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Synergistic Computational and Cellular Investigation of Berberine's Therapeutic Potential in Triple-Negative Breast Cancer

[1]Vummini Krishnamurthy Sri Keerti, [2]Monisha Prasad, [3*]Shenbhagaraman Ramalingam

ABSTRACT:

Background: Breast cancer is a worldwide health issue, characterized by a lack of efficient treatment modalities, particularly for aggressive sub types of breast cancer like triple negative breast cancer (TNBC). Berberine is a phyto-alkaloid molecule that has been shown as a promising oncological agent. In the present work in silico network analysis, molecular docking and in vitro studies are employed to examine potential of berberine as a therapeutic agent in breast cancer. **Methodology:** Differentially expressed genes (DEGs) in breast cancer were identified using GEO datasets Network pharmacology, PPI analysis and pathway enrichment revealed berberine's impact on key biological processes. Molecular docking (AutoDock and SwissDock) evaluated binding of berberine's to breast cancer targets. ADME analysis was evaluated its pharmacokinetic properties. In vitro studies on breast cancer cell lines was performed to examine berberine's effects on cell viability, proliferation and apoptosis using dose-dependent MTT, and ATP based assays highlighting its potential as a therapeutic agent. **Results:** In silico network analyses identified that berberine impacts key signaling pathway and interact with significant differentially expressed genes of breast cancer significance. Molecular docking studies demonstrate high affinities of berberine binding to specific target proteins, which were substantiated by good pharmacokinetic potential identified through ADME study. In vitro studies revealed the dose dependent effect of berberine in inhibiting cell viability inducing apoptosis and causing morphological alteration in TNBC cells thereby validating its drug potential. **Conclusion:** This holistic investigation emphasizes the cancer therapeutic efficacy of berberine using network reconstruction, direct protein interaction with cancer proteins, and apoptosis induction in cancer cells. Although these findings emphasize the therapeutic efficacy of berberine experimental validation and clinical trials are required prior to the development of targeted and personalized drug which will likely enhance efficacy of breast cancer therapy.

KEYWORDS: Breast cancer, berberine, network pharmacology, molecular docking, apoptosis, ADME analysis, cell viability

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Corresponding Author Email: shenbhagaraman@gmail.com

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1. INTRODUCTION

Breast cancer, especially invasive ductal carcinoma remains the most primary and prevalent cause of cancer related deaths in women globally. [1] Even though screening, early detection and treatment has improved, breast cancer is still rising all over the world which clearly indicates its complex nature. One of the key developments for improving outcome is developing personalized therapy approaches including early tumor detection, real time disease tracking, and tailored treatments. [2] While targeted therapies changed modern oncology by focusing on molecular genetic targets; still breast cancer heterogeneity brings many challenges due to chromosomal variations, histo-pathologic diversity and various environment factors. [3]

Different types of breast cancer exist such as luminal A luminal B, HER2 enriched, normal like and triple negative (also called basal like). Each of them has unique biological features and distinct response to treatment. [4] Triple negative breast cancer (TNBC) makes up around 15 to 20 percent of all cases and is marked by aggressive tumors fast spreading, poor patient outcomes and fewer treatment options. [5] TNBC lacks estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which makes hormonal and HER2 based therapies useless. Although chemotherapy is still the standard care, resistance both internal and gained over time and the high systemic toxicity limits its usefulness. These limitations highlight an urgent need for new therapy that overcome resistance and provide multiple advantages.

Breast cancer especially TNBC is shaped by both inside and outside risk factors. Things like age sex hormonal fluctuations, mutations in genes (like BRCA1 or BRCA2), lifestyle choices such as diet, exercise and socioeconomic factors play a key role in disease susceptibility and severity. [6, 7] Also evidence from epidemiology shows that exposure to environmental toxic substances, metabolic imbalance from being overweight and long term inflammation can cause or worsen breast cancer. [8] As these risk factors become clearer over time, natural bioactive compounds have gained attention for therapy due to their safety profiles and range of useful biological roles. [9 10]

Berberine (BBR), an isoquinoline alkaloid derived from *Coptis chinensis* and plants of *Berberis* genus, is widely known for its varied pharmacologic benefits, like antimicrobial, anti-inflammatory, anti-diabetes and also antiobesity activities (Fig 1). [11,12] Recently, berberine’s anti-cancer potential has been explored due to how it can affect many different pathways that relate to cancer growth and spread. [13] It can inhibit cell proliferation by causing G1 or G2/M arrest and this happens through suppression of cyclins and CDKs, while promoting cell death through both outside and inside mechanisms. [14] This includes the increased Bax and p53 levels, reduced Bcl2 and activation of caspase 9 with caspase 3 [15]. Also berberine can reduce metastasis by inhibiting theg epithelial to mesenchymal transition (EMT), lowering MMP-2 and MMP-9 levels and blocking factors that support angiogenesis. [16]

