International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 4, 2022

Review Article

A REVIEW OF ADVANCED NANOTECHNOLOGIES AND DRUG DELIVERY SYSTEMS OF SALINOMYCIN AND THEIR ROLE IN TRIPLE-NEGATIVE BREAST CANCER

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Received: 15 Dec 2021, Revised and Accepted: 04 Mar 2022

ABSTRACT

Cancer cells spread to other tissues and organs when they divide incorrectly. Breast cancer (BC) is the main cause of cancer-related mortality globally. Some recent studies on cancer stem cells (CSCs), drug resistance, tumor recurrence and metastasis, and the significance of $CD44^{+}$in targeted treatment for breast cancer are covered. Breast cancer stem cells (BCSCs) and bulk BC cells must be eliminated for the disease to be eliminated. Researchers have shown that Streptomyces Albus-derived monocarboxylic polyether antibiotic salinomycin kills human cancer stem cells (CSCs) and prevents the spread and growth of breast cancer cells. Several drug and apoptosis resistance mechanisms may also trigger apoptosis in breast cancer cells when salinomycin is used in combination with the treatment. Apoptosis-resistant cancer cells and cancer stem cells are both susceptible to the anticancer drug salinomycin. Salinomycin may be able to inhibit CSCs, as well as the source and structure modification of salinomycin analog exhibit potent anticancer activity, the effect of salinomycin on chemotherapeutic-resistant CSCs, and the various mechanisms by which salinomycin inhibits cancer stem cells in this study. Method and delivery technique for salinomycin Nano formulation in triple-negative breast cancer and also contains pharmacokinetics and toxicity of salinomycin. Salinomycin-based drug delivery system is the subject of the patent information. Tumor genesis, development, and invasion are all aided by salinomycin. It's possible to boost the effectiveness of cancer therapy by focusing on cancer stem cells (CSCs).

Keywords: Salinomycin, Breast cancer, Nanotechnology, Drug delivery system

DOI: https://dx.doi.org/10.22159/ijap.2022v14i4.44237. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Cancer is globally the next cause of mortality after cardiovascular diseases [1]. The global cancer mortality rate is estimated to be 2.5 million per year, accounting for more than one-third of all deaths worldwide, according to estimates [2-4]. It is estimated that over 42000 women die each year in the United States [5] and 76,000 women in India from breast cancer, making it the second main reason for death globally [6, 7]. Ductal carcinoma and lobular carcinoma are two types of breast cancer that are distinguished by the location of their origin in the ducts (the pathway that takes milk from the glands to the nipple) or the lobules (the tissue that surrounds the ducts) (milk-producing gland). Invasive breast cancer refers to breast cancer cells that have spread outside of the breast tissue and are no longer contained within it [8]. It occurs when cancer cells break out from their primary home within the lobules or ducts and infect neighboring tissue, a condition known as metastasis [9]. The probability of cancer spreading to other places of the body increases as a result of this. A non-invasive form of breast cancer arises when cancer has not spread beyond the location of its origin [10]. Nonetheless, in certain cases, these cells can progress to the point of causing serious breast cancer. The most important factors that influence the development and progression of breast cancer are reproductive factors [11], hereditary factors [12], lifestyle-related variables [13], and environmental exposure factors [14, 15]. The advancements in breast cancer treatment have been made over the years as shown in fig. 1 including the use of estrogen receptors in hormone therapy for specific patient subgroups, as well as the development of other personalized therapies. Despite these advancements, breast cancer remains the major reason for mortality among women globally [16, 17].

