



A REVIEW ON ANTICANCER ACTIVITY OF BERBERINE AND ITS NOVEL DERIVATIVES

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ABSTRACT:

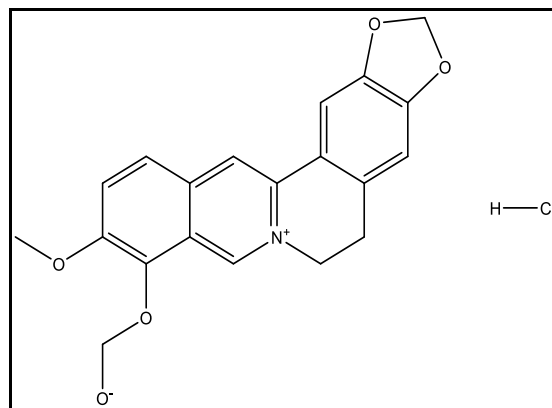
Berberine is a plant alkaloid and has been used since ages for treating innumerable health conditions as a part of Ayurvedic and traditional Chinese medicine. It has been proven to be a medicine with multiple uses, among them anticancer activity is of prime importance. Many researches have been conducted to study the anticancer activity on various cell lines namely hepatocellular carcinoma cell line, lung carcinoma cell line, mammary epithelial cell line, esophageal cancer cells, breast cancer cell lines, colon cancer cell lines, prostate cancer cell lines etc. This review has been carried out to scrutinize different assays that recounts berberine's anti-proliferative property, cell apoptosis, and cell cycle arrest caused in cancerous cells. Currently, novel derivatives of berberine are being developed which are suspected to be potent antitumour agents with less toxicological effects.

Index Terms: Berberine, anticancer, antiproliferation, cell cycle arrest, cell apoptosis, assays, cancerous cell lines.

I. INTRODUCTION:

Berberine, a quaternary ammonium salt, is discovered in the bark, rhizomes, stems, and roots of various plants including berberis, goldthread, Oregon grape, and barberry. The most popular type of berberine that is sold without the prescription is berberine hydrochloride (BBR HCl) which is a supplement encompassing the berberine component and HCl created to address various medical ailments. This substance is a member of the class of benzyloisoquinoline alkaloids. Due to the yellow tinge of Berberis species, they are employed as wood, leather, and wood dye.^[1] Berberine hydrochloride is essential for the metabolism of blood sugar (glucose) and cardiovascular health. It is an antimicrobial and anti-inflammatory drug used to treat bacteria, fungus, viruses, and other microbes and the people with gastrointestinal problems, diarrhoea, and bacterial overgrowth brought on by irritable bowel syndrome, ulcerative colitis, and other inflammatory bowel illnesses may be benefited by these properties of berberine. In addition, they are also used as antidiabetic agent to treat type-2 diabetes, cholesterol lowering agent to treat heart diseases, and to prevent recurrence of UTIs and cystitis.^[2] Owing to its well-known capacity to interact with nucleic acids, berberine hydrochloride's possible anticancer action has always been a topic of substantial research.

Structure



5,6-Dihydro-9,10-dimethyl benzo[g]-1,3-benzodioxole[5,6-a]quinolizinium hydrochloride

Anticancer mechanism of Berberine Hydrochloride

The antitumor effect of berberine is seen against lung cancer, cervical cancer, breast cancer, liver cancer, leukaemia, and other malignancies. It includes induction of apoptosis and cell cycle arrest, as well as inhibiting cell invasion and migration by modulating several pathways. Reactive oxygen species production, mitochondrial function, DNA topoisomerase inhibition, DNA or RNA binding, the oestrogen receptor, matrix metalloproteinase modulation, p53 activation, and NF-kappa B signal activation are some of the therapeutic targets of berberine hydrochloride.^[2,3] The antiproliferative activity of berberine is due to its capacity to bind specifically to oligonucleotides and stabilize DNA triplexes or G-quadruplexes by inhibiting telomerase and topoisomerase.^[3]

II. METHODS:

1. Lu Wang *et al.*, have reported the enhanced antitumor activity of berberine hydrochloride by solid lipid nanoparticle encapsulation on human breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HepG 2), human lung carcinoma cell line (A549), and human mammary epithelial cell line (MCF-10A) under the title “Enhancing the antitumor activity of berberine hydrochloride by solid lipid nanoparticle encapsulation”. They have developed a solid lipid nanoparticle (SLN)-based system for methodical incorporation and continuous release of berberine hydrochloride (BH). The cell viability was evaluated by MTT colorimetric assay where the decrease in yellow tetrazolium salt to purple formazan was detected. Here, MCF-7 cells (0.6×10^4), HEG 2 cells (0.8×10^4), A549 Cells (0.8×10^4), and MCF-10A cells (0.8×10^4) were added to a 96-well plate and incubated for 24 hr, following this the cells were treated with free BH, BH-loaded SLNs, and free BH spiked with blank SLNs at varying concentrations to get the IC₅₀ values and detected the cell viabilities using MTT medium. The formazan crystals which were formed were determined by a microplate reader at 570 nm after dissolving in dimethylsulfoxide. Flow cytometry was employed to check the cell cycle arrest caused by the formulated BBR on the cell cycle arrest. The study showed the anti-proliferation effect of MCF-7 cells, HepG 2 cells, and A549 cells by both free BH and BH-loaded SLNs. Furthermore clone formation, cellular uptake, cell cycle arrest, and cell apoptosis studies indicated the BH-induced SLNs enhanced antitumor activity on MCF-7 cells.^[4]
2. Ganesh Chandra Jagetia *et al.*, have reported the chemo-modulatory activity of *Alstoniascholaris* extract (ASE) in combination with berberine hydrochloride (BCL) in Ehrlich ascites carcinoma – bearing mice under the title “Effect of *Alstoniascholaris* in enhancing the anticancer activity of berberine in the Ehrlich ascites carcinoma bearing-mice”. They have combined 8 mg/kg of BCL with different doses of ASE in the range of 60-240 mg/kg and administered in tumour-bearing animals. It was observed that 180 mg/kg of ASE with 8 mg/mg of BCL showed the highest antitumor effect. Similarly, a dose-dependent increase in the anticancer efficacy was seen up to 8 mg/kg of BCL when 180 mg/kg of ASE was coupled with various dosages of BCL (2-12 mg/kg) whereas severe side effects appeared when the BCL dose was increased to 10 and 12 mg/kg. The

