Review article

Thymoquinone: A novel treatment option for triple negative breast cancer

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(Received: November 2022 Revised: October 2023 Accepted: December 2023)

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ABSTRACT

The most common treatment modes of cancer include chemotherapies along with other modes of treatment. But often resistance is developed and gruels towards the discovery of new modes of treatment for cancer. Natural products are used for ages in the treatment. One such component is thymoquinone (TQ). It has been extensively studied against cancer, especially in treating breast cancer. The review article updates its status on research against breast cancer and triple negative breast cancer. The paper describes various breast cancer (BC) treatment options in combination with TQ or alone such as heat shock 70-kDa protein 6 (HSPA6) situated on human chromosome in inhibition of BC growth, MMP-9 and MMP-2 as biomarkers in BC, use of cancer stem cells in vascular formation in solid tumor by VM (vascular mimicry) via MMP-2 and MT1-MMP genes upregulation, use of exosomes extracted from adipocyte-derived mesenchymal stem cells, cancer immunotherapy or immuno-oncology where natural killer lymphocyte cells are used to enhance innate immune response against tumors and pathogens, use of (TRAIL) tumor necrosis factor-related apoptosis-inducing ligand agonists, drugs with synergistic activity, identifying targeted genes IL17RD, and diet having natural molecules that can act potentially against cancer. The article also updates on research associated with TQ in overcoming hindrances related to its poor bioavailability making it an effective clinically acting drug molecule. Recent research indicates that TQ can be a successful candidate to eradicate cancer through its clinical interventions followed by drug design studies.

Keywords: Thymoquinone; triple negative breast cancer; therapy.

INTRODUCTION

Cancer ranks one of the leading causes of mortality all-inclusive after myocardial localized necrosis. Radiation therapy, hormonal therapy, chemotherapy, targeted therapy, and traditional pharmaceutical adjuvant therapy are all used in the treatment of cancer, however, viability strides to advantage the patients yet. Tumour metastasis process and treatment options have emerged as the foremost broadly investigated theme. In line with the World Health Organisation reports 2021, it is the foremost common cancer globally in women responsible for 12% of all newer cancer cases. It was anticipated to be more than 3 million by 2040 (1). The essential reason incorporates stagnation of the estrogen levels which may be due to terrible natural conditions, undesirable lifestyle and particularly after menopause (2). Moreover, other variables such as frequent use of liquor, cigarette smoking, contraceptives, trigger the improvement of BC (1). Being metastatic the cancer spreads to various body parts, such as liver, lungs, bone, and brain (3). It is heterogenous in origin which may be due to hereditary, epigenetic, and transcriptomic changes at the biological and histological levels (4). This phenotypic contrast impacts breast cancer prognosis, and treatment. Initially, the course of BC treatment was determined by the tumor's biomarker profile, clinical stage, and histologic findings (5). The medications used to treat BC prevent cancer from growing by obstructing particular chemicals that are necessary for tumour cells to proliferate and survive.

However, it is classified into five subtypes based on biological and molecular characteristics such as atomic profiling, growth factor expressions, and hormone profiling (5). Luminal A and B, triple-negative or basal-like (BL), human epidermal growth factor receptor 2 (HER2), and usual normal like BC are among them. The expression of progesterone (PR), oestrogen receptor (ER), luminal, and hormone receptor genes is a characteristic of the luminal A subtype. The expression of more luminal genes, followed by both ER/PR genes, is indicative of the luminal B subtype. The HER2 subgroup is distinguished by strong HER2 expression and low expression of ER and associated genes. Triple negative or BL patients have low expression of HER2 over time and strong expression of basal cytokeratins and epithelial cells (5). Compared to the luminal B subtype, the luminal A subtype has a more favourable prognosis and a greater survival rate. However, it has been shown that the HER2 subtype, which is defined by positive for HER2, is associated with strong histological features and a lower survival rate. This outcome may change dramatically if chemotherapy is combined with tyrosine kinase inhibitor and anti-HER2 monoclonal antibodies. ER, PR, and HER2 are therefore well-built breast cancer biomarkers that aid

in the diagnosis and treatment (5). Clinically, TNBC patients are more frequently unresponsive, even with the availability of new medications for treatment, treating TNBC remains difficult (5).