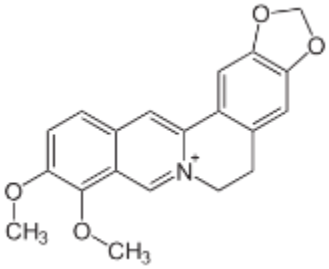


Fig 1: Structure of Berberine

Berberine affects a range of signaling routes like PI3K/Akt/mTOR, MAPK/ERK, NF-κBB and Wnt/β catenin which are all relevant in cancers. It also turns on AMPK, and this helps shut down mTOR which results in autophagy driven cell death especially when metabolism is stressed. [17] It also modulates the oxidative stress by boosting antioxidant defenses, protecting the mitochondria and lowering ROS buildup which stops DNA damage and tumour formation. [18] Furthermore it affects gut microbes, altering their proportion in ways that improves immune system activity and reduces inflammation signals linked to cancer formation. [19]

With recent improvements in computational biology, researchers now have new tools to study how berberine works at molecular level. Tools like molecular docking, dynamic simulations and network pharmacology enables detailed analysis of berberine's properties such as binding affinity to cancer targets, its absorption and behavior in body, and its potential interaction with other drugs. These computers based results support lab studies in cells and animals that confirm berberine's toxicity against TNBC, its influence on apoptosis and autophagy, and its power to stop cancer spreading and migration. Combining both computer modeling and lab work helps improve the chances of translating findings into real treatments.

This study aims to provide a comprehensive laboratory based and computational analysis of how berberine may be used against TNBC with the work flow diagram was presented in the fig 2. The main objective are to identify the molecular targets of berberine, understand its mechanism of action in human body and evaluate its overcome the drug-resistance. This study explores berberine's role in boosting cell death (apoptosis), controlling cell-cleaning process like autophagy, and stopping the spread of cancerous cells through metastasis, while also testing if it helps when used with current treatments. Because TNBC is a fast growing cancer with few reliable therapies, a compound like berberine that hits many biological path ways might become a helpful part of creating more personal cancer treatments.

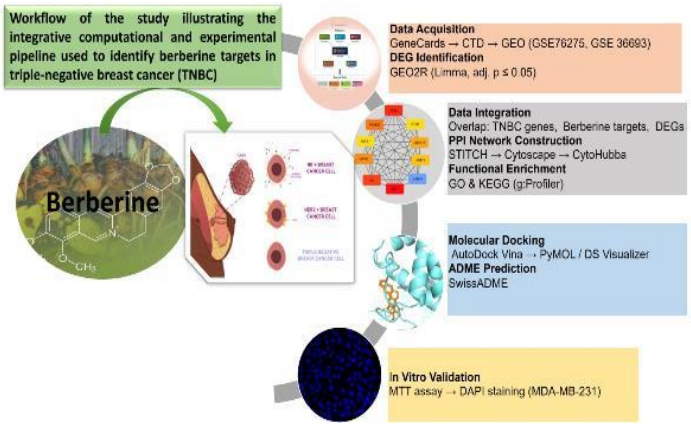


Fig.2 Work flow diagram for integrative computational and experimental pipeline

2. MATERIALS AND METHODS

Dataset used for the study:

All data used in this study were derive from publically available sources and confirmed to NPRS criteria and

FAIR data principle. Microarray dataset GSE76275 & GSE36693 was downloaded from the GEO data base (https://www.ncbi.nlm.nih.gov/geo/), berberine target were obtain from Comparative Toxicogenomics DataBase (CTD; https://ctdbase.org/), and TNBC related gene were retrieved the GeneCards database (https://www.genecards.org/). Protein-Protein and chemical - chemical interaction analyses were perform with help of STICH data base (https://stitch.embl.de/) . None of these dataset was generating specifically for this study and all this resources is freely accessible.

Target Identification, DEG integration and PPI network construction

Genes related to TNBC were down loaded from GeneCards database using filter set for relevant score, and berberine targets were retrieved from the Comparative Toxicogenomics Data base (CTD) by searching “berberine” & selecting curated chemical - gene interactions. At same time, microarray datas GSE76275, GSE36693 were processed using GEO2R under Limma package, where adj p-value ≤0.05 used to identify DEGs. Common DEGs from both the data set were selected for robust then cross checked with GeneCards TNBC genes and CTD berberine targets. Gene Intersection was mapped by FunRich v3 to find overlapped genes linking TNBC signature with berberin pharmacological footprints. Share genes were taken for protein– protein and chemical-protein interaction through STICH database at thresh hold of 0.08 and network interactions drawn into Cytoscape v3.5.1. Hub genes recognized through Cytohubba with topological parameters, while cluster dens areas captured through MCODE plug in (max depth=100, k score=2, node score cut off =0.2). This way, regulatory modules was could be pointed out.

