Parthenolide Induces Oxidative Stress and Impedes Cell Migration by Suppressing Wnt Pathway and Epithelial-Mesenchymal-Transition (EMT) in HCT-116 Metastatic Colorectal Cancer Cells

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INTRODUCTION

Colorectal cancer (CRC) causes a global crisis in terms of morbidity and mortality. It is the 3rd most fatal and 4th most recurrently identified cancer worldwide. 50% of CRC patients suffer from the metastatic hepatic disease over their lifetime, which ultimately fall out in death for more than two-thirds of these patients (Bray et al., 2018). Since there has been no remarkable development for metastatic CRC management, healing rate has remained near to the ground over the decades. Aggressive local invasion and metastasis have made colon cancer challenging to deal. Therefore, illuminating the mechanism of invasive metastasis of CRC and novel drug discovery that target colorectal cancer and its metastasis-related diseases, is urgently needed. Metastasis is a complex process during which cancer cells aid their migration, invasion, and finally colonisation in distant sites by degrading their anchorage with the extracellular matrix (ECM). Matrix metal...
Sesquiterpene lactones are secondary metabolites isolated from plants of Asteraceae family, *Tanacetum parthenium* and have been used to treat certain ailments traditionally. Parthenolide (PTL) is one of the active sesquiterpene lactones of this plant. PTL contains two functional groups, one epoxide group and one α-methylene-γ-lactone ring, which readily react at the nucleophilic region of biomolecules (Dey et al., 2016). Parthenolide has shown their cytotoxicity or induces apoptosis in different cancer cells. PTL shows its anti-cancer activity via different cellular signalling pathways viz inhibition of NFκB, STAT3, MAPK, JNK pathways, activation of p53, suppression of nucleic acid synthesis pathway, depletion of intercellular thiols, induction of oxidative stress, promoting mitochondrial dysfunction, interference of cellular calcium homeostasis, cell cycle arrest at G2/M phase, depletion of HDAC1 and inhibits tubulin carboxypeptidase activity (Zhang et al., 2004; Siyuan et al., 2004). Literature data showed that PTL selectively targets cancer cells. It has been reported that PTL specifically targets stem cells of acute myelogenous leukaemia (AML) and their progenitor cells without damaging normal hematopoietic cells (Guzman et al., 2005; Baranello et al., 2015). However, how Parthenolide affects cancer metastasis is not well understood. In this present study, we have assessed the anti-migratory/anti-metastatic potential of PTL against human HCT-116 colorectal cancer cells.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Parthenolide was procured from Sigma-Aldrich (India). DMEM (high glucose) culture media was procured from GIBCO (Thermo Fisher Scientific USA), antibiotics, fetal bovine serum (FBS) and non-fat dried milk were purchased from HiMedia Laboratories (India); MTT (Thiazolyl Blue Tetrazolium Bromide) was from Abcam (USA) and Bio-Rad, USA. The cell line was maintained in DMEM (high glucose) complete media added with 10% heat-inactivated FBS, along with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) in 5% CO2 and 95% humidified conditions maintaining 37°C. Cells were cultured to the exponential phase until the number of cells grown up to 1.0 x 10⁶ cells/ml.

**Cell viability assay**

The cytotoxicity of PTL on HCT-116 was quantitatively determined by colourimetric MTT assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) according to our previous study (Kole et al., 2011). The amount of drug, which inhibits 50% healthy cell viability (IC₅₀) was estimated by plotting graph. Data were compared by using multiple linear regression in Statistica, India (version 5.0) software.

**Fluorescence microscopic study using Acridine Orange and Ethidium Bromide**

To determine cell death, we used Acridine Orange (AO) and Ethidium Bromide (EtBr). In each well, 2x10⁴ HCT-116 cells were seeded in a 6-well plate and kept at 37°C for 24 hrs CO₂ incubator. PTL treatment was done at 5μg/ml dose and 10μg/ml dose for 48 hours. After the treatment, cells were trypsinised and washed with PBS. 10μl of the cells were then pipetted and placed on a clean glass slide and mixed with 10μl each of acridine orange (50μg/ml) and ethidium bromide (50μg/ml) (Gomes et al., 2011).

