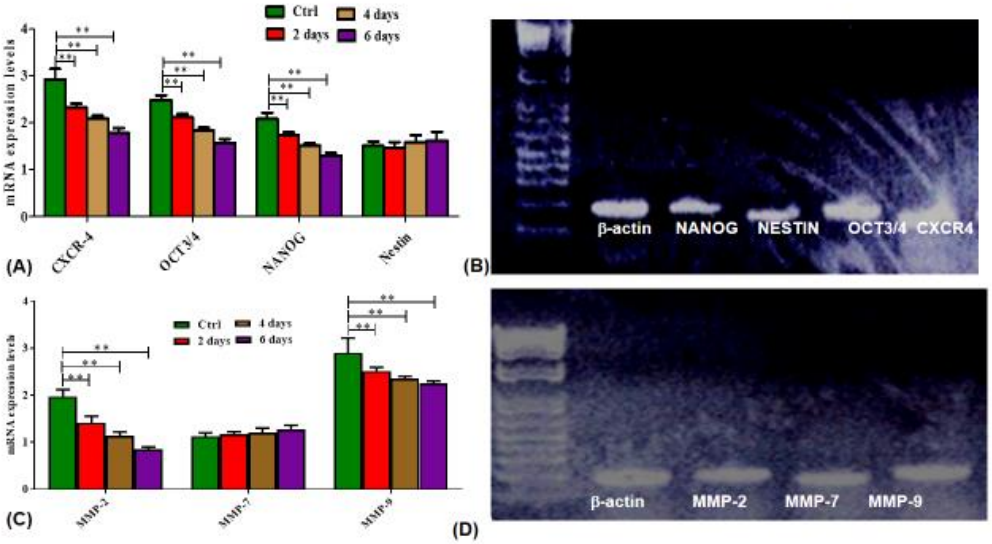
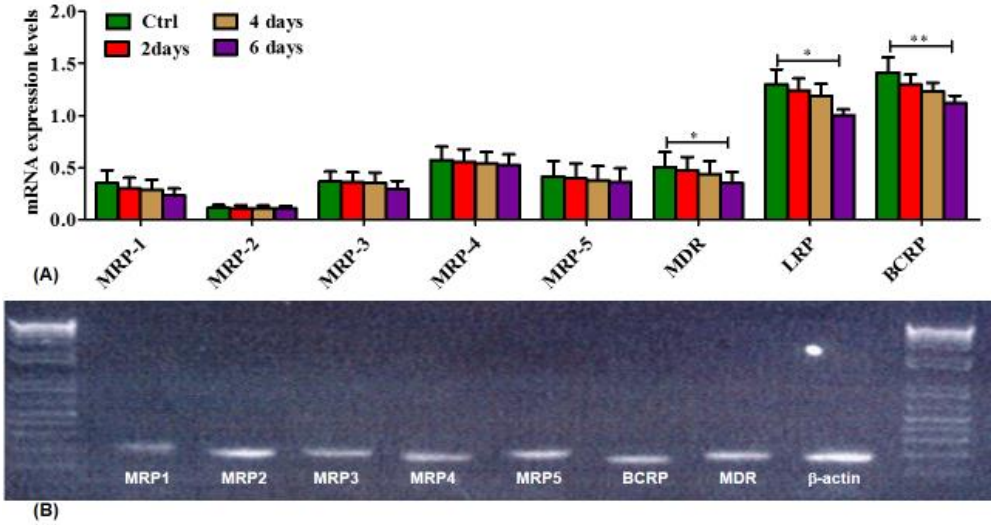


Figure 4



DISCUSSIONS

Breast cancer is a heterogeneous disease with variable history and it is estimated that small proportion of patients suffer from tumour metastasis that require aggressive management. The current study evaluated a significance of breast cancer cell line MCF-7 that has been represented in the breast cancer, incident rate of which is increasing day by day. MCF7 is largely employed as an in vitro model for the biology of breast cancer. Because there are so many variations, it may be used to develop chemotherapeutic treatments and understand drug resistance (Senthilraja and Kathiresan, 2015). Research has been conducted using MCF-7 cell line to address anti-proliferative potential of suramin and to highlight the advances in our understanding of the metastasis and its contribution to the severity of breast cancer.