Functional Enrichment Analysis

GO annotation and KEGG enrichment pathway were performed through g: Profiler (g:Gost). Genes were classified into three GO classes Biological process BP, Cellular Component CC, and Molecular Function MF. KEGG shows enriched signalling pathways TNBC. Benjamini–Hochberg corrections applied & those with adjusted-p values ≤0.05 considered significant.

Molecular Docking

Using AutoDock vina v1.20, molecular docking was conduct to estimate the binding affinity of berberin with certain hub protein (TP53, mTOR, Caspase3 and β-carotene binding protein).

Protein and Ligand Preparation

Protein crystal structures was download from Protein DataBank (PDB), and water molecule & hetero atoms where removed. Kollman charge added and polar hydrogen was appended with AutoDock Tools1.5.7. The 3-D structures of berberin were downloaded from PubChem, and MMFF94 force field was employ in OpenBabel for energy minimisations.

Docking parameters

Co-crystalized ligand binding site of each target protein or previously document active site, residues were employ to defined docking grid. For grid spacing 1.0Å and grid box size 40×40 ×40Å the grid centres was set at protein active site coordinate. Eight was exhaustiveness parameter, 3 kcal/mole was energy range and ten

binding mode was the numbers. It considered ligand as flexible while proteins were rigid

Verification & Display

Top ranking docking poses with least bind free energy (ΔG kcal/mol), were selected. PyMol and Discovery studio visualizer was use for analysis of hydrogen bond, hydrophobic interactions, and π-π interaction. Three docking per compound was done too ensure repetition.

ADME and Pharmaco kinetic prediction

ADME features of berberine were estimate by SwissADME webtool. Parameters such as molecular weight, TPSA, miLogP, Hbond donor/acceptors & rotatable bonds compare to Lipinski's rule of five. Intestinal absorption, brain barrier pass, Pgp substrate and CYP450 inhibit chances also predicted.

Compliance with Reporting Standards

All computational and network pharmacology analyses in this study were conducted under NPRS (2021-2023) from Chinese Pharmacological Society & International Network Pharmacology Society (INPS), as well as MINPS 2022 draft. Data sources (GeneCard, CTD, GEO, STICH & STRING) with thresholds, (adjust p ≤0.05, relevance score; confidence 0.08) were clearly defined. Software tool and their versions mentioned (FunRich, Cytoscape, Vina 1.2, SwissADME), and parameters standardized to make reproducibility. Study also follows FAIR principles (Findible, Accessible, Interoperable, and Reusable) by using document tools datasets workflows clear. Network topologies, (degree, betweeness, clusterings) done via CytoHubba, MCODE plug-in, validated with molecular dock and vitro cytotoxic tests, align with NPRS & MINPS guideline.

In vitro Analysis

Compound Handling

Berberine (≥98% purity) was purchased from Sigma Aldrich and stored cool, dry & dark. Stock made in DMSO then diluted with culture media at required concentrations. DMSO final concentration not exceeds 0.1%, with vehicle controls included.

Cell Culture

MDA MB231 TNBC cell line was obtained from NCCS Pune India. Cells were kept in DMEM plus 10% FBS and 1% antibiotic antimycotic, grown 37°C with 5% CO_2. Subcultures made at 80-90% confluence with trypsin EDTA, Cultures were checked for mycoplasma frequently. This study involves in vitro experiments using established human cell lines and does not include any direct human or animal subjects. Therefore, it is exempt from review by the Institutional Ethics Committee (IEC)

MTT Cytotoxic Assay

Cells (5×10^3/well) seeded into 96well plates then treated with berberine (10-100 µM) for 24 hr. After treatment, 20 µL MTT (5mg/mL) was added/well incubated 4 h. Formazan crystals dissolved in 150µL DMSO, absorbance measured 570nm. Cell viability calculated vs control and IC_{50} derived. All tests were done in triplicates for statistical significance.

DAPI staining

To examine nuclear changes, berberine treated cells were washed with PBS, harvested then stained with DAPI for 15min at room temp. Nuclear shapes observed under fluorescence scope for chromatin condensation

and fragmentation. This qualitative stain gave support evidences to quantitative MTT data.

Statistical Analysis

All experiment repeated thrice with triplicates. Data were written as mean ± SD. One-way ANOVA with Tukey posthoc used in Graphpad Prism v8. Significance $p < 0.05$ considered.