The stained cells were observed using the EVOS® FL Cell Imaging System (Life Technologies, USA) at epoxide group and one α-methylene-γ-lactone ring, which readily react at the nucleophilic region of biomolecules (Gonzalez-Avila et al., 2019). Epithelial-mesenchymal transition (EMT), a process, which plays a vital role in cancer progression and metastasis. EMT ease the metastasis by progressing the epithelial cancer cells to impair their cell-cell and cell-ECM adhesion characteristics and to acquire migratory and invasive characteristics (Lin and Wu, 2020).

**Preparation of Drug**

A primary stock solution of Parthenolide was prepared using absolute ethanol as a solvent and kept at -20°C. The final drug concentrations were prepared at the time of treatment by diluting the stock solution with media.

**Cell lines maintenance and culture**

The HCT-116 colon cancer cell line was a gift from Dr Sanjay Ghosh, Professor, University of Calcutta (India). The cell line was maintained in DMEM (high glucose) complete media added with 10% heat-inactivated FBS, along with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) in 5% CO₂ and 95% humidified conditions maintaining 37°C. Cells were cultured to the exponential phase until the number of cells grown up to 1.0 x 10⁶ cells/ml.
400X magnification.

**Intracellular ROS measurement by Fluorescence microscopy**

The quantity of reactive oxygen species generation was assessed using H$_2$DCFDA staining as per the method by Chattopadhyay et al. (2014). In brief, experimental cells (2 x10$^5$ cells/mL) were treated with PTL for 48 h. There was a positive control set where experimental cells were treated with H$_2$O$_2$ (100 mM) for 30 min before estimation. After 48 h of treatment, cells were washed, followed by 30 min incubation with H$_2$DCFDA (1 mg/ml ) at 37°C. Next, the cells were washed thrice with the media. Cells were observed under the EVOS® FL Cell Imaging System (Life Technologies, USA). All measurements were done in triplicate.

**Reduced Glutathione (GSH) measurement**

Reduced GSH of the cell lysates were estimated as stated by Halder et al. (2018). To the cell lysate, 25% of trichloroacetic acid was mixed with and centrifuged at 2000 RPM for 15 min, and the protein sediments were collected. The supernatants were aspirated, and they were diluted up to 1 ml using 0.2 M sodium phosphate buffer (pH 8.0). Then, 2 ml of 0.6 mM DTNB (Ellman’s reagent) was added to each of the vials. The experimental set up was incubated at 37°C for 10 min. After the reaction of GSH with DTNB, the absorbance was observed at 405 nm. Readings were taken using a standard curve prepared with different doses of standard GSH and expressed as µg of GSH per mg of protein. All measurements were done in triplicate.

**Oxidised Glutathione (GSSG) measurement**

The oxidised glutathione (GSSG) levels of the cell lysates were evaluated on the production of derivatised intermediate after the reaction of GSH with 2-vinyl pyridine as per Mahapatra et al. (2009). In brief, 0.5 ml sample and 2 µL, 2-vinyl pyridine reagent was added and kept at 37°C for 1 h. 4% sulfosalicylic acid (SSA) was added to this for deproteinisation and centrifuged for 1 min at 1,000 × g. The supernatants were collected, and GSSG concentrations were determined after reacting them with DTNB at 412 nm and compared with standard GSSG curve. Respective GSSG concentrations were calculated and expressed as µg of GSSG per mg of protein. All measurements were done in triplicate.

**Wound Healing Assay**

On an 80% confluent six-well plate, straight scratches were made across the diameter of the well using P100 pipette tip. Cells, that detached were washed using PBS, and fresh serum-free medium were added with different doses of PTL. Cell migration towards the wound areas was observed after 48 h. The ability of the cells to heal the wounds were assessed by measuring the ultimate areas of healed wounds. Images were captured in Olympus IX71 microscope at 100x magnification. Each scratch area was measured thrice using Image J software.