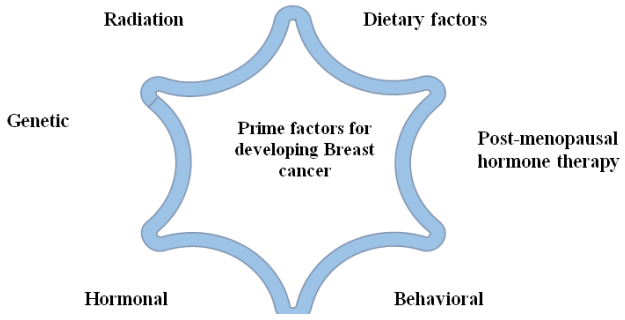

Fig. 1: The prime factors accountable for the growth of breast cancer

Triple-Negative Breast Cancer (TNBC) is responsible for 10%-15% of breast cancer reports. TNBC is recognized from other types of cancer by the absence of estrogen receptors (ER), progesterone receptors (PR), and the human epidermal growth factor receptor 2 (HER-2) [18]. It is characterized by a deficiency in hormone receptors (ER/PR/HER-2), which makes endocrine therapy and other treatments are ineffective [19]. Treatment with chemotherapy, palpation, mammography, ultrasonography, ultrasonography, magnetic resonance imaging (MRI), and immunohistochemistry are the most effective approaches for diagnosing TNBCs (IHC). The application of a non-specific contrast agent in the diagnostic process, as well as the presence of highly qualified professionals (oncopathologists and physicians), does not have reliable findings in the identification of TNBC [20].

Salinomycin, an antibiotic used in veterinary medicine that is 100 times more powerful than Taxol (paclitaxel), a commonly used breast cancer chemotherapy treatment, the breast cancer stems cells such as MDA-MB 231 cells [21]. Salinomycin, a non-hormonal ruminant growth promoter, is approved for its use in veterinary medicine with five other carboxylic polyether ionophores (lasalocid acid, narasin, maduramicin, monensin, and semduramicin), all of which have been commercially successful [22]. Because it is a mildly acidic compound with antibacterial activity against gram+ve bacteria, including mycobacteria, along with certain filamentous fungi, it is categorized as an ionophore (which means "ion carriers"). X-ray crystallography is used to determine the structure of salinomycin's p-iodophenacyl ester, which was previously unknown [23]. Developed by a strain of the bacteria Streptomyces albus, salinomycin is a polyether antibiotic with antibacterial, antifungal, antiparasitic, antiviral, and cytotoxic properties against tumor cells properties that have been tested (ATCC 21838) [24, 25]. Silica gel chromatography was used to purify the product after it had been purified using solvent extraction. In fig. 2, the results of salinomycin on numerous cancer types and cancer stem cells (CSCs) are summarized, depicting convincing indications for the drug's anti-cancer potential [26].

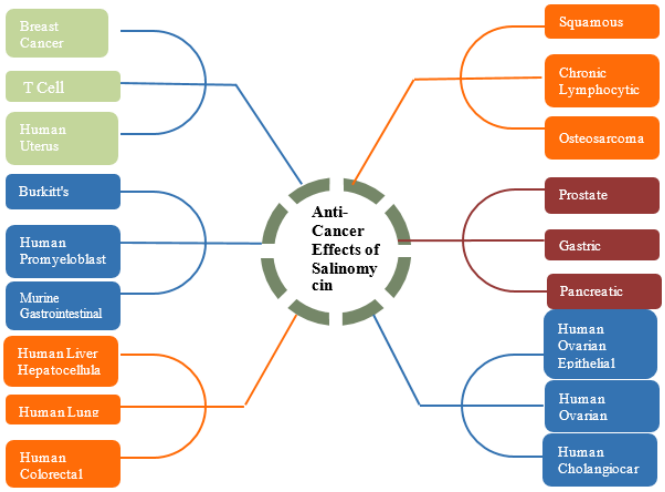

Fig. 2: Salinomycin's anticancer effects on numerous cancer types are summarized

It belongs to class IV of BCS classification with low solubility and low permeability while it appears as a white amorphous powder and detailed physicochemical properties have been explained in table 1. Structural modification is one of the approaches to enhance the solubility and permeability of BCS Class IV compounds [27]. This method entails going back to the step of drug development, which is complex, time-consuming, and costly, thereby limiting the number of drugs reaching the market. Due to its impecunious aqueous solubility (17 mg/ml), salinomycin cannot be administered by intraperitoneal injection without the aid of ethanol [28]. Several research studies have demonstrated the substantial toxicity of salinomycin in various forms of mammals, including humans, following unintended oral or inhaled intake. Significant efforts have been made to surmount these issues. The increasing evidence in the literature indicates attempts to create formulations with improved solubility and bioavailability, efficient therapeutic performance, and enhanced stability [29]. Hence, it is necessary to develop a suitable formulation and drug delivery strategies of BCS class IV compounds to improve their therapeutic potential in various diseases. Nanotechnology provides many unique characteristics, such as Nanometric size, longer half-lives of circulation. This technology's capabilities include the ability to link multiple targeting moieties, controlled release, and site-specific targeting, as well as greater drug entrapment and surface modification. The advances in nanotechnology bring a new paradigm in therapeutic approaches available for the treatment of TNBC [30]. The objective of this review paper is to summarize and offer a general perspective on salinomycin as a candidate for Nano formulation development. It offers source, chemical modifications, and various mechanisms of action. A comprehensive analysis of different formulations and drug delivery approaches has also been presented, with advances made in recent years to improve the solubility, bioavailability, and therapeutic effectiveness of salinomycin. Finally, the toxicity of salinomycin, pharmacokinetic parameters, and patents related to the formulation of salinomycin has been provided. The manuscript description/the word salinomycin is searched, excluding the citations in google scholar, Pubmed, Scopus, Science direct, Clinical key, Web of science, and all related articles, including patents, are downloaded and information is summarized.