3. RESULTS

Identification of Consistently Dysregulated Genes and Overlap of Targets

Analysis of TNBC data sets GSE76275 & GSE36693 showed 125 genes that were consistently dysregulated, indicating strong transcriptional changes common in TNBC (Fig 3). At the same time the CTD search for berberine yielded 127 chemical gene interaction targets. When compared using Fun Rich, 27 overlapping genes were found between TNBC related list and berberine targets, suggesting possible candidates that link TNBC biology with berberine pharmacology. These 27 shared genes were ranked as important group for later protein-protein interaction, path way enrichment, and functional analysis.

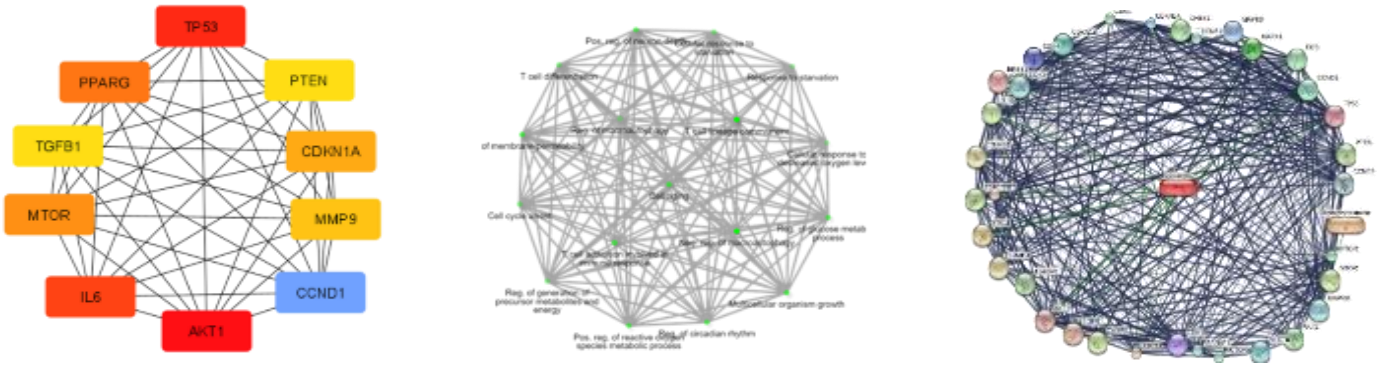


Fig 3: Gene inter action networks of overlap targets between berberine and TNBC, showing main regulatory genes along with their functonal link s and associations.

GO and KEGG Pathway Enrichment Analysis

GO analysis showed that up regulated DEGs were enriched in DNA replication, G2/M transition and mitotic spindle organization, while down regulated DEGs were mostly in immune response regulation, lipid metabolism and cell adhesion (fig 4 and Fig 5) . KEGG path ways enriched included PI3K Akt, p53 and MAPK signaling.

Fig 4: Venn diagram showing overlap between GeneCards derived TNBC related genes (125), CTD berberine targets (127) and the common intersecting genes. In total 27 overlap genes were found at the inter section, pointing to possible targets of berberine linked with TNBC patho biology