**Analysis of Gene Expression by q-RT-PCR**

Total cellular RNA was isolated using 'RNeasy® Mini Kit (250)' (Qiagen, Netherlands) and RNA was measured using Eppendorf BioSpectrometer®. Then an equal volume of each experimental samples was reverse-transcribed to form cDNA using ‘High-Capacity cDNA Reverse Transcription Kit' (Invitrogen™, USA) following the manufacturer’s instruction. The cDNAs were then used for the qPCR analysis of gene expression using GAPDH as housekeeping control. The list of primers used for the qPCR reactions was mentioned in Table 1, and amplification genes were performed in CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, USA) for 30 cycles. The threshold cycle (CT) readings were determined using the equation of $\Delta$CT = CT (target) - CT (endogenous control) and fold changes were measured as $2^{-\Delta(\Delta CT)}$. The results were plotted on the graph for the presentation.

**Western blot analysis**

Western Blot study was done by our previously standardised lab protocol (Sarkar et al., 2017). Experimental cells were seeded for treatment on 60 mm plates (Himedia, India), washed with Phosphate Buffer Saline before lysis with RIPA buffer consisting of protease inhibitor cocktail. The cell lysates were then centrifuged for 30 minutes at 10000 rpm at 4°C and supernatants were collected. Protein concentrations were estimated using Bradford reagent taking bovine serum albumin as standard, and samples were diluted at 1:1 ratio using sample buffer consisting of 4% SDS, 0.5 M Tris-HCl (pH 6.8), 20% glycerol and 0.002% bromophenol blue. Samples containing 50 μg of total protein (solubilised in sample buffer) were resolved SDS-PAGE gel and then electrotransblotted to a Polyvinylidene difluoride (PVDF) membrane. Then the PVDF membrane was blocked with 5% non-fat dried milk (Himedia, India). Membranes were incubated with the DKK1 and MMP-9 antibodies. After washing, membranes were incubated with HRP-conjugated secondary antibody and developed with ECL reagent and observed under ChemiDoc XRS+ System (Bio-Rad, USA).

**Statistical Analysis**
The data were represented as mean ± SEM, n=3. One-way ANOVA test (using a statistical package, Origin 6.1, Northampton, MA 01060 USA) was done to compare among the means of control and treated groups. P < 0.05 was considered as significance limit.

RESULTS

Cytotoxicity of PTL against HCT-116 cells was tested by MTT assay after 48 h treatment. The percentage of HCT-116 cancer cell death significantly reduced upon treatment of PTL. The 50% inhibitory concentration (IC50) value of PTL in HCT-116 cells was determined using different doses (1, 5, 10, 50, 100 µg/ml) of the drug for 48 h. Respective cell death percentages were plotted in a graph using Statistics software. The IC50 value of PTL in HCT-116 cells was noted as 12.19 µg/ml. After 48 h, morphologies of cells were altered drastically. PTL induced dose-dependent cell death which was observed at 48 h under phase-contrast microscopy (Magnification 200x). (Figure 1A, Figure 1B)

Fluorescence microscopic analysis

The fluorescence images after PTL treatment observed with EtBr-AO staining. These typical fluorescent dye stains in such a way that the healthy cells with undamaged DNA give green fluorescence, late apoptotic/necrotic cells having fragmented DNA emit orange/red-coloured fluorescence. Our experimental result shows that PTL treatment decreased the number of viable cells and an increased number of apoptotic/necrotic cell population. The significant population of HCT-116 cells stained with orange colour indicates cellular apoptosis in the PTL treated groups in a dose (5µg/ml and 10µg/ml) dependent manner. (Figure 1C)

Determination of Cellular Reactive Oxygen Species

The fluorescence intensity of Dichloro-dihydro-fluorescein diacetate (DCFH2-DA) represents ROS generation. In fluorescence microscopic image analysis, it was observed that in HCT-116 cells, DCFH2-DA fluorescence intensity was elevated significantly (p<0.05) by PTL treatment. Results presented as the mean of three experiments. Values were represented Mean ± SEM (p<0.05 significant). (Figure 2A, Figure 2B)