Heparanase is an endo-beta-glucuronidase that damages heparanase sulphate proteoglycans (HSPGs) and contributes in destruction and remodeling extracellular matrix (Masola et al., 2018). When expression of heparanase is highly elevated it lead to pathological situations thereby, increasing cleavage of HS and degradation of ECM. Degradation of ECM causes increased discharge of cytokines and inflammatory mediators and increases the process of tumour invasion, proliferation, inflammation and formation of blood vessels to tumour cells. Therefore, heparanase is critical promoter of the hallmarks of cancer. High expression of Heparanase in cancer results in poor survival rate in patients and provides the basis of aim for invention of anti-cancer drugs (Jayatilleke et al., 2018). Suramin being potent inhibitor of heparanase possesses anti-tumour and anti-angiogenic characteristics (Jia et al., 2017).

Suramin is a multiple sulfonic acid naphthoquinone salt with a broad range of IC50 values that has anti-proliferative effect against various malignancies. Weekly administration of suramin at 275 μM has been associated to a number of acute toxicities, such as dermatological toxicity, immune- and anticoagulant-mediated blood dyscrasias, adrenal insufficiency, and renal toxicity (La Rocca et al., 1990; Kobayashi et al., 1996; Figg et al., 1994). In many breast cancer cell lines, suramin therapy dramatically reduces cell proliferation in a dose- and time-dependent way (MDA-MB231, SK-BR-3, ZR/HERc, and T-47 D, ZR-75-1) consistent with previous reports (Vignon et al., 1992; Foekens et al., 1992; Mirza et al., 1997). Even yet, the suramin dosage was less than the lethal amounts >275 μM in human blood serum, as 275 μM is linked to several adverse outcomes. For this reason, we chose a 200μM dosage of suramin, which is safer and also inhibits the development of cancer cells in addition to down-regulating the production of heparanase protein and mRNA.

Suramin medium was shown to have an inhibitory effect on drug-resistant proteins, stem cell marker expression, epithelial mesenchymal transition, and MCF-7 proliferation. The increased heparanase expression in the MCF-7 cell line was likewise expressively downregulated by this medication.

The goal of the study was to focus on the regulation of EMT by altering the levels of EMT markers Snail, slug, vimentin, alpha- smooth muscle actin and twist with the treatment of suramin. This was investigated in MCF-7 cell line, and the results showed that suramin significantly inhibited both the production of heparanase and EMT as well as cell proliferation. The present results are in agreement with those of Masola et al 2018, who reported association of heparanase in EMT and the cell proliferation of breast cells.

The present study identified the different markers of cancer stem cells such as Slug, Snail, vimentin, NANOG, NESTIN, OCT3/4 and CXCR-4. Current studies have suggested that these cancer stem cells are resistant, and exhibitdormantaptitudes in a dormant state, and are involved in initiation of process of angiogenesis in tumor, increased proliferation rate in cancer cells, apoptotic resistance, differentiation and self-renewal (Bao t al., 2013; Soltysova et al 2005). To the best of our knowledge, the current work demonstrated for the first time in a human MCF-7 cell line an association between heparanase and epithelial mesenchymal transition in breast cancer, which leads to chemoresistance in breast cancer.

The current study also found that suramin induced a significant level of inhibition of multidrug resistance proteins in the MCF-7 cell line. Drug resistance is caused by multidrug resistant proteins such as multidrug resistance-associated proteins (MRPs), breast cancer resistance proteins (BCRPs), and lung resistance-related protein (LRP) (Borst et al., 2000; Burger et al., 2003).

In conclusion, Suramin has been shown to be beneficial in the prevention and treatment of EMTassociated metastases and chemoresistance in breast cancer. As a result, it is critical to continue attempts to find therapeutics that may be utilised in the treatment of tumours for which there are no existing treatments. The findings of this study can be used to generate more successful and less hazardous treatment techniques for breast cancer in the future.

LEGEND OF FIGURES

Figure 1:

(A) Diagram showing time dependent effect of Suramin (200µM) on cell proliferation of breast cancer cell line (MCF-7). One way analysis of variance (ANOVA) for cell proliferation was used.Cells continue to proliferate in control group (without suramin). Cell proliferation was inhibited in MCF-7 cells when treated with suramin. One way analysis of variance (ANOVA) for cell proliferation was used (*P<0.05, **P<0.01, ***P<0.001). The data was expressed as mean ± SEM with n = 6.