In the USA, 287,850 TNBC cases were reported which was high compared to 51,400 BC cases (6). The HER2, PR, and ER genes are not expressed in TNBC cells. The recently analyzed TNBC cases account for 15% to 20% of all. They contain unstable genomes, which are connected to characteristics, such as higher inclination for metastasis and more frequent diagnosis in more young females. TNBC has become highly conspicuous subject clinical studies for numerous reasons. It is more associated to be pathogenic, found more in premenopausal females. It was more often noticed by an African American female youth and became a prominent cause of death in them (7). An inflammatory cytokine, a mutation, and a more proliferative tumour marker were seen in women with TNBC (8). Chemotherapy is still the most widely used treatment for TNBC in modern times. Cisplatin and doxorubicin are the medications that are commonly used to treat TNBC (9). Chemotherapeutic agents act through injurious cellular nucleic acid. However, treatment with chemotherapeutic drugs develops acquired resistance, toxicity and many side effects over time (10). This may affect a patient's life psychologically, physically, and economically. As a result, novel approaches against pharmaceutical resistance are an immediate requirement. It would therefore be beneficial to identify lead chemicals that inhibit TNBC cell growth. Chemotherapies are among the most popular cancer therapy modalities. There have been several initiatives undertaken to lower the breast cancer death rate among affected individuals (11).

Treatment options to breast cancer and TNBC

Subsequent research indicates that one of the cancer treatment options is warm shock 70-kDa protein 6 (HSPA6) (OMIM: 140555). It codes for a 70-kDa protein found cytogenetically on human chromosome 1q23.3. HSPA6 was first identified as a stress-induced heat-shock gene. Although HSPA7 has no coding potential, HSPA6 and HSPA7 share more than 90% of their nucleotide coding. Even though HSPA6 was discovered thirty years ago, its function in the advancement of cancer is still unknown (12). However, the results for autophagy and apoptosis mediated by withaferin A suggest that HSPA6 is the reason for breast cancer's impediment (13). Gelatinase A and B, or lattice metalloproteinases 2 and 9, respectively, are also referred to as oncogenes and important tumor-suppressors during the early phases of carcinogenesis. These enzymes are known to play a dynamic role in breast cancer. Consequently, MMP- and MMP-9 could be used as potential prognostic indicators in the therapy of TNBC (14). Angiogenesis, vasculogenesis through vascular mimicry (VM) is another treatment choice for cancer. In cancer cells, the dissemination of oxygen and supplements is basic to advance the extension and improvement of cancer. In this regard, so-called angiogenesis—the development of new blood vessels—is crucial for providing the needs of tumour cells. Apart from that, the organisation of vascular structure inside the tumour stroma may be facilitated by a supplementary angiogenic process known as vasculogenesis and/or VM (vascular mimicry). Because resistance to chemotherapies is a serious problem, conventional medicines may not always be able to completely eradicate cancer cells. It has been noted that through VM, CSCs (cancer stem cells) increase microvascular thickness within the target malignant cells (15). Numerous signaling pathways PI3K and MT1-MMP and MMP-2 are closely related with the VM. Subsequently, one effective strategy to prevent tumour spread and metastasis may be to regulate the angiogenesis signalling pathways in CSCs or cancer ECs. Cancer immunotherapy or immuno-oncology (16) is quickly advancing and has become one of the treatment alternatives alongside restorative surgery, radiation, and cytotoxic chemotherapy. It can be utilized at the same time with different medicines by the reports. NK (Normal executioner) cells are a sort of lymphocyte that play a major part against tumors and pathogens. These cells can quickly recognize and assault tumor cells. CD56 and CD16 cells contain perforin and granzyme, make up over 90% of the NK cell population in circulation, and they use cytolytic granules to start cell lyses. They cooperate with interferon-α (IFN-α) and cause apoptosis in target cells. They also improve antibody mediated cytotoxicity, by discharge of antibodies that recognize antigens within the attacking pathogens. They also function in other ways, such as tumour rot calculation, which involves the interaction of NK cell-based FAS ligands with target cell passing receptors (FAS/ CD95). NK cell–based immunotherapy may be a viable therapeutic approach for both aggressive and hematologic tumours. Surgery, chemotherapy, radiation therapy, and monoclonal antibodies (mAb) may all be used in this treatment regimen. NK cell activation inhibits the growth of many tumours, with breast cancer specifically targeting oestrogen receptors. Oestrogen suppresses NK cell function by preventing NK cells from expressing receptors such CD69, NKG2D, NKp46, and CD244, and by reducing the release of granzyme B and FasL.