Measurement of Cellular GSH and GSSG

Reduced Glutathione (GSH) level was estimated using cell lysate of different groups. The result shows a 33.33% decrease in GSH level in 5µg/ml PTL treated group while comparing with the control. In 10µg/ml PTL treatment group, the level of GSH was decreased by 58.3% compared to that of control. PTL treatment increased GSSG level significantly (p<0.05) in HCT-116 cell lysate. It has been observed that PTL (5µg/ml and 10µg/ml) treatment upsurge GSSG level by 75% and 87.5% respectively while comparing with the control. (Figure 2C, Figure 2D)

Wound Healing Assay

PTL at concentrations of 5µg/ml and 10µg/ml promote dose-dependent inhibition of migratory properties of HCT-116 cells. The gap did not achieve full wound closure for both doses. PTL treatments after 48 h with a dose of 5µg/ml and 10µg/ml demonstrated that the percentage of recovery was decreased with respect to control. The cell migration assay was performed by wound closure by the migrated cells initial (0 h) and final (48 h) wound area of the scratches, and they were measured by ImageJ (NIH) software. In 5µg/ml of PTL treatment in migrating cells, 30% of the wound healing was inhibited as compared with the control. But in 10µg/ml treatment setup, the wound area was increased by 40% as compared to the initial wound created at zero hour of the experiment due to extensive cell death. (Figure 3)

qRT-PCR analysis of the expression of transcription factors and genes associated with EMT

Treatment with PTL (10µg/ml) for 48 h in HCT-116 cells showed a down-regulated expression of c-fos, c-jun and N-cadherin whereas it induces up-regulated expression of E-cadherin. The figure showed expression of different genes in the RNA level when the results of PTL treated HCT-116 cells were compared to that of their respective controls. Results are represented as levels of mRNA expressions equalised with GAPDH as the control. Our data showed that PTL (10µg/ml) decreased 40% of c-jun expression and 50% of c-fos expression in HCT-116 cells (Figure 4A, Figure 4B). The same dose of the treatment showed 50% up-regulated E-cadherin expression (Figure 4C) and 30% down regulation of N-cadherin expression (Figure 4D).

Western Immunoblot analysis of DKK-1 and MMP-9

The effect of PTL on DKK-1 and MMP-9 expression in HCT-116 colon cancer cells were determined quantitatively using Western immunoblot analysis. The data were further analysed by Image-J software. We measured the expression levels of DKK-1, a key negative regulator of the Wnt signalling pathway, after treatment with varying concentrations of PTL. Here, we observed that PTL treatment at 5µg/ml dose showed 1.2 fold expression of

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Figure 1: Cytotoxicity of PTL on HCT-116 colon cancer cell.

(A) Determination of IC$_{50}$ of Parthenolide, (B) Morphology study: PTL induced dose-dependent cell death observed under phase-contrast microscopy (Magnification 200x), (C) AO/EtBr staining and fluorescent microscopy (Magnification 400x).

Table 1: List and Sequence of Primers used in the qRT-PCR analysis.

| Gene    | Forward                     | Primer                     |
|---------|-----------------------------|----------------------------|
| GAPDH   | 5'GGTCTCCTCTGACTTTCAA-3'    | 5'-AGCCAAATTCGTGTTCAT-3'   |
| E-CADHERIN | 5'TTGCTACTGGAACAGGG -3.'   | 5'-GTTCTGCTGTGAAAGGA -3'   |
| N-CADHERIN | 5'-ACAGCTCACCACATGACTCC -3' | 5'-ACCTCCACCACATGTCAGC-3'  |
| C-FOS   | 5'-GAACCTGTCAAGAGCATCAG-3'  | 5'-TCCAGTCTGCTGCATAGAAG-3' |
| C-JUN   | 5'-ATCCTGAACAGAGCATGACC-3'  | 5'-TTGCTGGACTGGATTACGG-3'  |
DKK-1 whereas 10µg/ml PTL treatment showed 2.0 fold DKK-1 expression as compared with the control after 48 h of treatment. Next, we measured MMP-9 expressions. MMP-9 is a vital biomarker of invasion and metastatic properties of cancer. Data showed that 5µg/ml and 10µg/ml of PTL treatments lower MMP-9 expression by 20% and 30% respectively as compared with the control indicating the anti-metastatic effect of PTL in a dose-dependent fashion. (Figure 5)