effective results were seen on combining 180 mg/kg of ASE with 6 or 8 mg/kg of BCL which showed increase in anticancer activity.^[5]

3. Shu-Xian Jiang *et al.*, have reported the antitumor effect of berberine on esophageal cancer (EC) cells under the title “Berberine displays antitumor activity in esophageal cancer cells *in vitro*”. They have conducted study on human ESCC cell line KYSE-70 and EAC cell line SKGT4 and reported that berberine induced strong growth inhibition of these cell lines at different concentrations (20, 40, 60 and 80 $\mu\text{mol/L}$) at different time (12, 24 and 48 h) where KYSE-70 cells were more liable to the inhibitory effects of berberine in a dose-dependent and time-dependent manner. To evaluate the cell viability, MTT assay was carried out where the KYSE-70 ($10^4/\text{well}$) and SKGT4 ($5000/\text{well}$) were added to a 96-well culture plates at 37°C for one night and then the cells treated with berberine HCl followed by addition of MTT dye (10 μL). After a period of 4 hr incubation, the blue formazan crystals were dissolved in DMSO (100 $\mu\text{L}/\text{well}$) and detected at 562 nm using Multiskan Spectrum microplate reader (Thermo Fisher Scientific). Flow cytometry was used to analyse the cell cycle distribution of KYSE-70 cells on application of berberine and it was seen that berberine caused cell cycle arrest in the KYSE-70 cells at G2/M phase of the cell cycle which may be due to regulation of cell cycle protein p21. In order to determine whether berberine’s antiproliferative activity was connected to its apoptotic effect, they treated KYSE-70 cells with 50 $\mu\text{mole/L}$ berberine and analysed using flow cytometry by double staining with Annexin-V FITC/PI. It was noted that the growth of treated cells was in a scattered way which resulted in loss of intercellular conjunction concluding that berberine increases apoptosis and decreases proliferation. Important targets of berberine activity includes AMPK activation and Akt-mTOR/p70S6K signalling pathway inhibition.^[6]
4. Iwona Popiołek *et al.*, have reported the enhanced anticancer activity of berberine (BBR) complexed with a cationic derivative of γ -cyclodextrin (GCD) modified with propylenediamine (PDA) in normal (NMuMG) and cancerous (4T1) murine mammary gland cells under the title “Cellular delivery and enhanced anticancer activity of berberine complexed with a cationic derivative of γ -cyclodextrin”. They have added PDA to GCD, creating an inclusion complex that is a successful carrier for the delivery of BBR. A cell proliferation assay called Crystal violet (CV) staining test was utilized to study the antiproliferative property of BBR complexes on NMuMG, B16-F10, and 4T1 cells by implanting the cells into 24-well plates. The BBB complexes were added, incubated for 24 hr, washed with PBS, fixed with 1 mL of 4% v/v formaldehyde/PBS for 15 mins, treated with CV solution and finally the absorbance was measured at 540 nm. Murine melanoma (B16-F10) and murine mammary gland cancer (4T1) were employed to study the anticancer activity of the GCD-PDA/BBR complex, with normal NMuMG cells serving as a standard for 4T1 cells. GCD-PDA was proven to be non-toxic for NMuMG and B16-F10 cells at concentrations up to 400 mg/L, but at concentrations over 200 mg/L for 4T1 cells, it reduced the fraction of live cells by roughly 25%. They have reported that up to about 300 and 150 μM , neither free BBR nor that complexed with GCD-PDA were toxic for NMuMG cells whereas both free BBR and the GCD-PDA/BBR complex were hazardous for the 4T1 cells. By complexing with GCD-PDA, free BBR’s toxicity for cancer cells (B16-F10 and 4T1) was increased. The complex’s efficacy against murine mammary gland cancer was selective because it was not harmful to healthy NMuMG cells. Consequently, the GCD-PDA/BBR complex can be viewed as a promising tool in the treatment of cancer.^[7]
5. K. V. Anis *et al.*, have reported the inhibition of tumours in mice produced by Dalton’s lymphoma ascites tumour cells by berberine in a dose-dependent manner under the title “Role of berberine as an adjuvant response modifier during tumour therapy in mice”. Using L929 cells, the cytotoxicity of berberine hydrochloride in culture was assessed. It was observed that in-vitro, berberine hydrochloride was only cytotoxic (44%) to DLA tumour cells at concentration of 1 mg/ml while it had a dose-dependent cytotoxic effect on DLA cells at lower doses. It also showed cytotoxicity to L929 cells in vitro at lower concentrations and nearly 40 mg/ml of berberine hydrochloride was required to cytotoxicate L929 cells in culture by 50%. Elevation in the life span of mice ($P < 0.001$) by 32% was seen after administration of 10 mg/kg berberine hydrochloride intraperitoneally although, a small increase in the life span of tumour-bearing mice was noticed on oral administration even at 25 mg/kg which caused only 5% increase in the life span. A marked synergistic effects of berberine hydrochloride in conjugation with cyclophosphamide, radiation and hyperthermia lead to the reduction in the volume of solid tumours of mice. Thereby stressing on the fact that berberine acts as an adjuvant response modifier in chemotherapy.^[8]