Table 1: Physicochemical characteristics of salinomycin

Characteristics	Description	References
Occurrence	Polyether antibiotics isolated from *Streptomyces albus* DSM 41398	[31, 32]
Chemical class	Antibiotics	
IUPAC	(2R)-2-[(5S,6R)-6-[(1S,2S,3S,5R)-5-[(2S,5R,7S,9S,10S,12R,15R)-2-[(2R,5R,6S)-5-ethyl-5-hydroxy-6-methyl-2-tetrahydropyranyl]-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5^{7}.3^{5}]pentadec-13-en-9-yl]-2-hydroxy-1,3-dimethyl-4-oxoheptyl]-5-methyl-2-tetrahydropyranyl]butanoic acid	
Molecular formula	$C_{42}H_{70}O_{11}$	
Molecular weight	751.00 g/mol	
Melting point	112.5-113.5 °c	
Purity	≥ 98%	
Appearance	White amorphous powder	
Stability	Stable for at least 2 y at–20 °C.	
UV absorption	285 nm	
pKa	6.4 (DMF)	
Optical rotation	Levorotatory of-63° in ethanol solution.	
Solubility	Alcohols, acetone, ethyl acetate, benzene, chloroform, carbon tetrachloride, ether, petroleum ether, and hexane,	
BCS classification	BCS-IV	

Salinomycin-source, chemical modification

The manufacture of salinomycin was accomplished through tank fermentation with Streptomyces albus in a rotary shaker, and the product was concentrated in a vacuum chamber [33]. As a result of this method, salinomycin was obtained in the form of a colorless sodium salt prism. Salinomycin is composed of 11 oxygen atoms that are preserved in a variety of functional groups on the side chains, including seven methyls, three ethyl's, three hydroxyls, ketone, and carboxyl groups, as shown in fig. 3. [34]. Salinomycin is a synthetic antibiotic that is used to treat bacterial infections [35]. The salinomycin molecule is composed of a hydrophilic cavity (pocket) that is filled with oxygen atoms from several functional groups, including ether, carbonyl, and hydroxyl, among others [36]. This ensures that significant monovalent and divalent cation selectivity is preserved. However, because their molecules are predominantly formed of hydrophobic hydrocarbon skeletons, the exterior half of their molecules are non-polar, indicating that they are polar on the inside of their molecules [37]. It is because of the presence of a hydrophobic surface that the cell has high lipophilicity and permits the extracellular environment to enter the cell through the cell membranes [38].

When a molecule has an open-chain structure with a hydroxyl group on one edge and a carboxylic group on the other, intramolecular 'head-to-tail hydrogen bonding occurs [39]. As a result of this, oxygen atoms are drawn closer to the center of the molecule, allowing salinomycin to easily bind cations, especially potassium and sodium. In contradiction, the alkyl groups are scattered over the exterior surface and provide the host-guest system highly lipophilic, which allows its transport beyond the biological membranes [40]. This allows salinomycin to enter lipid bilayers, and to transport cations across them down their concentration gradients by passive diffusion [41].