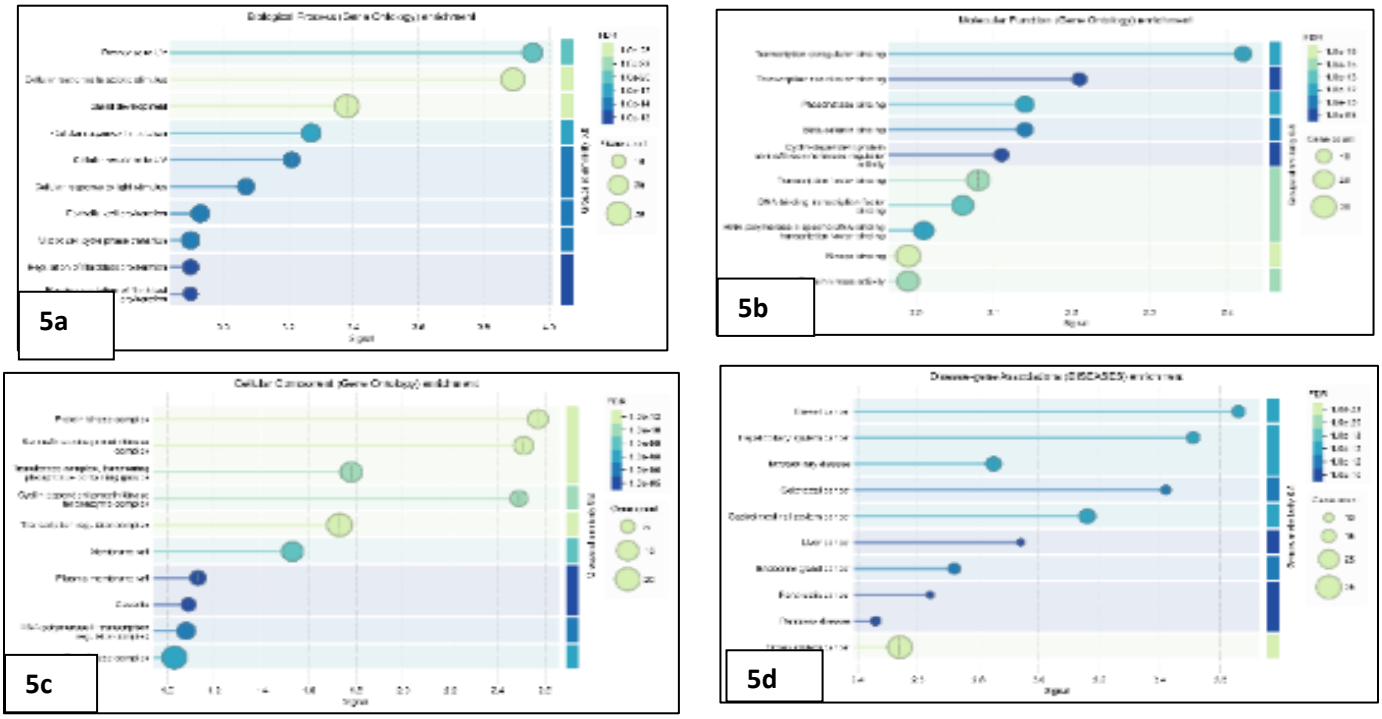
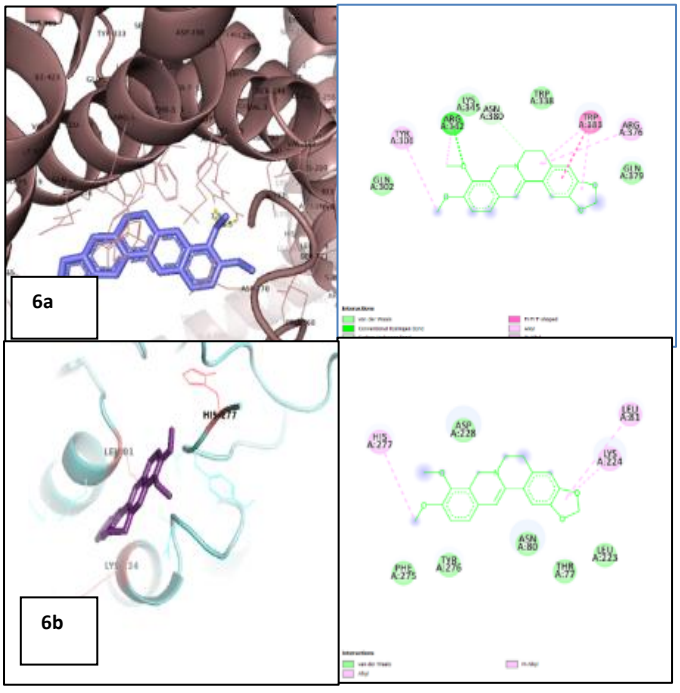


Fig 5: Gene Ontology (GO) and KEGG path way enrichment of the 27 over lapping targets . Bar charts shows significant (a) enriched biological process, (b) molecular functions (c) cellular components, and (d) pathways such as PI3K / Akt, MAPK and p53 signalings

Molecular Docking Analysis of Berberine with TNBC Targets

Molecular docking experiments were done to check the interaction between berberine and TNBC protein targets such as mTOR, TP53, caspase3 and beta carotene binding protein. Docking runs done by AutoDock Vina revealed binding scores −7.8 kcal/mol for mTOR, −6.8 kcal/mol for TP53, −6.8 kcal/mol for caspase3 and −5.8 kcal/mol for beta carotene binding protein (Fig 6). Hydro gen bonds and hydrophobic contacts at binding pocket checked in PyMOL, which supported berberine ability to bind key protein regions. The inter action nature indicate possible role in cell growth and apoptosis pathway modulations, agreeing with its known anti-cancer effect.