(B) Bar chart showing time dependent effect of suramin (200µM) on Wound healing assay in MCF-7 cells.Mann-Whitney test for gene expression and wound healing assay was used. Wound healing rate of MCF-7 cells were determined after treatment with 200µM suramin in a time-dependent manner (6hrs, 12hrs, 24hrs, 36hrs) with significant statistics of *P<0.05, **P<0.01. The data was expressed as mean ± SEM with n = 6. Inhibition of cancer cells was started at time period of 6 hours with the closing rate of 145µm/hr. while maximum inhibition was observed at 36 hours with closing rate of 80µm/hr. Suramin inhibited the migration at 6, 12, 24, 36 hours with respective value of 80µm/hr ± 4.4 vs 175µm/hr ± 5.

(C) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of HPSE in MCF-7 cell lines in control and treated group. Mann-Whitney test for gene expression of Heparanase was used. The outcomes were expressed as average ± SEM of six independent experiments *P < 0.05, **P < 0.01, ***P<0.001. The statistics was expressed as mean ± SEM with n = 6.

Figure 2:

(A) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of E.cadherin in MCF-7 cell lines in control and treated group. Expression of epithelial markers like E.cadherin was elevated in MCF-7 cells after treatment of suramin (200µM) in a time dependent manner. Mann-Whitney test for gene expression of E.cadherin was used the results were compared with control in 6 independent experiments **P < 0.01. The statistics was expressed as average ± SEM with n = 6.

(B) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of E.cadherin in MCF-7 cell lines in control and treated groups:Expression of mesenchymal marker were downregulated among treated cancer cells of breast cancer in contrast to the cell lines in control group. Mann-Whitney test for gene expression of mesenchymal markers was used. The consequences were compared with control group and expressed in six independent experiments *P < 0.05, **P < 0.01, ***P<0.001. The statistics was expressed as average ± SEM with n = 6.

(C) mRNA expression of epithelial mesenchymal markers (E.cadherin, snail, slug, vimentin) after treatment with 200µM suramin in gel electrophoresis showing downregulation of snail, slug and vimentin whereas increase in levels of E.cadherin.

Figure 3:

(A) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of cancer stem cell markers CXCR4, OCT3/4, NANOG and NESTIN in MCF-7 cell lines in control and treated groups: Levels of cancer stem cell markers (CXC-4, OCT3/4, and NANOG) were higher except Nestin in MCF-7 cell lines in control group whereas levels of cancer stem cells were lowered in MCF-7 cell lines when treated with 200µM of suramin. Mann-Whitney test for gene expression of cancer stem cell markers was used. The outcomes are expressed in six independent experiments **P < 0.01. The statistics was expressed as average ± SEM with n = 6.

(B) mRNA levels of cancer stem cells markers after treatment with 200µM suramin in gel electrophoresis expressing downregulation of stemness markers i.e. NANOG, NESTIN, OCT3/4, CXCR4.
(C) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of matrix metalloproteinase in MCF-7 cell lines in control and treated groups. Expression of matrix metalloproteinase were higher in MCF-7 cell lines in control group whereas expression of drug transporters were lowered in MCF-7 cell lines when treated with 200uM of suramin for 2, 4 and 6 days. Mann-Whitney test for gene expression of drug resistant proteins was used. The outcomes are expressed in six independent experiments (**P < 0.01). The statistics was expressed as average ± SEM with n = 6.
(D) mRNA expression of matrix metalloproteinase after treatment with 200µM suramin in gel electrophoresis showing inhibition of expression levels of MMP-2, MMP-7, and MMP-9.

Figure 4:
(A) Bar diagram showing time dependent effect of suramin (200µM) on mRNA expression of drug transporters (MRP-1, MRP-2, MRP-3, MRP-4, MRP-5, BCRP, MDR-1 and LRP) in MCF-7 cell lines in control and treated groups. Expression of drug transporter transporters (MRP-1, MRP-2, MRP-3, MRP-4, MRP-5, BCRP, MDR-1 and LRP) was higher in MCF-7 cell lines in control group whereas expression of drug transporters were lowered in MCF-7 cells when cured with 200uM of suramin for 2, 4 and 6 days. Mann-Whiteney test for gene expression of drug resistant proteins was used. The results are expressed in six independent experiments *P < 0.05, **P < 0.01, ***P<0.001. Results with MRP-1, MRP-2, MRP-3, MMP-4, and MMP-5 were non-significant. The statistics was expressed as average ± SEM with n = 6.
(B) mRNA levels of multidrug resistant proteins after treatment with 200µM suramin in gel electrophoresis showing decrease in expression levels of MRP1, MRP2, MRP3, MRP4, MRP5, and BCRP.