6. Zhi-Cheng Wang *et al.*, have synthesized novel 9,13-disubstituted berberine derivative and evaluated their antiproliferative activities against human prostate cancer cell lines (PC3 and DU145), breast cancer cell line (MDA-MB-231) and human colon cancer cell lines (HT29 and HCT116) under the title “Synthesis and anticancer activity of novel 9,13-disubstituted berberine derivatives”. The study shows that the synthesized 9, 13-disubstituted berberine derivatives have intensified antiproliferative properties when compared to berberine and addition of lipophilic groups at positions 9 and 13 promoted anticancer activity. To analyse the antiproliferative properties of compounds 15a-b and 18a-g, SRB assay was carried out on PC3, DU145, MDA-MB-231, HT29, HCT116 and human aortic fibroblasts (HAF) taking berberine as a positive control and calculated using Graphpad software where the results were expressed as IC₅₀ values. On administering these compounds, highest cytotoxicity was seen in PC3 cells whereas insensitivity in case of MDA-MB-231 cells. Among them compound 18e was more potent with intense antiproliferative property and acceptable selectivity as well as inhibition of cell migration and colony forming at low concentrations. Furthermore, it also arrests the cell cycle in the G1 phase and could also induce cytoplasmic vacuolation which is quite dissimilar to the berberine.^[9]
7. Elisa Pierpaoli *et al.*, have reported the novel mechanisms involved in the anticancer effects of berberine (BBR) and also demonstrated the greater effectiveness of NAX012 and NAX014 analogs in inducing apoptosis and cellular senescence in HER-2/neu overexpressing tumour cell lines (breast cancer cells) under the title “Antitumor effect of novel berberine derivatives in breast cancer cells”. In order to assess the antiproliferative property of berberine derivatives on breast cancer cells, Alamar Blue assay was carried out to determine the viability of treated cells followed by supplementing HER-2/neu-overexpressing SK-BR-3 cells with BBR, NAX012, NAX013, NAX014, or NAX035 over a concentration range of 0.4 to 50 µM for 24, 48, and 72 hr. In cells treated with BBR, dose- and time-dependent inhibition of cell viability was observed, whereas, the cells were found to be more sensitive to NAX014 treatment when compared to that on BBR-treatment with IC₅₀ value at 72 hr of 26.5±2.2 µM and 36.0±1.8 µM, respectively ($P < 0.05$). NAX012 and BBR showed similar effects at same time points. Flow cytometry was employed to measure the apoptosis of human breast cells induced by NAX compounds after exposing the cells to 50 µM of BBR, NAX012, or NAX014 at 24, 48 and 72 hr. The cytotoxic effect was noted in BBR-treated SK-BR-3 cells which experienced apoptosis in a time-dependent manner (44% dead cells) with increase in number of apoptotic cells starting from 24 hr time-point while the NAX012 (71.6% dead cells) and NAX014 (68.4% dead cells) treated cells experienced elevation in the number of dead cells on increasing the time of exposure. They have also studied the effect of BBR and NAX compounds on senescence-like growth arrest by measuring p53, p21^{WAF1}, p16^{INK4a}, and PAI-1 mRNA expression in SK-BR-3 treated cells. BBR treated cells showed rapid increase in the p16 and PAI-1 mRNAs on treating with 100 µM BBR for 24 hr and PAI-1 remained elevated over 24 hr treatment followed by increase in p53 and p21 mRNAs only after 48 hr treatment. Likewise, NAX012 and NAX014 showed the same effect as BBR but without increase in PAI-1 over 24 hr treatment. p53, p21^{WAF1} and p16^{INK4a} may be important mediators of BBR- and NAX compounds-induced senescence. The study concluded that derivatives of BBR i.e., NAX012 and NAX014 exhibited more cytotoxicity on human breast cells than BBR and a decrease in HER-2/neu expression was linked to both apoptosis and senescent-like growth arrest in SK-BR-3 treated with either BBR or NAX drugs.^[10]
8. Viktor Milata *et al.*, have synthesized a series of 4 novel 9-*O*-substituted berberine derivatives with improved antiproliferative and apoptosis-inducing activities on human HeLa (human cervical cancer) and HL-60 tumour cell lines under the title “Synthesis and anticancer activity of novel 9-*O*-substituted berberine derivatives”. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to test the antiproliferative activity of berberine derivatives and measured as half-maximal inhibitory concentration, which is an assessment of how well a substance inhibits cell growth as well as viability of cell. Compared to berberine, increase in antiproliferative activity of derivatives 1-4 was observed when tested on HeLa and HL-60 tumour cell lines. The IC₅₀ values for berberine derivatives 1-4 ranged from 95.0 to >200 µM for HeLa cells and from 11.2 to 23.4 µM for HL-60 cells, indicating cytotoxic action. Berberine derivative-4 showed the lowest antiproliferative effect, with IC₅₀ values ranging from 15.7 to >200 µM whereas highest antiproliferative effect was exhibited by derivative-3 with IC₅₀ values of 58.0 and 36.0 µM for HeLa cells and 3.7 and 0.7 µM for HL-60 cells treated for 24 and 48 hrs, respectively. The ability of these derivatives to induce apoptosis were compared by using DNA laddering and caspase-3 activation assays and only HL-60 cells treated with berberine derivative-3 were positive for