Ligand associated with tumour rot factor that induces apoptosis (Path) or Path agonists, which are entirely separate types of cancer treatment for the liver and breast, are used. Because of its official capacity to the passing receptors DR4 and DR5, it was shown to be safe and therapeutically efficacious. Path may cause cancer cells to undergo apoptosis in a certain way without affecting normal solid cells. As a result, one of the promising anticancer therapies for several types

of human cancer is recombinant human TRAIL (rhTRAIL). When compared to other TNF family ligands, TRAIL's receptor expression in tissues such as the breast, ovary, prostate, placenta, and colon is thought to have restorative potential. It is regarded as a promising and compelling operator for cancer treatment in clinical settings. Chemotherapeutic drug resistance represents the primary drawback of the successful TRAIL cancer treatment. This significant limitation can be addressed by controlling a synergistic medication combination. Food can reduce the risk of cancer by reducing the expression and movement of the oestrogen receptor-α, inhibiting metastasis, growth, migration, angiogenesis, cell cycle arrest, inducing apoptosis, and increasing the susceptibility of breast tumour cells to chemotherapy and radiation therapy. Phytochemicals diminish aggravation and interrupt processes that encourage cancer generation within the body. Intake of flax seeds, allium, cruciferous vegetables, broccoli, food that contains selenium, vitamin D, folic acid, chlorophyll, vitamin B-12, and carotenoids (antioxidants) and probiotics are advantageous as anticancer dietary measures. This droves numerous analysts to research common food for the avoidance of cancer (17). Of them, curcumin, the active component of *Curcuma longa*, is the most widely studied substance suggested to be an anticancer agent (18–20).Curcumin targets several signalling/molecular pathways, including nuclear factor kappa B cells (NF-κB), mitogen-activated protein kinase, p53, Rb, and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) (18–21).

Thymoquinone in the treatment of cancer

Bioactive species from common food items have gained prime consideration as they have phytochemicals which are safe, affordable, and effective and have long been used in therapeutics owing to their medicinal properties. They are copiously dispersed all through plants and marine living beings (22). But they have not been completely acknowledged clinically, primarily due to their undefined molecular mechanisms and cost. Additionally, tumors are creating resistance or side effects within due to different treatment alternatives used (23). Hence, finding a natural product that is exceedingly compelling with no harmfulness has become a critical issue. The plant *Nigella sativa* L. seeds commonly called as dark cumin (family: *Ranunculaceae*), is one of the foremost promising drug, which has been utilized for a long time from Mediterranean local to Western, South-Eastern Asia, Middle east, and Africa to treat different maladies (24). Its pharmacological effects reported were anti-bacterial, anti-inflammatory, anti-neoplastic, anti-asthmatic, pain-relieving, anti-hypertensive, and antioxidant. In Europe, Asia, and Africa, it's also used as a nutraceutical, flavouring, appetiser, and feeding ingredient. Through immune-modulatory effects that stimulate human NK cells, it has anticancer effects. The intended mechanism of action was a variety of suppressive effects on free radicals, proliferating cells, mutagens, and metastasis (14). It has been reported that thymoquinone (TQ), the primary active component of dark seed oil (*Nigella sativa* L.), may offer an alternative to conventional cancer treatment (23).