**DISCUSSION**

Metastasis is the most complicated stage of neoplasm being the major lethal cause due to cancer. Epithelial to mesenchymal transition is the process through which tumour cells migrates and metastasise into the circulation and distant organs. Colorectal cancer has become the most common gastrointestinal malignancy in the recent decade. Therefore, search for a potent anti-cancer drug which can potentially prevent proliferation and migration of cancer cells and downregulate EMT markers is going on. Parthenolide is a key component among sesquiterpene lactones present in Feverfew. PTL has an epoxide functional group and an α-methylene-γ-lactone ring were having nucleophilic reaction ability with biological molecules, especially having cysteine thiol (-SH) groups by 'Michael addition reaction' (Dey et al., 2016). It has been shown to inhibit growth or induce apoptosis in several tumour cell lines (Guzman et al., 2005; Carlisi et al., 2016). In the present study, we have examined the therapeutic efficacy of PTL against migration and metastatic properties of HCT-116 colorectal cancer cells in vitro. This study showed that PTL significantly killed the HCT-116 cells in a dose-dependent fashion. Collectively, 5µg/ml and 10µg/ml dose of PTL with 48 h incubation period were selected. Being free radicals, ROS molecules are highly reactive and show a crucial role in cell signalling regulation, causing oxidative cell damage and ultimately cell death. In normal physiological homeostasis, the amount of ROS remains lower during metabolism, which is effectively quenched by many antioxidant enzymes of the glutathione system. Cellular ROS is generated via different enzymatic relations, viz. the mitochondrial respiratory chain reaction, membrane-bound superoxide generating enzyme, NADPH oxidase etc. (Carlisi et al., 2016).
Wound closure was checked at 48 h of treatment, and photographs were taken at 0h and 48h (Magnification 100x). Percentage of wound recovery is shown in graphical representation.

Figure 3: Effect of PTL on HCT-116 cells migration.

Figure 4: Effect of PTL on gene expression in HCT-116 cells.
From the fluorescence microscopic image analysis, it has been observed that in HCT-116 cells, DCFH$_2$-DA fluorescence intensity was significantly elevated (p<0.05) by PTL treatment. Increased level of ROS excites the release of several inflammatory molecules, including TNF-α. TNF-α stimulates NF-κB and JNK, which eventually increases apoptotic and necrotic cell death (Shen and Pervaiz, 2006).

To observe the cell death pattern due to PTL treatment, we stained the cancer cells with EtBr and AO and the fluorescence images after PTL treatment were observed. These typical staining stains that the healthy cells with undamaged DNA and give green fluorescence, and late apoptotic/necrotic cells’ having fragmented DNA emits orange/red-coloured fluorescence. In our experiment, it is evident that PTL treatment decreases the number of viable cells and increases the apoptotic cell population in a dose-dependent manner. Glutathione, an important cellular antioxidant, protects cells from cellular peroxides and different free radicals. This study showed that GSH levels in HCT-116 cancer cell line were significantly decreased (p<0.05) when treated with PTL. Conversely GSSG level in cancer cells significantly increased (p<0.5) when treated with PTL. Therefore, it can be said that PTL treatment can alter the cellular redox balance and directs cells towards oxidative damage.

Most of the cancer patients died due to metastasis. Tumour cell migration and invasion to the circulation from the surrounding tissue through epithelial to mesenchymal transition is considered as an initial step of the metastasis. To examine the effect of PTL in colon cancer migration, an in vitro wound healing study was carried out. We have measured the percentage of inhibition of wound healing which was calculated using ImageJ software analysis. After treatment with 5μg/ml PTL for 48 h, we observed that the wound closure rate decreased by 30%.