apoptotic DNA fragmentation and had active caspase-3 after being incubated with 10 μ M doses of berberine derivatives for 48 hrs. Derivative-3 also showed 30-fold superior antiproliferative activity than berberine on treating the HL-60 cells with 30 μ M berberine and 10 μ M derivative-3. It was also reported that derivative 1, 2 and 3 arrests the HL-60 cells at the G₂/M phase of the cell cycle with a corresponding decline in the number of cells in G₀/G₁ and S phases on the other hand derivative-4 showed cell cycle arrest in G₂/M and S phases. Derivative-3 exhibited 6-fold increase in the number of cells than berberine. Thus concluding that derivative-3 is a potent candidate for anticancer therapy with highest cytotoxicity and apoptosis-inducing potential.^[11]

9. Elizaveta D Gladkova *et al.*, have discovered new type of berberine derivatives with a fused sultone ring which were obtained by reacting berberrubine with sulfochloride as well as with classical sulphonates and tested their inhibitory activity against Tdp1 along with cytotoxicity on HeLa (human cervical cancer) cell line under the title “Discovery of novel sultone fused berberine derivatives as promising Tdp1 inhibitors”. Sultones and their 12-brominated analogues were checked for their inhibitory activity against DNA-repair enzyme tyrosyl-DNA phosphodiesterase (Tdp1), a promising target for antitumor therapy. The reaction was carried out at micromolar or submicromolar concentrations with a reaction mixture containing Tdp1 buffer, 50 nM biosensor, and an inhibitor being tested and POLARstar OPTIMA fluorimeter was used to measure fluorescence every 55 s (ex. 485/em, 520 nm) and all the above synthesized compounds were found to be active against Tdp1. EZ4U Cell Proliferation and Cytotoxicity Assay was carried out to scrutinize the cytotoxic effect of synthesized compounds on HeLa cell line. Due to its superior Tdp1 inhibition, non-toxicity, and enhancement of topotecan’s cytotoxic action on HeLa cells, compound 18c can be regarded as lead compound.^[12]
10. Akio Hoshi *et al.*, have reported the anticancer activity of berberine, berberrubine, and their derivatives on sarcoma-180 ascites using total packed cell volume method under the title “Antitumor activity of berberrubine derivatives”. In total packed cell volume method, a female mice was intraperitoneally administered with 10⁷ cells of seven day old sarcoma-180 ascites and after 24hrs berberine or berberrubine derivatives were administered intraperitoneally at 30, 10, or 3 mg/kg dose whereas for tetrahydroberberine derivatives 100, 10, or 1 mg/kg doses were administered and results were noted after a week. The results obtained indicated that 13-alkyl-, 13-hydroxy-berberine derivatives and tetrahydroberberine derivatives have no antitumor activity, while berberrubine (9-dimethyl berberine) and esters of berberrubine have excessive antitumor activity. The relative ED₉₀ for berberrubine, its acetate, and its benzoate were 15, 23, and 44 mg/kg whereas LD₅₀ were 100-141, 174-200, and 150-216 mg/kg, respectively. According to the TPCV technique, these substances had the following therapeutic indices (LD₁₀/ED₉₀): berberrubine hydrochloride (6.7-9.4), 9-acetyl-9-dimethyl berberine (9-acetyl berberrubine) (7.6-8.7), 9-benzoyl-9-demethyl-berberine (9-benzoyl berberrubine) chloride (3.4-4.9). It is possible to draw the conclusion that one of the chemical structures with anticancer activity is of the berberrubine type.^[13]
11. Scordino A. *et al.*, have reported the cell death induced by berberine hydrochloride using extra vesicles under the title “Delayed luminescence to monitor programmed cell death induced by berberine on thyroid cancer cells” and is described as follows: Firstly the preliminary experiments were carried out using two types of cell cultures namely 8305C cell lines and FTC-133 thyroid cancer cell lines for which MTT analysis was carried out. The cell lines were exposed to different concentrations and the effect was dependent on the duration of cell treatment and the dose. So further to analyse the apoptosis, the TUNEL assay was carried out and the cell lines treated with berberine were compared with untreated ones and thus evaluating the anti-cancer activity of berberine. Thus, these test results showed that the cells with berberine treatment successfully avoided the cell cycle arrest and inhibited the tumour formation in thyroid cells when compared to untreated cells. Thus the study enables us to understand the delayed luminescence of both FTC-133 and 8305C cell lines showing anticancer activity of berberine using thyroid cell lines showed accurate and precise results of berberine.^[14]
12. Ortiz LM. *et al.*, have reported the anticancer activity of berberine hydrochloride using colon cancer cells with the title “Multiple derivatives of Berberine derivatives on colon cancer cells” and is described as follows: Berberine and its derivatives like NAX012, NAX014 & NAX018 were designed, synthesized and characterised by Naxospharma. The human colon cells and fibroblast were grown in Eagle's medium and some drug supplements were given. Morphology analysis, Cell cycle analysis, Western blotting technique were performed. By MTT analysis the NAX012, NAX014 and NAX018 were administered to the cells which were grown for about 24 hrs in a

medium. It was seen that berberine was ineffective on cancer cells but after administering NAXs they altered or inhibited the cell viability. The immunofluorescent test was carried out using paraformaldehyde and the images were captured with a digital camera. So the current experiment helps to study the effect of berberine along with its derivatives on human colon cancer cells effectively and accurately.^[15]