Fig. 3: Structure of salinomycin

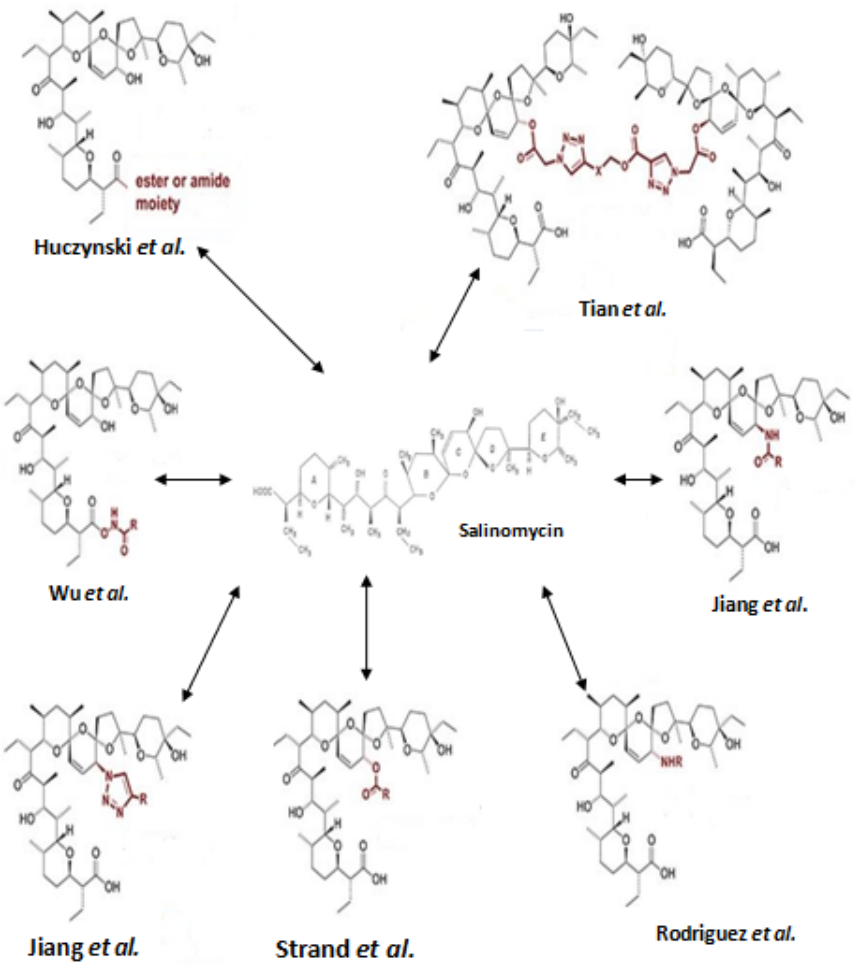

Fig. 4: Chemical modification of salinomycin

Chemical modification of salinomycin

Salinomycin analogs show potent anticancer activity [42], it is essential to make chemical modifications that won't deteriorate the compound and will yield the required products with satisfactory results without changing its pharmacological effect. It is observed by modifying the C-1 carboxyl group of salinomycin, its esters and amides were obtained [43]. Most SAL C-1 derivatives have been shown to have anticancer activity micromolar doses around the IC50 and to be capable of overcoming multidrug resistance in tumor cells, particularly LoVo/DX cells, indicating that salinomycin analogs

should be used in cancer treatment [44]. Wu *et al.*, have synthesized 10 salinomycin conjugates that include hydroxamic acid linked to ester linkages [45]. Using region selective O-acylation of the three hydroxyl groups in salinomycin, strand *et al.*, were able to synthesize salinomycin [46]. The drug was effective in destroying breast CSCs at Nanomolar concentrations while also rendering salinomycin ineffective [47]. To their knowledge, no one has ever demonstrated before that flipping the structure at C20 could provide a favorable place for the chemical modification of salinomycin, particularly by sterically volatile molecules. They were shown to be substantially less toxic to normal cells than the beginning chemical and to have significantly better cytostatic activity than salinomycin, which was previously thought to be toxic [48]. Recent research demonstrated that the photocycloaddition process [2s+2s] can be used to successfully functionalize the C18=C19 double bond of salinomycin after producing a polar alkyl derivative. When compared to salinomycin, this chemical, on the other hand, displayed moderate anticancer activity while preserving some selectivity for cancer cells [48]. They also develop several salinomycin triazole derivatives, including four dimers connected at the C20 site, which were also discovered by Tian and colleagues. After extensive research, it was justified with quite advantageous in treating breast cancer as shown in fig. 4 [49].