TQ has been the subject of several in vitro and in vivo investigations due to its demonstrated anti-inflammatory, anti-parasitic, anti-microbial, immunomodulatory, anti-diabetic, hypotensive, hepatoprotective, and anti-cancer qualities. TQ can prevent the metastasis of cancer cells in a variety of malignant tumours (25, 26). Nine out of the eleven cancer hallmarks were modifiable by TQ. TQ is affordable, readily obtainable from plants, and harmless to normal cells and tissues (27). TQ has been *in vitro* studied using cancer cell lines for its inhibitory impacts on cell expansion. The molecular pathways of TQ as anti-cancer agent incorporate anti-proliferation, apoptosis, and acceptance of cell cycle capture and anti-metastasis or anti-angiogenesis, avoidance of aggravation and oxidative push, upregulation of tumor silencer qualities, and tumor-promoting qualities inhibition or down regulation (28). TQ's anticancer effects are mostly brought about via oncogene downregulation, NF-κB inhibition, responsive oxygen species control, hypoxia, and anti-metastasis movement (29). Additionally, TQ functions by disrupting the microtubule structure and reducing the production of the cell survival protein (14).

Additionally, it functions through downregulating VEGF and the NF-kappa B pathway. TQ showed promise in the treatment of cancer by sensitising cancer cells to radiation and amplifying the anticancer effects of certain chemotherapeutic drugs. Moreover, it targets different proteins in many signalling pathways and alters DNA structure through its immunomodulatory action. Additional mechanisms pertaining to tumour organisation, including cell migration, assault, and epigenetic modification in malignant cells, have been documented and have demonstrated cytoprotective effects. TQ suppresses breast cancer both when taken alone and in combination. TQ in conjunction with piperine reduced vascular endothelial development, calculated expression, shifted T helper1 responses, and enhanced serum interferon-γ levels and apoptosis acceptance against EMT6 epithelial breast cancer. In conjunction with amoxifen, TQ induced apoptosis via the Akt signalling pathway in breast cancer cell lines (30).

Recent studies on thymoquinone in treating cancer

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has the ability to cause malignant cells to undergo apoptosis. As a result, recombinant human TAIL (rhTRAIL) has been improved as a

treatment for various types of human cancer. It was evaluated how well the combination treatment of TRAIL+TQ inhibited human breast cancer cells. The MDA-MB-231 and MCF-7 breast cancer cell lines were employed in the investigations. The MTT test, flow cytometry, and cytotoxicity, apoptosis, and cell cycle were investigated. According to the findings, TRAIL caused apoptosis and cell cycle arrest. More interactions were seen with MDA-MB-231 cells than MCF-7 cells. When combined with TRAIL, TQ increased MDA-MB-231's chemosensitivity while lowering MCF-7's resistance to TRAIL. This suggests that TQ and TRAIL work together to destroy breast cancer cells in a synergistic manner. When used together, these two showed promise as breast cancer medications (31). The hereditarily different human breast cancer cell lines MDA-MB-231 and MDA-MB-468 were used to evaluate the mechanism of TQ. The component showed effects on MDA-MB-468 and MDA-MB-231 cells that were cytotoxic. In MDA-MB-231 TNBC cells, it increased the expression of 15 apoptotic genes, including caspases like GADD45A, TP53, DIABLO, BNIP3, DFFA, TRAF2/3, and TNFRSF10A. Sixteen apoptotic properties, including TNFRSF10A, TNF, TNFRSF10B, TNFRSF11B, FADD CASP2, and TRAF2 were increased in MDA-MB-468 cells. The expression of one anti-apoptotic characteristic, BIRC5, in MDA MB-231 cells, is covered, and they are all fundamental to the apoptotic process. Increased sensitivity to TQ-induced apoptosis may be observed in response to elevated TNF and their receptor protein levels. One could argue that TQ has an unanticipated effect on the MDA-MB-231 and MDA-MB-468 cells. The apoptotic quality profile of TQ may indicate TQ as a viable pharmacological candidate for TNBC treatment, given the high requirement of medications in chemoresistant TNBC (32). Using the chromatin immunoprecipitation sequence (ChIP-Seq) and the MBD1 (methyl-CpG binding domain protein 1) antibody, the genome-wide methylation regions impacted by TQ were investigated. Among the 136 genes that ChIP-seq identified, the tumour suppressor IL17RD may be a new target for TQ. TQ epigenetically upregulates it in the TNBC cell lines BT-549 and MDA-MB-231. The Kaplan–Meier analysis method was applied to examine the relationship between survival outcomes and IL17RD expression. It was discovered that the combination of TQ therapy with IL17RD overexpression was more effective against TNBC cells. Longer TNBC patient survival is correlated with higher expression of IL17RD, which also increases TQ and IL17RD's anti-TNBC capability. The study's findings indicate that IL17RD for TQ is regarded as a new target in TNBC. As suggested by the study's findings, TQ may epigenetically upregulate IL17RD in TNBC cell lines. This is likely to be one of the main ways that TQ affects TNBC cells in an anticancer and antimetastatic capacity (33).