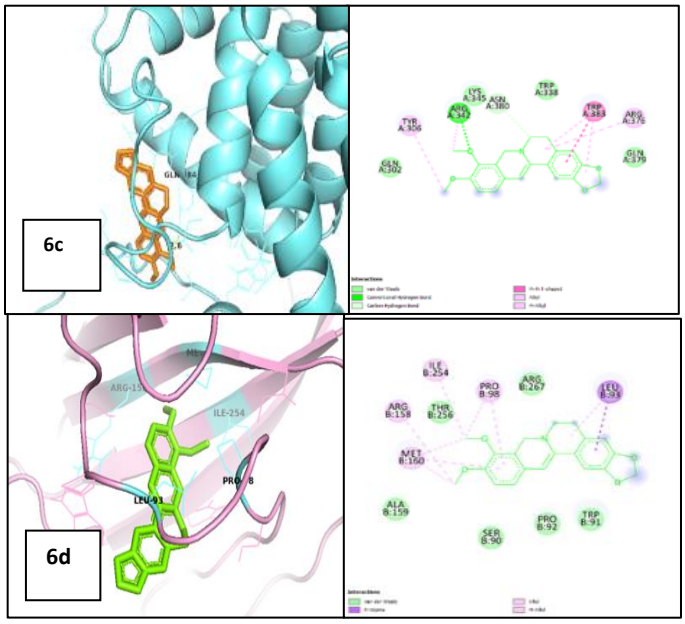


Fig 6: Moleculer docking inter actions of berberine with TNBC relatd targets . (a) mTOR, (b) TP53, (c) caspase3 and (d) beta carotene bindng protien, viewed in PyMOL / Discovery studio, showing hydro gen bonds plus hydro phobic inter action at the active binding sites

ADME and Pharmacokinetic profile of Berberine

Swiss ADME study showed berberine fits Lipinski rule of five, with high gastro intestinal absorption, poor brain barrier passing and few CYP450 effects. These findings, point to its drug likeness and translational potential.

In Vitro Cytotoxic Effects of Berberine in TNBC Cells

Berberine cytotoxicity was checked in triple negative breast cancer cell line MDA-MB231 using the MTT test (Fig 7). Berberine (10–100 µM) exposure for 24h caused dose dependent decrease in cell viable, with IC_{50} value 77.133 ±6.741 µM. Significantly lower cell numbers ($p<0.05$) were seen at ≥77 µM compared to untreated controls, showing berberine anti proliferative action.

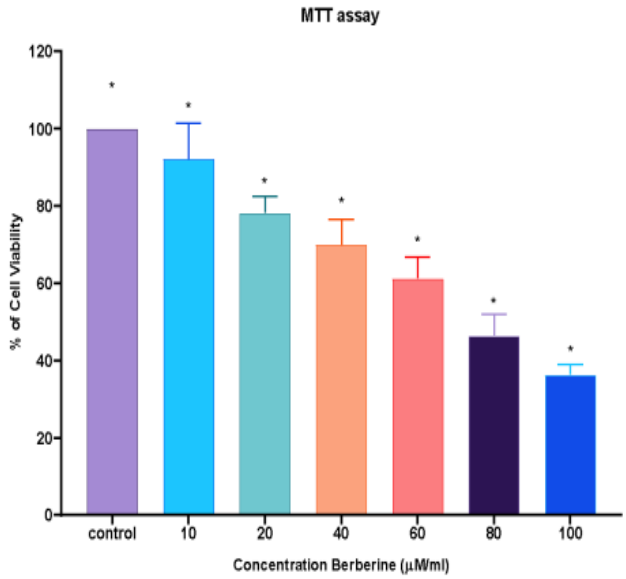


Fig 7: Dose-dependent cytotoxicity of berberine in MDA-MB-231 TNBC cells measured by MTT assay. Cell viability decreased significantly with increasing berberine concentrations (10–100 µM), with IC_{50} of 77.13 µM

Visualization of Cells through DAPI Staining

To further examine these changes on cell alterations after treatment, MDA MB231 cells were treated with berberine for 24h, collected and kept in ice cold PBS. Later the cells were resuspended in DAPI staining solution, and observed under fluorescence microscope (Fig 8). Berberine Treated cells showed strong nuclear fluorescence while control had weak signal, pointing nuclear damages typical of cytotoxic stress. Even without a detailed apoptosis assay, the DAPI staining gave morphologic evidence of nuclear alteration due to berberine.

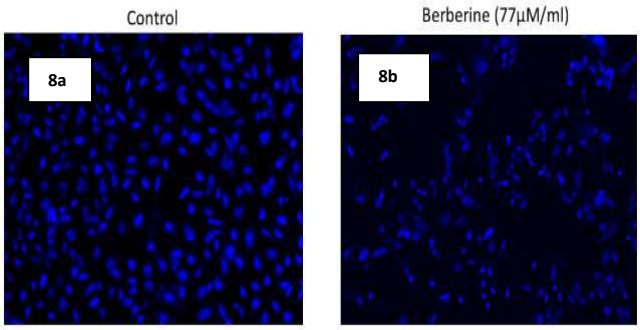


Fig 8: DAPI staining of MDA MB231 TNBC cells after 24 h berberine treatment. (b)Treated cells showed nuclear condensation and fragmentation compared to un treated controls (a), indicating nuclear damage linked with apoptosis