REFERNCES

1. Buch, K., Gunmalm, V., Andersson, M., Schwarz, P., & Brons, C. (2019). Effect of chemotherapy and aromatase inhibitors in the adjuvant treatment of breast cancer on glucose and insulin metabolism-A systematic review. [Review]. *Cancer Med, 8*(1), 238-245.
2. Kitamura, T., Qian, B. Z., Soong, D., Cassetta, L., Noy, R., Sugano, G., Pollard, J. W. (2015). CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages. *J Exp Med, 212*(7), 1043-1059.
3. Majeed, A. I., Jadoon, M., Riazuddin, S., & Akram, J. (2016). Awareness and screening of breast cancer among rural areas of Islamabad capital territory, Pakistan. *Annals of PIMS ISSN, 1815*, 2287.
4. Amjad, A., Khan, I., Kausar, Z., Saeed, F., & Azhar, L. (2018). Risk Factors in Breast Cancer Progression and Current Advances in Therapeutic Approaches to Knockdown Breast Cancer. *Clin Med Biochem, 4*(137), 2471-2663.1000137.
5. Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. [Review]. *Genes Dis, 5*(2), 77-106.
6. Tariq, R., Huma, S., Butt, M. Z., & Amin, F. (2013). Risk factors and prevalence of breast cancer-a review. *Journal of the Pakistan medical association, 63*(8), 1075-1078.
7. Giordano, S. H., Lin, Y. L., Kuo, Y. F., Hortobagyi, G. N., & Goodwin, J. S. (2012). Decline in the use of anthracyclines for breast cancer. *Journal of clinical oncology, 30*(18), 2232.
8. Hung, C. M., Hsu, Y. C., Chen, T. Y., Chang, C. C., & Lee, M. J. (2017). Cyclophosphamide promotes breast cancer cell migration through CXCR4 and matrix metalloproteinases. *Cell biology international, 41*(3), 345-352.
9. Deveci, H. A., Nazıroğlu, M., & Nur, G. (2018). 5-Fluorouracil-induced mitochondrial oxidative cytotoxicity and apoptosis are increased in MCF-7 human breast cancer cells by TRPV1 channel