The structural activity of salinomycin

The pseudo-cyclic structure of salinomycin and its salts is thought to be due to hydrogen bonds formed between the carboxylic group on one side and the two hydroxyl groups on the other [50]. The researchers claim that salinomycin's hydroxyl groups' targeted O-acylation could greatly increase its anti-cancer activity. Using both marker-based and functional experiments, selected 20-O-acylated analogs demonstrated substantial CSC activity initially at 50 nM doses where salinomycin was inert. The carboxylate group is likewise ineffective against CSCs when converted to hydroxamic acid derivatives that strongly coordinate alkali metal ions but have lesser ionophores activity. Salinomycin's biological and synthetic characteristics have also been studied extensively. The carboxylate group of salinomycin can be modified by esters, amides, and bioactive chemical conjugates [51]. Wu *et al.* investigated C17 and C21 epimerization as well as 20-epi-ester. In our previous work, we have synthesized and tested the biological activities of 17-epi-salinomycin and 17,21-di-epi-salinomycin as well as their benzoylated derivatives. The results showed that the 17-epi-salinomycin and its analog almost lost activity but 17,21-di-epi-salinomycin and its ananalognhanced the activity, indicating the important roles of the spatial configurations of the salinomycin. To elucidate the effect of C20-hydroxyl configuration on the biological activities, herein we reported the synthesis and evaluation of anti-tumor activities of 20-epi-salinomycin and its 20-O-acyl derivatives.

Effect of salinomycin on chemotherapeutic resistant csc's

Salinomycin has anticancer activity against CSCs that have developed resistance to other chemotherapeutic drugs such as Oxaliplatin, Imatinib, Etoposide, Doxorubicin, Gemcitabine, and 5-fluorouracil as shown in table 2 [52]. Salinomycin has anticancer activity against CSCs that have developed resistance to other chemotherapeutic drugs. $CD133^{+}$cancer cells that had gained resistance to oxaliplatin were treated with salinomycin, which inhibited the epithelial-mesenchymal transition (EMT) pathway [53]. When imatinib was used to treat gastrointestinal stromal tumors, it was discovered that $KitlowCD44^{+}CD34^{-}$cells were resistant. Salinomycin inhibited the proliferation of $KitlowCD44^{+}CD34^{-}$cells, allowing them to self-renew and specialize, hence enhancing their susceptibility to chemotherapy [54]. MDA-MB-231 is specific Triple-negative breast tumor cell lines showing how the mechanism of salinomycin inhibition by various analyses method such as immunofluorescence staining, flow cytometry assessment, western blot study, trypan blue exclusion assay.

Table 2: Effect of salinomycin on specific cancer stem cells which are resistant to chemotherapic drugs

Cell lines	Present in cancer cells	Resistance to cancer cells	Effect of salinomycin	Reference
KG-1a	Human leukemia stem cell	Etoposide, Doxorubincin, gemcitabine, 5-fluorouracil	Treatment resistance in KG-1a cells was proven through the expression of functional ABC transporters. However, salinomycin showed the ability to overcome ABC transporter.	[53]
P-glycoprotein overexpression multiple drug resistance (MDR)	Cancer cell lines	Doxorubicin	Salinomycin showed inhibition of the cell growth in P-glycoprotein overexpressing MDR cancer cell lines.	[55]
Tumor stem cells	Osteosarcoma	Methotrexate, Adriamycin, and Cisplatin	Salinomycin showed a selective cytotoxic effect and sensitized the CSCs to conventional chemotherapeutic drugs	[56]
NCI-N87 and SNU-1, (ALDH expressing stem cell lines)	Gastric cancer cell lines	5-fluorouracil, cisplatin	ALDH-expressing stem-like gastric cancer cell lines demonstrated enhanced cytotoxicity to salinomycin, which gives clues for selective chemotherapy on gastric carcinoma.	[57]
MDA-MB-231	breast cancer cell lines	Doxorubicin	Salinomycin causes a G2 arrest and senescence, produces a p53-individual upregulation of $p21^{waf/cip}$	[58]
leukemic cells	chronic lymphocytic leukemic cells	Mitoxantrone	Salinomycin restrains Wnt signaling causes apoptosis in prolonged lymphocytic leukemic cells	[59]
Prostate cancer cells	Prostate cancer	Cabazitaxel	Salinomycin causes death in VCaP and LNCaP prostate tumor cells by increasing intracellular reactive oxygen species (ROS) amounts and depolarizing the mitochondrial membrane.	[48]