Discussion

Bioinformatics, molecular docking, pharmaco-kinetic prediction, and in vitro checking was all applied in this study to explain, berberine anti-cancer actions, on triple negative breast cancer (TNBC) . The multi layered approach showed that berberine binds with key protein targets, affecting many tumour pathways, and lowers TNBC cell viable. In line with earlier works, our finding agrees with that berberine anticancer effects come from complex molecular networks not just one mechanism. [20]

The exact ways by which berberine shows anticancer activity against many cancers, including breast cancer (BRCA), and TNBC are still unclear. [21] A total of110 overlap DEGs were found in TNBC by comparing two different data sets; most were up regulated enriched for mitotic progress and DNA replication, which match with the highly proliferative nature of this subtype. Importantly, 17 of these altered genes also matched with berberine targets, pointing that berberine may block transcriptional process, that enhance the tumor growth. Our data indicates that berberine blocks proliferative gene signature at transcriptome levels in TNBC, agreeing with reports that it reprogram oncogenic transcription by lowering DNA synthesis gene and altering cyclin expression.

With bioinformatics and docking investigation, 29 autophagy related genes regulated by berberine (AMGRBs) is identified in BRCA. These are included CASP3, MTOR, AKT1, GSK3B and PIK3CA. KEGG pathway analysis revealed EGFR inhibitor resist, PI3K/Akt and JAK STAT signaling, while functions annotation highlighted their role in autophagy and catabolic down regulation. Survival tests found ATM, HTR2B, LRRK2, PIK3CA, CDK5 and IFNG had prognosis value in BRCA, and docking confirmed strong berberine binding. [22] Together, our results indicate berberine has two anti-cancer actions: stopping proliferative transcriptional programs in TNBC and modulating autophagy signals in BRCA. This gives broader explanation for its potential therapeutic roles.

Our GO enrichment found down regulated DEGs linked to immune response, lipid metabolisms and cell adhesion, while up regulated ones enriched in G2/M transition, DNA replication and spindle organizations. PPI network also found main controllers of apoptosis and cell cycle check points. Such twofold pattern suggests TNBC driven by proliferative force plus weak immune and metabolic control. PI3K Akt, p53 and MAPK path ways again came by KEGG as key in TNBC. CHK1 and PLK1 were hub genes with poor prognosis, while GABPB1 and JUN stood as important upstream regulators, each hinting control over mitosis and

survival. [23] AKT mTOR path way, TP53, MAPKs and CDKs were all strongly linked in co expression data, supporting shared role in growths. So TNBC aggression, arise from survival and cell cycle driver activating hub regulator like PLK1 and CHK1. Berberine suppresses CDK, increase p21/p27, and triggers apoptosis by p53 activation, and Bcl2 down regulation as shown before. [24, 25] This leads to G0/G1 or G2/M arrest. Putting these mechanistic and network data together, berberine can targets hub regulators such as PLK1, and CHK1 to stop uncontrolled mitosis and induce apoptosis.

Enrichments also pointed PI3K Akt, MAPK, p53 dysregulations in TNBC; these pathways are central for growth and evasions of apoptosis. Nearly 60% of TNBCs show disruption in PI3K/Akt/mTOR, which block apoptosis, disturb autophagy and improve DNA repair, all supporting tumor survival and drug resistance. [26] Loss of p53 plus MAPK activation gives other escape path. Our results agree with studies that berberine shift balance towards cell deaths, by blocking PI3K/Akt, and restoring p53 mediated apoptosis. These findings show proliferation and apoptosis control converges in TNBC and can be evidenced further at molecular level.

Docking provide extra mechanistic view of how berberine controls TNBC growth and apoptosis. As seen earlier in breast cancer, berberine affects autophagy by hitting CASP3, MTOR, AKT1, GSK3B and PIK3CA. [22] Our data also showed strong bindings to mTOR, p53 and caspase3. By activating caspase and stabilizing the p53, berberine improves tumour suppressive and pro apoptotic processes while blocking PI3K/Akt/mTOR cascades. Based on enrichments and docking, berberine act as dual modulator in TNBC by slowing proliferations and encouraging apoptosis. This is similar with its reported, action linked to auto phagy and survivals in breast cancer.