activation but not Hypericum perforatum treatment. *Molecular and cellular biochemistry*, *439*(1-2), 189-198.
10. Sharma, P., López-Tarruella, S., García-Saenz, J. A., Ward, C., Connor, C. S., Gómez, H. L., & Picornell, A. C. (2017). Efficacy of neoadjuvant carboplatin plus docetaxel in triple-negative breast cancer: combined analysis of two cohorts. *Clinical cancer research*, *23*(3), 649-657.
11. Remesh, A. (2012). Toxicities of anticancer drugs and its management.
12. Ji, X., Lu, Y., Tian, H., Meng, X., Wei, M., & Cho, W. C. (2019). Chemoresistance mechanisms of breast cancer and their countermeasures. *Biomedicine & Pharmacotherapy*, *114*, 108800.
13. Zheng, H. C. (2017). The molecular mechanisms of chemoresistance in cancers. *Oncotarget*, *8*(35), 59950.
14. Huh, H. J., Park, C. J., Jang, S., Seo, E. J., Chi, H. S., Lee, J. H.,& Ghim, T. (2006). Prognostic significance of multidrug resistance gene 1 (MDR1), multidrug resistance-related protein (MRP) and lung resistance protein (LRP) mRNA expression in acute leukemia. *Journal of Korean medical science*, *21*(2), 253-258.
15. Vtorushin, S. V., Khristenko, K. Y., Zavyalova, M. V., Perelmuter, V. M., Litviakov, N. V., Denisov, E. V., & Cherdyntseva, N. V. (2014). The phenomenon of multi-drug resistance in the treatment of malignant tumors. *Experimental oncology*.
16. Sui, H., Zhu, L., Deng, W., & Li, Q. (2014). Epithelial-mesenchymal transition and drug resistance: role, molecular mechanisms, and therapeutic strategies. *Oncology research and treatment*, *37*(10), 584-589.
17. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646-674.
18. Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu Rev Cell Dev Biol. 2011; 27:347–76.
19. Thiery, J. P., Acloque, H., Huang, R. Y., & Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *cell*, *139*(5), 871-890.
20. Secchi, M. F., Masola, V., Zaza, G., Lupo, A., Gambaro, G., & Onisto, M. (2015). Recent data concerning heparanase: focus on fibrosis, inflammation and cancer. *Biomolecular concepts*, *6*(5-6), 415-421.
21. Wood, R. J., & Hulett, M. D. (2008). Cell surface-expressed cation-independent mannose 6-phosphate receptor (CD222) binds enzymatically active heparanase independently of mannose 6-phosphate to promote extracellular matrix degradation. *Journal of Biological Chemistry*, *283*(7), 4165-4176.
22. Nakajima, M., Irimura, T., & Nicolson, G. L. (1988). Heparanases and tumor metastasis. *Journal of cellular biochemistry*, *36*(2), 157-167.
23. Masola, V., Gambaro, G., Tibaldi, E., Brunati, A. M., Gastaldello, A., D'Angelo, A.,& Lupo, A. (2012). Heparanase and syndecan-1 interplay orchestrates fibroblast growth factor-2-induced epithelial-mesenchymal transition in renal tubular cells. *Journal of Biological Chemistry*, *287*(2), 1478-1488.
24. Masola, V., Zaza, G., Secchi, M. F., Gambaro, G., Lupo, A., & Onisto, M. (2014). Heparanase is a key player in renal fibrosis by regulating TGF-β expression and activity. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(9), 2122-2128.
25. Steverding, D. (2010). The development of drugs for treatment of sleeping sickness: a historical review. *Parasites & vectors*, *3*(1), 15.
26. Nakajima, M., DeChavigny, A., Johnson, C. E., Hamada, J. I., Stein, C. A., & Nicolson, G. L. (1991). Suramin. A potent inhibitor of melanoma heparanase and invasion. *Journal of Biological Chemistry*, *266*(15), 9661-9666.
27. Li, H., Qu, H., Zhao, M., Yuan, B., Cao, M., & Cui, J. (2015). Suramin inhibits cell proliferation in ovarian and cervical cancer by downregulating heparanase expression. *Cancer Cell Int*, *15*, 52.
28. Mirza, M. R., Jakobsen, E., Pfeiffer, P., Lindebjerg-Clasen, B., Bergh, J., & Rose, C. (1997). Suramin in non-small cell lung cancer and advanced breast cancer: two parallel phase II studies. *Acta Oncologica*, *36*(2), 171-174.