The mechanisms of salinomycin against cancer stem cells

CSCs have been discovered at a wide range of humanoid malignant cancers, that consists of blood, breast, brain, bone, skin, liver, lung, bladder, ovary, prostate, colon, pancreas cancers, states that physiological proliferative tissues, been hierarchically prepared and broadcasted by a small number of stem cells [60]. CSCs are cells inside a tumor that may self-renew and result in many extractions of cancer cells that make up the tumor. CSCs can be identified experimentally in repeated xenotransplantation procedures by their capacity to boost the growth of a growing tumor. Although it is questionable if tumorigenic cells recovered from hematological and solid tumors based on the manifestation of particular cell surface indications are the true "stem cells" of the tumor. CSCs also exist significantly in treatment settings, according to current findings. Although there are various mechanisms underlying salinomycin's eradication of CSCs as shown in fig. 5 are unclear, new research has increased our understanding of salinomycin's mechanism and MOA in human CSCs and cancer cells [53].

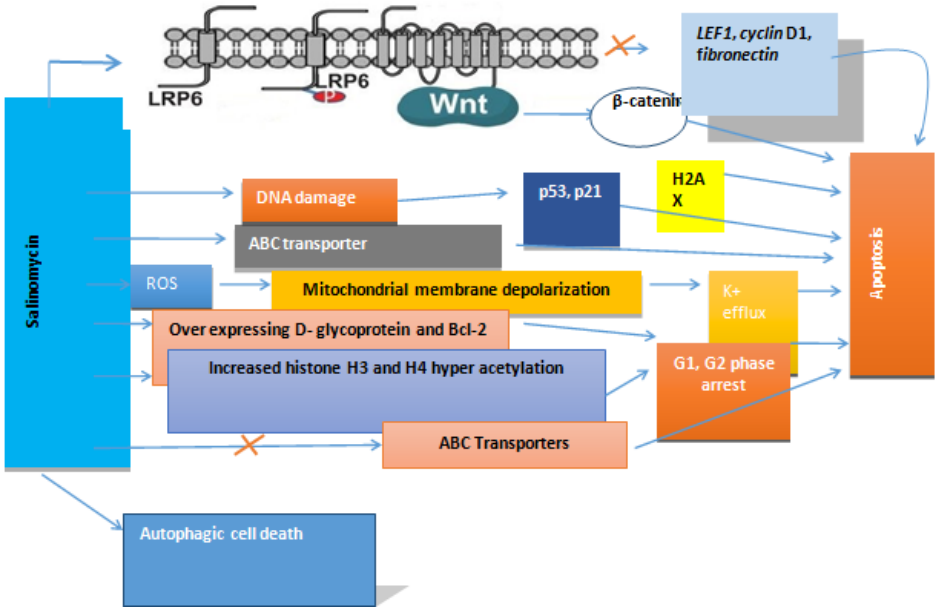

Fig. 5: **The mechanisms of salinomycin**

Salinomycin exerts its inhibition on the Wnt signaling pathway through its suppression of the phosphorylation of LRP6 and the expression of β-catenin. It also induces the production of ROS and mitochondrial membrane depolarization, which consequently results in the activation of caspase-3, the induction of PARP-1 cleavage, and the elicitation of DNA damage. These events subsequently induce tumor cell death and inhibit cancer cell growth. Moreover, salinomycin can induce Autophagic cell death.

Molecular mechanisms of salinomycin against cancer stem cells

CSCs have been discovered at a wide range of humanoid malignant cancers, that consists of blood, breast, brain, bone, skin, liver, lung, bladder, ovary, prostate, colon, pancreas cancers, states that physiological proliferative tissues, been hierarchically prepared and broadcasted by a small number of stem cells [60]. CSCs are cells inside a tumor that may self-renew and result in many extractions of cancer cells that make up the tumor. CSCs can be identified experimentally in repeated xenotransplantation procedures by their capacity to boost the growth of a growing tumor. Although it is questionable if tumorigenic cells recovered from hematological and solid tumors based on the manifestation of particular cell surface indications are the true "stem cells" of the tumor. Salinomycin is more effective than paclitaxel, the conventional chemotherapeutic medication for the treatment of BCSCs [61]. Salinomycin causes BC mammosphere cells to apoptosis, which is associated with Bcl-2 expression downregulation and lowers their migration potentially, which is accompanied by c-Myc and Snail expression downregulation [62]. Salinomycin inhibits mammosphere development, induces cell death, and inhibits cell proliferation in BC cells through influencing stem cell signaling, such as Wnt and Hedgehog signalling, or ALDH1 activity [63–65]. Although the specific processes governing salinomycin's actions on BCSCs are unknown, the current review has given insight on its molecular mechanisms of action of salinomycin, underlying eradication of CSCs as shown in fig. 5.