13. Salek A. *et al.*, have reported the antitumor effects of berberine hydrochloride using extra vesicles with the title “Enhancement of the in vitro antitumor effects of berberine chloride when encapsulated within small extracellular vesicles” and is described as follows: The small extracellular vesicles were taken from immature dendritic cells and were loaded with berberine. Proliferation was carried on both free berberine and berberine loaded-small extracellular cells. So the berberine loaded cells were 4 times more potent than free berberine. Also cell cycle analysis and western blotting techniques were carried out on breast cancer cells to analyse the anti-tumour activity of berberine. It was seen that the berberine loaded small extracellular cells reduced the formation of small capillary like structures and also inhibited NO release. The berberine mainly inhibited the two main processes angiogenesis and tumorigenesis and showed the anti-cancer activity accurately. So thus using small immature dendritic extracellular cells this study will give the accurate, precise information about anticancer activity of berberine.^[16]
14. Li C. *et al.*, have reported the physiological impact of berberine in nasopharyngeal carcinoma cells with the title “Berberine Hydrochloride Impact on Physiological Processes and Modulation of Twist Levels in Nasopharyngeal Carcinoma CNE-1 Cells” which is described as follows: The nasopharyngeal carcinoma cells were cultured in DMEM medium supplied by penicillin-streptomycin and kept at 37 °C. The wound healing analysis was carried out with introducing uniform wounds and scratches were made and two concentrations of berberine were replaced and the scratches were evaluated. Next RT-PCR was carried out using Trizol reagent with different series of conditions. Further western blotting technique was carried out wherein the cells were washed with a buffer containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS 50 mM Tris-Cl with pH 7.4. And were incubated on ice for 30 min. The result was detected by the Bio-Rad ChemiDoc XRS method. Through cytometry analysis the apoptosis detection was carried out of berberine. So the berberine with different concentrations inhibited the proliferation of CNE-1 cells and also seen that by increasing the concentration the intensity of result was also increased. So in this study at very small concentrations the shorter suppression of proliferation of CNE-1 cells and inducing apoptosis was seen. So it concluded that berberine showed anti-cancer activity effectively on nasopharyngeal carcinoma cells in all conditions which was demonstrated by performing different experiments accurately and precisely.^[17]
15. Seo Y *et al.*, have reported anticancer activities of berberine using neck carcinoma cells with the title “Berberine-induced anticancer activities in FaDu head and neck squamous cell carcinoma cells” which is described as follows: FaDu cells were given by American Type Culture Collection (ATCC) and were kept in a medium containing fetal bovine serum which were cultured in 96 culture plates for 24 hrs after which they were treated with berberine and other essential chemicals. To check the apoptosis these cells were added in 6-well plates and incubated for 24 hrs then treated with berberine. Cells were collected and washed with PBS and were analysed. Further western blot technique was carried out using a buffer containing protease and phosphatase inhibitors. Migration analysis along with gelatin zymography assay was carried out to analyse the activity of the drug. The viability of berberine was tested along with MTT to analyse the cytotoxicity. Thus the berberine was cytotoxic for FaDu cells depending on concentration. Test was made to analyse whether berberine reduces the phosphorylation of MAPK in FaDu cells thus the drug suppressed the phosphorylation of MAPK in FaDu cells. So thus the study showed the anticancer activity with apoptosis, viability, western blotting and cytotoxic analysis of berberine in FaDu cells very efficiently and effectively.^[18]
16. Pierpaoli E. *et al.*, have reported the antitumor activity of berberine using breast cancer cells under the title “Antitumor activity of NAX060: a novel semisynthetic berberine derivative in breast cancer cells” which is described as follows: The berberine was supplied by Naxospharma and the derivatives like NAX060, NAX103 and NAX114 were used. Further cell viability analysis was carried out using 96-well culture plates using Alamar Blue Solution. To determine apoptosis and cell cycle determination the cells were treated with trypsin, ice-cold ethanol and incubated for some time. So results were noted by counting the number of hypodiploid cells in the sub-g1 region. Western blotting technique was carried out using a buffer containing 150 mM NaCl, 50 mM Tris-HCl and 0.1% SDS. Antibodies were diluted in 3% skimmed milk. Thus the intensity was measured