Through AMPK, PI3K/Akt, MAPK and NFkB pathway change, berberine an isoquinoline alkaloids has many effects – anti diabetic, anti-inflammatory, anticancer and cardio protective. Moderate absorption, poor brain penetrations and CYP3A4 metabolism are seen in silico ADMET predictions with ADMETlab 3.0. This means possible drugs interaction but low toxicity. Its multi-targeting and antioxidant power strengthens protection against cancer and metabolic disorders. A drawback are weak oral bioavailability, which may refined by novel drug delivery. With broad activity and generally good pharmaco kinetics and safety profile, berberine may act as promising therapeutic drug. [27]

Natural compounds have been deeply studied as therapy, and cytokines driven inflammatory changes in tumour micro environment are linked to breast cancer growths. Plant derived berberine is an isoquinoline alkaloid used in Chinese, and North American traditional medicine for its anti-inflammation, anti-cancer and antioxidant value. In this study, we have combined *in vitro* tests with *in silico* tools including GO, PPI and dockings to check berberine impact on TNBC cell line MDA MB231. Our works showed dose dependent cytotoxic with IC_{50} 77.133 µM, similar to its effect in MDA MB468, and BT549 cells. Normal epithelial cells were, not affected by berberine toxic effect on cancer cells viability. [28] In scratch assay, it stopped migrations and slowed wound healing. In scratched cells, tests

showed low phospho of cJun and cFos, NFkB blocked via IkBα stabilizations, and TNFα, IL6 suppression under LPS induction. These finding suggest that berberine may treat aggressive breast cancer by blocking migration, growths and inflammatory signals.

Nuclear density also decreased after berberine treatment, which indicates higher death or lower growth. Altered nuclear morphology activates apoptotic, and cytotoxic process, matching known disruption of growths and survival signaling. These nuclear changes were consistent with microscopy and apoptosis in T47D and MCF7. Specifically, berberine induced G2/M arrests in T47D, and G0/G1 arrests in MCF7, while doxorubicin caused G2/M arrest in both. The combo of doxorubicin plus berberine showed stronger effect, proving berberine disrupts cell cycle depending on cell types. [29] Overall, these result confirms that berberine inhibit proliferation, induce apoptosis, and shift cell cycle in TNBC breast cancer cell, with nuclear changes as morphologic proofs of toxicity that need more preclinical investigations.

4. CONCLUSION

Our study shows that berberine exerts anticancer actions through network mediated way. Proliferative genes are suppressed at the transcriptome level; central signaling proteins are targeted at protein level; oncogenic changes are modulated at pathway level; pharmacologically it show good drug like character; and at cell level it cause cytotoxic and nuclear changes. In vitro study also confirms its ability to inhibit tumor growth, and extend survival. On the whole, berberine is promising drugs for TNBC, since our data show that it targets both survival and apoptosis regulatory mechanism.

Ethical Statement

This study involves in vitro experiments using established human cell lines and does not include any direct human or animal subjects. Therefore, it is exempt from review by the Institutional Ethics Committee (IEC) and Institutional Animal Ethics Committee (IAEC) as per the guidelines of the Indian Council of Medical Research (ICMR, 2017) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India).

Abbreviations with expansions

µM- Micromolar

AKT1- RAC-alpha serine/threonine-protein kinase

AMPK- 5' AMP-activated protein kinase

ATM- Ataxia-telangiectasia mutated (ATM) gene

BCL2- B-cell lymphoma 2

CASP-3- Caspase-3

CHK1 -Checkpoint kinase 1

CTD- Comparative toxicogenomics database

CYP3A4-Cytochrome P450 3A4

DEGs- Differentially expressed genes

DMEM-Dulbecco's Modified Eagle Medium

DMSO- Dimethyl sulfoxide

EGFR- epidermal growth factor receptor

ERK-Extracellular signal-regulated kinase cascade

GEO-Gene Expression Omnibus

GSK3B- Glycogen Synthase Kinase 3 beta

HER2-Human epidermal growth factor receptor 2

HTR2B - 5-hydroxytryptamine (serotonin) receptor 2B

IC_{50}- 50% inhibitory concentration

IL-6- Interleukin -6

INPS -International Network Pharmacology Society

LPS- Lipopolysaccharide

LRRK2- Leucine-rich repeat kinase 2

MAPK- mitogen-activated protein kinase

MTOR - Mammalian target of rapamycin

MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NCCS-National Centre for Cell Science

NFkB- Nuclear Factor kappa-light-chain-enhancer of activated B cells

PI3K/Akt- Phosphoinositide 3-kinase (PI3K)/Protein Kinase B (Akt)

PLK1- Polo-like kinase 1

TNBC- Triple negative Breast cancer cell line

TNF-α- Tumour necrosis factor alpha

Authors Details:

[1]Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai–602105, India

[2]bMolecular Nutrition and genomics Lab, Department of Community medicine, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai–602105, India

[3*]Department of ENT, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

Authors Contribution:

Conceptualization and clinical management: VKS, MP

Data collection and literature search: VKS, MP

Writing – original draft: VKS, MP, SR

Reviewing & editing: MP& SR

Approval of final manuscript: All authors

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Declaration of Generative AI

The authors declare this manuscript was written without the use of generative artificial intelligence tools. All the content, including text generation, data analysis and references was developed and reviewed by the author without assistance from AI technologies.

Conflict of Interest – The authors declare no conflicts of interest.

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