With the TNBC cell line BT-549, RNA-sequencing (RNA-seq) was carried out. Annotations and gene functions were examined using the KEGG (Kyoto Encyclopaedia of Genes and Genomes) catalogue. By using RT-PCR and western blot techniques, the regulated gene was proven. For HSPA6 and TQ to prevent the growth, advancement, and interference of TNBC cells, functional experiments by overexpression or knockdown were carried out using bioinformatics and internet databases. Online databases and bioinformatics were used to study the HSPA6 regulation mechanisms and prognosis for breast cancer survival. In patients, prolonged survival was actually correlated with robust HSPA6 expression. TQ inhibits invasion and migration by enhancing HSPA6's tumour suppressive function when it is overexpressed. HSPA6 appears to be a unique TQ targeted gene that regulates tumour inhibition, making it useful for the therapeutic management and treatment of TNBC (34). A lot of research was done to address this problem, including genetically altering NK cells to increase their resistance and using immune stimulants to provide synergistic effects. Since human normal killer (NK) cells are the body's first line of defence against infections and a type of lymphocyte, Nigella sativa has been shown to have safe modulatory effects that strengthen them and help prevent cancer. On NK cell cytotoxic pathways, the restorative effect of TQ was investigated. Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line was used to cultivate NK cells, and TQ was administered during the process. Measures were taken of the NK cells' cytotoxicity. The quantities of interferon-α (IFN-α), granzyme B, and perforin in the culture medium were measured using enzyme-linked immunosorbent assay. TQ enhanced the cytotoxic effect of NK cells on tumour cells by causing an increased release of perforin, granzyme B, and IFN-α. The findings demonstrate that TQ stimulates NK cell cytotoxic activity against breast cancer MCF-7 cells. One technique that might be used to start the creation of tubular structures in the internal tumour stroma is called vasculogenic mimicry (VM). Within the tumour microenvironment, cancer stem cells (CSCs) promote the development of new vascular structures, which leads to increased metastasis throughout all locations. It was taken into consideration that TQ inhibits the human breast MDA-MB-231 cell line via the Wnt/PI3K signalling pathway. TQ was added to MDA-MB-231 CSCs at different concentrations and left for 48 hours. The MTT test was used to investigate the CSCs' viability (35). In CSCs, the combination of TQ, PI3K, and Wnt3a inhibitors was studied. The Matrigel assay was used to determine tubulogenesis capability. The percentage of CD24− CSCs and the rhodamine 123 efflux capacity were investigated using flow cytometry. Protein levels of Akt, Wnt3a, p-Akt, and MMP2 were measured using the western blotting

technique, which was then followed by nine vascular endothelial cadherin (VE-cadherin). TQ may prevent VEGF, EGF, and FGF from stimulating CSCs. Following TQ treatment, CSCs' ability to form new blood vessels was reduced. Western blotting results demonstrate that inhibiting the metalloproteinases MMP 9 and MMP 2 reduced the metastasis of CSCs. TQ-treated CSCs showed reduced VEcadherin protein levels, which indicated suppression of the mesenchymal-endothelial transition (MendT). TQ inhibited PI3K and Wnt3a, which may be connected to the decline in the p-Akt/Akt ratio. It might have reduced the quantity of CD24-positive CSCs and the efflux capacity of rhodamine 123. Furthermore, it made the CSC less useful. The stimulatory effects of VEGF, EGF, and FGF on CSCs appear to be limited when TQ is combined with PI3K and Wnt3a inhibitors (36). Saddiq *et al.,* investigated the combined effects of two distinctive bioactive compounds on MCF7 and MDA-MB-231 breast cancer cell lines: Cur and TQ. We looked at Cur and TQ's combination record and Fa (fragmentary anisotropy) values. In this regard, experiments were conducted on cytotoxicity, growth, annexin-V, relocation, colony arrangement, and cell cycle analysis. In addition, ELISA was used to assess the levels of protein, caspase-3, phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT), and protein levels. The findings suggest that Cur, TQ, and Cur + TQ induced apoptosis by arresting the cell cycle, forming colonies, and reducing cell motility and expansion. The combination of Cur+TQ increased caspase-3 overall while lowering AKT and PI3K protein levels. These serve as proof of the Cur and TQ combination potentially effective anticancer action against breast cancer (37).