- individually and reported. The NAX060 showed inhibition of cell viability. Cells exposed to NAX060 were also analysed. This study enables us to understand the activity of berberine using the NAX060 derivative in breast cancer cells effectively.^[19]
17. Szeto S *et al.*, have reported the anticancer activity of berberine in human cancer cells under the title “Characterization of berberine on human cancer cells in culture” which is reported as follows: The stock solution was prepared dissolving berberine in PBS solution. Exclusion assay was carried out using trypan blue to study the cell viability. Cells were firstly washed with PBS then mixed with different concentrations of berberine. After which they examined the viable and non-viable cells and reported the same. At 37°C comet assay was carried out using different concentrations of berberine and mixture of 1% low MP Agarose gel and thus results were noted. Along with concentration the cell viability was decreased in berberine treated compared to untreated. Generally no apoptotic cells were seen but after 20 hrs treatment few cells were seen. Thus this study showed cell viability, cytotoxicity along with the anticancer activity it enables us to study the activity in human cancer cells in a very effective manner.^[20]
 18. Yip Novia *et al.*, have reported berberine induced apoptosis in liver cancer cells under the title “Berberine induces apoptosis via the mitochondrial pathway in liver cancer cells” which is described as follows: The berberine chloride was supplied by Sigma-Aldrich Inc. and the WRL68 and Huh7 liver cells were grown in a medium RPMI-40 and 10% FBS medium. Then cells were washed and centrifuged further used for MTT assay. 96-well cultured plates were taken and MTT solution was added. Further DMSO was added after incubation for 4 hrs. While testing the apoptosis the Huh7 cells were treated with different concentrations of berberine for 24, 48, 72 hrs. After which they were harvested with trypsin and followed by staining. In western blot technique the Huh7 cells were seeded in culture dishes and further treated with berberine and incubated for 72 hrs. These were treated with buffer and blocking solutions, further washed with TBST solution. So results were seen that the viability of cells were determined by MTT assay compared to normal cells. It was seen that berberine-treated Huh7 cells showed less viability after incubation. Also it was seen that the Huh7 cells induced apoptosis in liver cancer cells. Thus this study enables us to study the viability, apoptosis, toxicity and the anticancer activity of berberine chloride using liver cancer cells in a very effective manner and also results were up to the mark.^[21]
 19. Tuguzbaeva G. *et al.*, have reported berberine induced anticancer effects under the title “Berberine-induced anticancer effects in squamous cell carcinoma of oral tongue cells” which is described as follows: The berberine was supplied by SIGMA and the isoquinoline alkaloid was dissolved in DMSO to prepare stock solution. The SCOTT cell line and CAL-27 cell lines were purchased and stored in 10% bovine serum. 96-well plates were taken and CAL-27 cells were seeded and incubated. Berberine was added in three different concentrations i.e., 1, 10 and 100 µg/ml. The optical density was determined to study the cell viability. Boyden chamber cell invasion assay was carried out using FBS- free medium and incubated for 24 hrs. Invaded cells were treated with 4% paraformaldehyde solution and non-invaded cells were removed by cotton swab. So the berberine-treated CAL-27 cells were having decreased cell density after evaluation. These also inhibited oral cancer cell proliferation. Depending on dose the cell viability test showed that berberine treated cells inhibited cancer growth. Thus this study enables us to study the anti-cancer activity of berberine using oral tongue cells effectively.^[22]
 20. Lin Hong. *et al.*, have reported photocytotoxicity of berberine under the title “Synthesis and in vitro photocytotoxicity of 9-/13-Lipophilic substituted berberine derivatives as potential anticancer agents” which is described as follows: The chemicals were supplied by Sigma-Aldrich. Three human cancer cells were used to test the cytotoxicity. Chemically after increase in alkyl chain length the anticancer activity in the cells was also increased. The potential side effects were also checked by treating MDCK cell lines with berberine and no side effects or cell death was seen. Proliferation assay was carried out to determine photo cytotoxicity in HepG2 cells which were exposed to 420 nm visible light in presence of different concentrations of berberine. So the growth inhibition was seen in concentration and dose-dependent manner. After this to determine the cell viability the MTT assay was carried. Further apoptosis was checked and these cells induced the apoptosis. Thus this study enables us to understand the anticancer activity of berberine by studying photocytotoxicity, cell viability, and side effects along with proliferation study effectively and accurately.^[23]
 21. Shi-Hai Yan *et al.*, have reported anticancer activity of berberine hydrochloride using colorectal adenoma with the title “Chemoproteomics reveals berberine directly binds to PKM2 to inhibit the progression of colorectal cancer”. For undeviating study of ligands that bind proteins in cells and

tissues, a biophysical method called thermal transfer assay is used. Multiplication of colorectal cancer cells HT29 and HCT116 with EC50 of 4.62 mM and 9.39 mM are inhibited by berberine *In vitro*. Determination of berberine bound with PKM2 of HT29 cells nurture with berberine (5 mM) for 30 min and by bioinformatics examination, four types of proteins were recognized namely H6PD, PKM2, MBP-1, and GAPDH and to fabricate pharmacological effects, berberine can directly bind to PKM2. For *in vitro* observation of the activity HT29 cells were used and too for protein, and gene levels of PKM2 in the presence of berberine. The structure of 3gr4 was enlarged by homology modeling with 4jpg as a template on the SWISS-MODEL server and Western blotting showed that PKM2, p-STAT3, Bcl-2, and Cyclin D1 were significantly decreased in HWT HT29 cells after berberine intervention. The EC50 values to inhibit tumour cells were 4.71 and 1.59 mM, respectively (Figures S17B and S18B). Compared with berberine (4.62 mM), the biological activity of p-aminobenzoic acid-berberine ester was improved to some extent. Berberine is used in traditional Chinese medicine and has no side effects and used for prevention and treatment of disease.^[24]