Additionally, 10μg/ml PTL treatment increased the wound area by 39% due to extensive cell death. To further confirm we performed gene expression analysis by the real-time qPCR method of some genes that are essential modulator of epithelial to mesenchymal transition. c-fos is a proto-oncogene that is expressed in many cancers. c-fos overexpression promotes cancer cell growth and angiogenesis. c-fos has a DNA binding domain which remains in a dimer form with c-jun gene product and ultimately forms the transcription factor activating protein 1 (AP-1). Since c-fos is a member of the AP-1 family, it is associated primarily with signal transduction, cell proliferation and cellular differentiation (Milde-Langosch, 2005). In our experimental result, we found a significant decrease in c-fos and c-jun gene expression level, indicating the anti-proliferative and anti-angiogenic potential of PTL.

Additionally, we have studied gene expression of two important markers of EMT pathway, E-cadherin and N-cadherin. E-cadherin is an adhesion protein which represents the epithelial nature of cells, whereas N-cadherin is a marker of mesenchymal phenotype. E-cadherin downregulation and N-cadherin upregulation switch on the EMT pathway. Our experimental data revealed that PTL could significantly up-regulate E-cadherin gene expression.
and down-regulates N-cadherin gene expression in HCT-116 colon cancer cells, thus inhibited EMT.

The scientific report suggested that expression of E-cadherin modulates β-catenin translocation, thereby inhibiting the Wnt pathway (Loh et al., 2019). So, in this study PTL induced overexpression of E-cadherin may also take part in suppressing Wnt pathway in HCT-116 colon cancer cells. Wnt pathway is one of the major pathways that promote metastasis. In colon cancer cells, it was reported that the Wnt/β-catenin pathway is hyperactivated and nuclear translocation and accumulation of β-catenin promotes metastasis (Tenbaum et al., 2012).

Hence, the pharmacological intervention, which blocks canonical Wnt pathway holds promising indication to control metastatic migration of colon cancer cells. DICKKOPF-1 (DKK-1) is an antagonist of the Wnt pathway, and therefore, we have analysed the effect of PTL treatment on DKK-1 protein expression in HCT-116 cells.

Our present study, the up-regulated expression of DKK-1 by PTL treatment in HCT-116 cells, indicates inhibition of Wnt/β-catenin pathway-mediated EMT in this colon cancer cell. The mesenchymal phenotype marker N-cadherin form synergistic crosstalk with fibroblast growth factor receptor via the extracellular domains and aid the ERK 1/2 pathway activation and MMP-9 expression (Suyama et al., 2002).

Generally increased MMP-9 expression and activation is one of the hallmarks of tumour progression, including angiogenesis, invasion and metastasis. In our study, PTL repressed the MMP-9 protein expression in a dose-dependent fashion. Present data suggest that PTL treatment inhibits cell migration/invasion by regulating EMT markers (E-
CONCLUSIONS

In conclusion, this can be demonstrated that PTL inhibits cell proliferation and induces HCT-116 cell death through several pathways including oxidative stress, thiol depletion, DNA damage and protooncogene downregulation. Our findings also provide the shreds of evidence that PTL inhibits cell migration through the modulation of Wnt signalling, EMT pathway and MMP. These findings support that, PTL has a promising anti-cancer potential against metastatic HCT-116 colorectal carcinoma cells.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Baranello, M. P., Bauer, L., Jordan, C. T., Benoit, D. S. W. 2015. Micelle Delivery of Parthenolide to Acute Myeloid Leukemia Cells. *Cellular and Molecular Bioengineering*, 8(3):455–470.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., Jemal, A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.

Carlisi, D., Butttita, G., Fiore, R. D., Scerri, C., Drago-Ferrante, R., Vento, R., Tesoriere, G. 2016. Parthenolide and DMAPT exert cytotoxic effects on breast cancer stem-like cells by inducing oxidative stress, mitochondrial dysfunction and necrosis. *Cell Death & Disease*, 7(4):e2194–e2194.

Chattopadhyay, S., Dash, S. K., Mahapatra, S. K., Tripathy, S., Ghosh, T., Das, B., Das, D., Pramanik, P., Roy, S. 2014. Chitosan-modified cobalt oxide nanoparticles stimulate TNF-α-mediated apoptosis in human leukemic cells. *JBIC Journal of Biological Inorganic Chemistry*, 19(3):399–414.