TNBC made combined treatment a crucial decision due to its resistance to chemotherapy and meta-stasis. Larvae and queen bees are fed royal jelly (RJ), the nutrient-rich hypopharyngeal secretion of worker honeybees, in order to extend their lives. It has been utilised since antiquity for advantageous purposes. The MDA-MB-231 cell line was therefore used to investigate the anticancer impact of TQ and RJ together against TNBC. The cell viability under various treatment conditions was observed using Trypan blue and 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays. The regulation of the cell cycle and cell death were studied using propidium iodide deoxyribonucleic acid and flow cytometry. The use of immunostaining allowed for the study of the expression of Ki67 and cleaved caspase 3. By increasing cancer cell death, the combination of TQ and RJ appears to have an inhibitory effect on cancer. The most potent apoptosis inducer was TQ, which dose-dependently triggered caspase 3 apoptosis. It was shown that the expression of Ki67 lacked a regulating mechanism. There was more emphasis on apoptosis rather than a decrease in cell division. Combining TQ and RJ against MDA-MB-231 breast cancer cells appears to provide a benefit for cancer therapy (38).

Drug design studies on thymoquinone

TQ can be employed as a possible therapeutic therapy alternative (39). However, while TQ's IC_{50} in some breast cancer cells is greater than 165μM and its effective drug concentration is apparently high, there are various restrictions when utilizing TQ (40). TQ's restricted water solubility (less than 1 mg/mL) and extremely lipophilic properties make oral administration of the medication still difficult. Its log P value is 2.55. Poor GI tract solubility and absorption from the small intestine contribute to its limited oral bioavailability (39). To enhance TQ's oral administration, a sophisticated delivery mechanism is thus required. TQ is not able to reach the tumor site in clinical trials because of its physical and chemical properties, which include its quick biotransformation and high potential to bind to plasma (41). Even while nanotechnology has been successful in enhancing the bioavailability of medicinal substances, the rapid clearance of most of the deleterious effects of MPS nano-formulations remains a major obstacle to their use as drug delivery systems.

Research on nano formulation, TQ bioavailability, cellular delivery, biodistribution, and cancer cell targeting has greatly improved. Exosomes with low cytotoxicity could be a helpful nano-delivery strategy for overcoming biological barriers and avoiding rapid clearance. Three distinct methods were combined to create a novel approach for loading Tq into exosomes recovered from adipocyte-derived mesenchymal stem cells (AdMSCs). These are surfactant treatment, incubation, and physical techniques like freeze-thaw cycles. Additionally, information was obtained using the MTT test to assess cellular uptake, cytotoxicity, and encapsulation efficiency of Tq-loaded exosomes in L929 and MCF7 cells. The results obtained showed 60% encapsulation efficiency. The findings of flow cytometry and fluorescence microscopy demonstrated the cells' effective absorption of TQ exosomes throughout a 4-hour period. According to MTT data, TQ exosomes substantially reduced MCF7 cell viability without harming L929, which are normal cells. The investigation showed that exosomes resolved the issues related to hydrophobicity and insolubility and functioned as efficient drug carriers for TQ-based breast cancer treatment (42). Due to low bioavailability and a lack of measurement techniques in tissues and blood, TQ was clinically hindered. Using emulsification homogenization, loaded cubosomes were produced in order to overcome it. Particle size, zeta potential, shape, entrapment effectiveness, and other physicochemical properties were all examined. Furthermore, the anticancer efficacy was investigated using MDA-MB-231 and MCF-7 breast cancer cell lines. The MCF-10A non-tumorigenic cell line was used to compare the