29. Tayel, A., Abd El Galil, K. H., Ebrahim, M. A., Ibrahim, A. S., El-Gayar, A. M., & Al-Gayyar, M. M. (2014). Suramin inhibits hepatic tissue damage in hepatocellular carcinoma through deactivation of heparanase enzyme. *Eur J Pharmacol, 728*, 151-160
30. Falcone, A., Pfanner, E., Cianci, C., Danesi, R., Brunetti, I., Del Tacca, M., & Conte, P. F. (1995). Suramin in patients with metastatic colorectal cancer pretreated with fluoropyrimidine-based chemotherapy. A phase II study. *Cancer, 75*(2), 440-443.
31. Shah, S., Fourgeaud, C., Derieux, S., Mirshahi, S., Contant, G., Pimpie, C., Mirshahi, M. (2018). The close relationship between heparanase and epithelial mesenchymal transition in gastric signet-ring cell adenocarcinoma. *Oncotarget, 9*(73), 33778.
32. Bhargava, S., Hotz, B., Hines, O. J., Reber, H. A., Buhr, H. J., & Hotz, H. G. (2007). Suramin inhibits not only tumor growth and metastasis but also angiogenesis in experimental pancreatic cancer. *J Gastrointest Surg, 11*(2), 171-178.
33. Wade, T. P., Kasid, A., Stein, C. A., LaRocca, R. V., Sargent, E. R., Gomella, L. G., Linehan, W. M. (1992). Suramin interference with transforming growth factor-beta inhibition of human renal cell carcinoma in culture. *J Surg Res, 53*(2), 195-198.
34. Shin, R., Naomoto, Y., Kamikawa, Y., Tanaka, N., & Orita, K. (1997). Effect of suramin on human esophageal cancer cells in vitro and in vivo. [Comparative Study]. *Scand J Gastroenterol, 32*(8), 824-828.
35. Gansler, T., Vaghmar, N., Olson, J. J., & Graham, S. D. (1992). Suramin inhibits growth factor binding and proliferation by urothelial carcinoma cell cultures. *J Urol, 148*(3), 910-914.
36. Hensey, C. E., Boscoboinik, D., & Azzi, A. (1989). Suramin, an anti-cancer drug, inhibits protein kinase C and induces differentiation in neuroblastoma cell clone NB2A. [Research Support, Non-U.S. Gov't]. *FEBS Lett, 258*(1), 156-158.
37. Eisenberger, M. A., Reyno, L. M., Jodrell, D. I., Sinibaldi, V. J., Tkaczuk, K. H., Sridhara, R., & Egorin, M. J. (1993). Suramin, an active drug for prostate cancer: interim observations in a phase I trial. *JNCI: Journal of the National Cancer Institute*, *85*(8), 611-621.
38. Li, J., Pan, Q., Rowan, P. D., Trotter, T. N., Peker, D., Regal, K. M., & Yang, Y. (2016). Heparanase promotes myeloma progression by inducing mesenchymal features and motility of myeloma cells. *Oncotarget*, *7*(10), 11299.
39. Senthilraja, P., & Kathiresan, K. (2015). In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *J Appl Pharm Sci*, *5*(3), 080-084.
40. Masola, V., Bellin, G., Gambaro, G., & Onisto, M. (2018). Heparanase: A multitasking protein involved in extracellular matrix (ECM) remodeling and intracellular events. *Cells, 7*(12), 236.
41. Jia, L., & Ma, S. (2016). Recent advances in the discovery of heparanase inhibitors as anti-cancer agents. *European journal of medicinal chemistry*, *121*, 209-220.
42. Jayatilleke, K., Duivenvoorden, H., Parker, B., & Hulett, M. (2018). PO-220 Defining the role of heparanase in breast cancer progression using the PyMT-MMTV mouse model: BMJ Publishing Group Limited.
43. La Rocca, R. V., Meer, J., Gilliatt, R. W., Stein, C. A., Cassidy, J., Myers, C. E., & Dalakas, M. C. (1990). Suramin-induced polyneuropathy. *Neurology*, *40*(6), 954-954.
44. Kobayashi, K., Weiss, R. E., Vogelzang, N. J., Vokes, E. E., Janisch, L., & Ratain, M. J. (1996). Mineralocorticoid insufficiency due to suramin therapy. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *78*(11), 2411-2420.
45. Figg, W. D., Cooper, M. R., Thibault, A., Headlee, D., Humphrey, J., Bergan, R. C.,& Sartor, O. (1994). Acute renal toxicity associated with suramin in the treatment of prostate cancer. *Cancer*, *74*(5), 1612-1614.
46. Foekens, J. A., Sieuwerts, A. M., Stuurman-Smeets, E. M., Dorssers, L. C., Berns, E. M., & Klijn, J. G. (1992). Pleiotropic actions of suramin on the proliferation of human breast-cancer cells in vitro. *International journal of cancer*, *51*(3), 439-444.
47. Vignon, F., Prebois, C., & Rochefort, H. (1992). Inhibition of breast cancer growth by suramin. *JNCI: Journal of the National Cancer Institute*, *84*(1), 38-42.

48. Bao, B., Ahmad, A., Azmi, A. S., Ali, S., & Sarkar, F. H. (2013). Overview of cancer stem cells (CSCs) and mechanisms of their regulation: implications for cancer therapy. *Current protocols in pharmacology*, *61*(1), 14-25.
49. Soltysova, A., Altanerova, V., & Altaner, C. (2005). Cancer stem cells. *Neoplasma*, *52*(6), 435.
50. Borst, P., Evers, R., Kool, M., & Wijnholds, J. (2000). A family of drug transporters: the multidrug resistance-associated proteins. *Journal of the National Cancer Institute*, *92*(16), 1295-1302.
51. Burger, H., Foekens, J. A., Look, M. P., Meijer-van Gelder, M. E., Klijn, J. G., Wiemer, E. A., Nooter, K. (2003). RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. *Clinical Cancer Research*, *9*(2), 827-836.