22. Lei Zhu *et al.*, have reported anticancer effects of berberine hydrochloride using colon tissues with the title “Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats”. 60 healthy male wistar rats weighing 200–230 g were purchased from Yangzhou University’s laboratory animal center and by administering 5% DSS in the drinking water for 7 days. After being conducted by berberine hydrochloride and from blood circum after the reperfusion, the whole blood samples were obtained. By using ELISA and PCR assay, the expression of cytokines were intended. At 12,000g for 10 min at 4°C, the blood samples were deviated and at 450 nm, the absorbance was calculated. By using SPSS 17.0 software, the results were examined and conveyed as mean ± standard deviation for *P < 0.05, **P < 0.01. P < 0.05 and were the significant differences. The rats showed increased DAI scores when the rats collected DSS administration and weight loss with a loose stool, depletion in daily tasks, anorexia, colourless / dull hair colour were seen in rats. All the results conclude that DSS-induced colitis in rats can be averted by using berberine hydrochloride and for treatment of ulcerative colitis it will be promising and functional approach.^[25]
23. Shuang Guo *et al.*, have reported antitumor activity of berberine hydrochloride using human colon cancer cells by the title “Solid dispersion of berberine hydrochloride and Eudragit® S100: Formulation, physicochemical characterization and Cytotoxicity evaluation”. By using MIT assay in human colon cancer cells, the *In-vitro* cytotoxicity studies were examined. 97.0% of pure berberine hydrochloride was prepared in the laboratory and at a mass ratio (1:2, 1:4, 1:6 and 1:8) HB-S100’s solid dispersion were processed. For 25-35 min at 45°C, at various ratio’s HB and S100 were liquefied into 95%-ethanol stirred using a magnetic stirrer and for 48 hours at a temperature of 40°C, HB and S100 were dried. By calibration curve: $Y = 70296 X + 52314$ and $R^2 = 0.99948$ of HB-S100 solid dispersion having berberine hydrochloride was premeditated. DIKMA Diamonsil-C18 column (200mm×4.60mm, i.d., 5µm particle size) (200 mm × 4.60 mm, i.d., 5 µm particle Size) was used for the observation of berberine hydrochloride content. Mobile phase mixture of acetonitrile: phosphate buffer (40:60 v/v) and has 1.0 mL/min of flow rate and 8 min is the retention time of berberine hydrochloride at a temperature of 25°C. At DMEM medium, Caco-2 HCT116 and SW480 which are human colorectal cancer cells are grown and cultured in MEM. FT-IR investigated the interconnection between HB and S100. Finally, the berberine hydrochloride is used for its anticancer effectiveness and used for colon cancer therapy and prevention.^[26]
24. Sunil Pandey *et al.*, have reported anticancer activity of berberine hydrochloride using folic acid with the title “Biogenic gold nanoparticles as fotillas to fire berberine hydrochloride using folic acid as molecular road map”. HAuCl₄, (BHC), (DMSO), n-hydroxysuccinimide (NHS), dicarboxy Aminocarbodimide (DCC) and (TEA) and folic acid are used in experiment and Nanopure water of 18 MΩ is used and in 20 ml of nanopure water 10 g of T. bispinosa peel was compressed dark pink colour is obtained in solution. For 15 min at 5000 rpm the solution was separated and to get a very clear light pink colored solution ultra-filtration having 0.22 µ filter is used. The molar ratio of GNP-FA: BHC: TEA is (1:1:2) and in a still environment for 4 hours, at 28 ± 2 °C the mixture is agitated. By using MTT assay, cytotoxic consequence of the BHC and GNP-BHC conjugate was deliberated Using Vero and folate receptor expressing HeLa. At 526 nm, a sharp peak appeared. 86 % is the BHC loading capacity of gold nanoparticles and BHC liberates pH 5.8 which is acidic. On the surface of GNP, Drug loading efficiency of BHC was estimated to be 86 % and BHC remains to be anchored on the surface of GNP at a pH of 7.2 and stays for a longer period of time. GNP-FA-

BHC associate is a suitable therapeutic effect in cancer cells showing the effectiveness of biogenic gold nanoparticles.^[27]

25. Fang Bai *et al.*, have reported the antitumor activity of berberine hydrochloride using rabbits under the title “Berberine hydrochloride inhibits inflammation and fibrosis after canalicular Laceration repair in rabbits” which is described as follows: 30 healthy male adults New Zealand white rabbits having body weight of 2.5–3.0 kg were used from research. Animals are nurtured at a room temperature of 20–25°C and a relative humidity of 40–70% with a light or dark cycle of 12 hours. Rabbits are assigned randomly in 6 groups. The rabbits were provided food for 7 days and 4 times a day; 30 ml of solutions were dispensed 30 µl. Before the 7 days of surgery, the rabbits are slaughtered. In Image-Pro Plus 6.0 software, Fibrosis and collagen content were examined. p-P38/P38 and p-ERK/ERK signaling pathways play a major role in reciprocation of inflammation. By using the BCA protein Assay kit, protein concentration was insisted. On the P38 pathway according to our data, substantial response is shown by berberine. Signaling pathways of TAK1/JNK and TAK1/NF-κB get involved in intestinal post-operative inflammation and adherence. The P38 pathway is associated with a diversity of inflammatory responses and pathological fibrosis procedures. After canalicular repair abscission, anti-inflammatory and anti-fibrosis effects of berberine were appeared in rabbits.^[28]
26. Xin Jin *et al.*, have reported Anticancer Activity of berberine hydrochloride by derivatives of phenyl-substituted berberine triazolyls on MCF-7 (breast), SW-1990 (pancreatic), and SMMC-7721 (liver) and the noncancerous human umbilical vein endothelial cell (HUVEC) under the title “Design, synthesis, and anticancer activity of novel Berberine derivatives prepared via CuAAC “click” Chemistry as potential anticancer agents”. Reactions were observed on analytical thin-layer chromatography utilizing silica gel 60 F254 plates and at 254 nm of ultraviolet light irradiation, the spots were envisaged. The concoction was agitated for 3 hours at room temperature, filtered, and then evaporated under vacuum, observed by TLC. The primitive mixture is decontaminated by column chromatography to give pure compounds 4–36 in 50%–90% yield. HUVEC cell lines were calculated by an assay was based on the cleavage of the yellow tetrazolium salt MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich) to configure purple formazan crystals in viable cells. Each experiment was carried out at the minimum in triplicate and by mean ± standard deviation, the data are introduced. Probability values of P<0.05 of mean values were considered as statistically remarkable. By using CuAAC “click” chemistry the establishment of a new sequence of triazole-contain inactive anticancer compounds. Active anticancer compounds manifested lower cytotoxicity to noncancerous HUVEC cells contrasted with berberine.^[29]
27. Chih-Yu Lo *et al.*, have reported Antitumor activity of berberine hydrochloride by lipophilic substitutes by the title “Synthesis and anticancer activity of a novel series of 9-O-substituted Berberine derivatives: A lipophilic substitute role” is described as follows. Berberine takes over the remarkable cytotoxicity against human cancer cell Lines, HepG2 and HT-29. By using MIT assay, the efficacy of anticancer activity of compounds was accomplished. Berberine and its derivatives had also been assessed as constraint of topoisomerase I and II and determination with anticancer activity. Berberine showed a low inhibiting effect on vanquished cancer cell growth because berberine preoccupies poorly in intestines and berberine is hydrophilic in nature. Flash column chromatography decontaminates all the compounds. NMR techniques including DEPT, COSY, HSQC, and HMBC Analyses were carried out with Full signal assignment of 1H and 13C. Maximal inhibitory concentration (IC50) was rated in half by the study of anticancer activity of the compounds. To evaluate their effectiveness as preferential cytotoxic agents against human cancer HepG2 and HT-29 Cells, many alkylated and ter-phenylated berberine derivatives are integrated. Human colon cancer HT-29 cell lines are considered as eligible candidate for new anticancer expansion.^[30]
28. Xiaojia Shi *et al.*, have reported anticancer activity of berberine hydrochloride using mitochondria under the title “Anti-cancer effects of honokiol via mitochondrial Dysfunction are strongly enhanced by the Mitochondria-targeting carrier berberine”. According to scheme 1, Ten berberine-linked honokiol derivatives were outlined and integrated and to experiment the cytotoxicity of target Derivatives in six human cancer cell lines and one normal hepatocyte cell line HL7702, MIT assays were used. Length of the linker between the moiety of BBR and HN influences Cytotoxicity was proposed by structure- activity investigation. Compound 6b was picked up as are emblematic compound given its structural similarity, Higher vacuole inducing potency, relatively good cytotoxicity and cell selectivity. Cytoplasmic vacuolation via dilation of mitochondria is conciliated