outcomes. The formulation's cellular uptake, apoptotic effects, and subcellular localization were identified. The cubosomal nanosystem was shown to be effective for encapsulating TQ, as demonstrated by the high entrapment efficiency and zeta potential seen in the data. Over the course of three months, there was also a rise in caspase 3 cleavage and apoptotic bodies in the research. The administration of TQ-loaded cubosomes, which include self-assembling cubic liquid crystalline nanoparticles, resulted in an additional decrease in cell viability and an increase in anticancer activity. Based on the results, TQ might be successfully employed in clinical trial investigations by encapsulating it in a unique cubosomal nanoparticle (43). The natural polymer chitosan (CHS) was used in a recent work to create THQ-loaded lipid-polymer hybrid nanoparticles (TQ-LPHNPs) by the nanoprecipitation technique. A three-factor, three-level Box-Behnken design was employed to optimize the nanoparticles. Under various environmental circumstances, TQ-LPHNPs demonstrated exceptional stability both in the gastrointestinal tract and during storage. Utilizing MTT assay, a cell viability test, MCF-7, and MDA-MB-231 cells were used in cell culture studies to investigate the effects of breast cancer. TQ loaded with nanoparticles was applied to cell lines, and the membrane integrity was assessed using the LDH test. Utilizing free TQ, the outcomes were contrasted. The stated technique was followed in an *ex vivo* TQ permeation investigation employing a rat gut to assess drug penetration from the small intestine following oral administration. Upon oral administration of highly lipophilic substances, the improved formulation demonstrated noteworthy properties. Following oral administration, intestinal permeability and bioavailability were improved by the natural polymer's stronger mucoadhesive qualities. More bioavailability 4.7 times higher than that of TQ suspension was shown by TQ-LPHNPs. Comparing the findings with free TQ, the results showed increased cytotoxicity against the breast cancer cell lines. With improved oral bioavailability and therapeutic efficiency, mucoadhesive LPHNP formulation may represent a fresh alternative strategy (44). As seen in several research using cell lines from breast cancer patients, TQ's IC_{50} is more than 165 µM, indicating lower bioactivity. By preparing derivatives, the structure of TQ was altered and refined to increase its bioactivity. TQFL12 ($C_{17}H_{16}ClNO_2$), a recently synthesised derivative known as (E)-3-4-chloro benzylidene amino-5-isopropyl-2-methylcylohex-2,5-diene-1,4-dione was investigated *in vitro* using flow cytometry, western blotting, and RT-PCR (reverse-transcribed-primer:5'-ggagccttgatgtggtagga-3', reverse primer: 5'-tttcatccagccttccattc-3'), and *in vivo* using mouse xenograft assays and molecular docking mechanism of action investigations. TQFL12 therapy affects TNBC cells more than TQ therapy does, and it affects cancer cell migration and invasion both *in vivo* and *in vitro*. The results showed that, as a novel TQ derivative, it had a higher antitumour effect on breast cancer cells with a less deleterious influence on various aspects of breast cancer progression, such as migration, cell proliferation, invasion, and apoptosis. Furthermore, in a mouse model of cancer cell-derived Xenograft, a TQ derivative reduced tumor development and metastasis while posing less toxicity than TQ itself. Through stabilizing the AMPK α protein, TQFL12 increases the activity of AMPK (5'-adenosine monophosphate–activated protein kinase alpha), and ACC (acetyl-CoA carboxylase). These findings were obtained *via* docking experiments. It is reasonable to assume that TQFL12 has a lot of promise as a targeted therapy adjuvant for patients with breast cancer. It may thus offer potential therapeutic benefits and be a potential chemical to cure TNBC (45).

CONCLUSION

In conclusion, it was discovered *via* examining current studies on thymoquinone that the substance has a bright future in the treatment of cancer. The compound is a possible contender because of the unique modes of action associated with newly identified targeted genes that control tumor development and apoptosis. In addition, the physical limitations of the molecule were surmounted by the synthesis of appropriate derivatives and their dispensing in innovative drug delivery systems, rendering it acceptable for clinical use.

ACKNOWLEDGEMENT

The authors are thankful to Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, 521108, Andhra Pradesh, India.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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