Dey, S., Sarkar, M., Giri, B. 2016. Anti-inflammatory and Anti-tumor Activities of Parthenolide: An Update. *Journal of Chemical Biology & Therapeutics*, 01(02):107–107.

Gomes, A., Giri, B., Alam, A., Mukherjee, S., Bhattacharjee, P., Gomes, A. 2011. Anticancer activity of a low immunogenic protein toxin (BMP1) from Indian toad (Bufo melanostictus, Schneider) skin extract. *Toxicon*, 58(1):85–92.

Gonzalez-Avila, G., Sommer, B., Mendoza-Posada, D. A., Ramos, C., Garcia-Hernandez, A. A., Falfan-Valencia, R. 2019. Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Critical Reviews in Oncology/Hematology*, 137:57–83.

Guzman, M. L., Rossi, R. M., Karnischky, L., Li, X., Peterson, D. R., Howard, D. S., Jordan, C. T. 2005. The sessquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. *Blood*, 105(11):4163–4169.

Halder, S., Sarkar, M., Dey, S., Bhunia, S. K., Koley, A. R., Giri, B. 2018. Protective effects of red grape ( *Vitis vinifera* ) juice through restoration of antioxidant defense, endocrine swing and Hsf1, Hsp72 levels in heat stress induced testicular dysregulation of Wister rat. *Journal of Thermal Biology*, 71:32–40.

Kole, L., Giri, B., Manna, S. K., Pal, B., Ghosh, S. 2011. Biochanin-A, isoﬂavones, showed anti-proliferative and anti-inflammatory activities through the inhibition of iNOS expression, p38-MAPK and ATF-2 phosphorylation and blocking NFκB nuclear translocation. *European Journal of Pharmacology*, 653(1-3):8–15.

Lin, Y. T., Wu, K. J. 2020. Epigenetic regulation of epithelial-mesenchymal transition: focusing on hypoxia and TGF-β signalling. *Journal of Biomedical Science*, 27(1):39–39.

Loh, C. Y., Chai, J. Y., Tang, T. F., Wong, W. F., Sethi, G., Shanmugam, M. K., Looi, C. Y. 2019. The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: signaling, therapeutic implications, and challenges. *Cells*, 8(10):1118–1118.

Mahapatra, S. K., Das, S., Bhattacharjee, S., Gautam, N., Majumdar, S., Roy, S. 2009. In Vitro Nicotine-Induced Oxidative Stress in Mouse Peritoneal Macrophages: A Dose-Dependent Approach. *Toxicology Mechanisms and Methods*, 19(2):100–108.

Milde-Langosch, K. 2005. The Fos family of transcription factors and their role in tumourigenesis. *European Journal of Cancer*, 41(16):2449–2461.
apoptogenic efficacy of antidiabetic drugs metformin and sitagliptin against MCF7 and HEPG2 cancer cells: A comparative molecular study. *Journal of Drug Delivery and Therapeutics*, 7(6):11–21.

Shen, H.-M., Pervaiz, S. 2006. TNF receptor superfamily-induced cell death: redox-dependent execution. *The FASEB Journal*, 20(10):1589–1598.

Siyuan, Z., Ong, C.-N., Shen, H.-M. 2004. Involvement of proapoptotic Bcl-2 family members in parthenolide-induced mitochondrial dysfunction and apoptosis. *Cancer Letters*, 211(2):175–188.

Suyama, K., Shapiro, I., Guttman, M., Hazan, R. B. 2002. N-cadherin and the FGF receptor control a signalling pathway is leading to metastasis. *Cancer Cell*, 2(4):150–152.

Tenbaum, S. P., Ordóñez-Morán, P., Puig, I., Chicote, I., Arqués, O., Landolfi, S. 2012. β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nature Medicine*, 18(6):892–901.

Zhang, S., Lin, Z. N., Yang, C. F., Shi, X., Ong, C. N., Shen, H. M. 2004. Suppressed NF-κB and sustained JNK activation contribute to the sensitization effect of parthenolide to TNF-α-induced apoptosis in human cancer cells. *Carcinogenesis*, 25(11):2191–2199.