by 6b and it is obligatory and necessary to transverse the formation of cytoplasmic vacuolation. Blue fluorescence is emitted by 6b according to the emission spectrum. BBR serves as an effective mitochondrial-targeting Cation carrier, and the antitumor efficacy and potency of honokiol is demonstrated.^[31]

29. Elisa Pierpaoli *et al.*, have reported anticancer activity of berberine hydrochloride using HER2/neu-positive mammary carcinoma under the title “Antiangiogenic and antitumor activities of berberine derivative NAX014 compound in a transgenic murine model of HER2/neu-positive mammary carcinoma”. Naxospharma provides BBR and NAX014. Using HPLC on Jasco system LC-2000 series and agilent Eclipse XDB-C18 (4.6×150×3.5mm) column, the purity of >97% of the derivative NAX014 was evaluated. 50% water, 50% acetonitrile plus 0.1% trifluoroacetic acid is a mobile phase whose flow rate was 1 ml/min and was maintained and 235, 265, 340 and 420 nm was where the absorbance was measured. From the American Type Culture Collection, Human breast adenocarcinoma SK-BR-3 cell line was acquired and SK-BR-3 cells were distinguished for cell morphology, growth rate and HER-2 expression before the experiment. According to the manufacturer’s directions, Total RNA was isolated from tumour tissues using the RNeasy kit. By using TUNEL assay, the existence of apoptosis in mammary tumour masses was then examined in situ. 10.9mg/kg was the LD50 in FVB mice and 30.9mg/kg for NAX014. Tumour tissues originated from treated mice by western blot analysis examined the effect of BBR and NAX014 on HER-2 and HPA2 expressions. Reduced tumour vascularity and inhibition of degradation and remodelling of the tumour extracellular matrix are the antitumor effect of NAX014.^[32]
30. Chen-Feng Chiu *et al.*, have reported anticancer activity of berberine hydrochloride using gold nanoparticles on alkaloid berberine (Au-Col-BB) on non-transformed bovine aortic endothelial cells (BAEC) and Her-2 breast cancer cell lines with the title “Delivery Capacity and Anticancer Ability of the Berberine-Loaded Gold Nanoparticles to Promote the Apoptosis Effect in Breast Cancer”. Au-Col-BB was less cytotoxic to BAEC vs. Her-2 cell line with regards to MTT assay and cell cycle behaviour. GNT Gold consists of no other heavy metals or poisonous compounds and GNT Gold has 99.99%. At 50:1 vol ratio for 8 hours at 4°C, Au-Col-BB were amalgamated with 0.5 mg/mL FITC. 190 to 1100 nm is typical absorption peak for Au is the typical absorption peak for Au. Origin Pro 8 software is used to examine and measure data. 6-well plates were used to plant Cells at the density of 2×10^5 . For 48 hours, the cells were incubated with dissimilar material for Western blotting assay. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ are the p values less than 0.05 was contemplated as statistically significant. UV-Vis spectrophotometer was used to examine the energy absorption shift for decorated particles. The study is used for nourishment and nutritional treatment by an inventive approach in developing cellular uptake of nutraceuticals.^[33]

III. CONCLUSION:

This review concludes that the berberine hydrochloride which acts as anti-cancer drug used in the experiments enables us to study the different types of actions using different carcinoma cells. The cell viability tests, proliferation test, different assays and other studies were performed in order to estimate the effect of the drug. So from the results obtained we can deduce the effect of berberine on cells or groups of cells and use the information for further studies and drug discovery. As some of these methods are eco-friendly and cost effective, they can widely be used in all aspects for accurate and effective research study. The study signifies that berberine as an anti-tumor agent can be used effectively in numerous cancer cell lines.

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