Salinomycin's effect on the induction of apoptosis

When it comes to embryonic development, tissue homeostasis, and immunological control, Apoptosis is the most important cell death mechanism. It is responsible for the removal of superfluous and dangerous cells during these processes. An intracellular cysteine protease named caspases is primarily responsible for the execution of apoptosis. Caspases are activated via death receptor-dependent (extrinsic) and death receptor-independent (intrinsic or mitochondrial) mechanisms [66]. Apoptosis can be induced by salinomycin in CSCs of various origins [67]. In terms of the specific mechanisms, however, the origin of CSCs has a significant impact. In Salinomycin-treated cells, both BC and Hs578T cells exhibit a large increase in DNA breaks, as well as an increase in both P53 and gH2AX expression. Salinomycin produces DNA damage, which has a considerable influence on the rate of apoptosis, according to research. In MDA-MB231 cells, Salinomycin enhances pro-apoptotic protein Bax expression while decreasing anti-apoptotic protein Bcl-2 expression, indicating that a rise in the ratio of Bas Bcl-2 is tangled in apoptosis which is caused due to salinomycin. Salinomycin reduced atomic translocation of NF-kB, resulting in a drop in the appearance of pro-survival proteins controlled by NF-kB. Salinomycin is also said to trigger a separate apoptotic pathway from p53, cysteine-aspartic proteases initiation, the CD95/CD95L organization, also prosome [68]. Surprisingly, Dhaheri *et al.* discovered in combining salinomycin along paclitaxel or docetaxel might induce programmed cell death at BC cell line MDA-MB231, even though salinomycin alone does not cause apoptosis [69]. Salinomycin can cause cancer cells to die in apoptotic or nonapoptotic ways, while the exact mechanisms of salinomycin-induced cell death in cancer cells persist unclearly.

Salinomycin's effect on autophagy

Autophagy degrades a cell's components. This catabolic process directs the breakdown of the components of its cell via the lysosomal mechanism. It's a physiological mechanism that recycles broken organelles, and supplies ATP throughout "slender periods," but it may also kill cells if it's overstimulated. Excessive and long-term autophagy activation leads to the degradation of vital proteins and organelles outside a confident threshold, eventually leading to cell demise. Autophagy is claimed to be both induced and inhibited by salinomycin [70]. Salinomycin causes a substantially higher Autophagic response than rapamycin in MDA-MB-468 and SKBR3 cells, as well as normal human dermal fibroblasts. Salinomycin has a long-term influence on BC cell ATP levels. Human normal dermal fibroblasts, lose mitochondrial mass after salinomycin therapy, although they are robust to ATP depletion. Salinomycin protects cancer cells by causing autophagy. The protective function of ATG7 can be undone using siRNA, causing cancer cells to die. In the clinic, using autophagy blockers combined will be more successful. Salinomycin's capacity to delay LC3 and long-lived protein degradation might be explained by a reduction in autophagy. The ALDH (+) population of HMLER cells had a higher rate of apoptosis than the ALDH (+) population. When ATG7 is diminished, salinomycin's proapoptotic activity is boosted in the ALDH (+) population [71].

Salinomycin's effect on necrosis

Unlike apoptosis, necrosis does not always include the activation of caspases. This syndrome causes ER, mitochondria, and cytoplasm expansion, followed by plasma membrane rupture and cell lysis. The interaction of multiple signaling cascades causes necrotic cell death. RIP3, calcium, and mitochondria are important contributors to necrosis. Enzymes involved in glucose and glutamine metabolism, as well as other proteins, bind to this protein. Calcium modulates PLA, calpain, and NOS activation, leading to necrotic cell death. Salinomycin induces an outspread protein retort and an abnormal Autophagic movement in glioblastoma cells, which results in necrosis as a result of mitochondrial and lysosomal alterations [72].

Formulation strategy and drug delivery system of salinomycin

Formulations have examined various Nano-technological and biological research for breast cancer therapy with contrast agents and drug delivery carriers during the previous two decades. This review provides an overview of several salinomycin formulation processes. Attempted to decrease the premature release of the drug while also ensuring intracellular drug delivery by enhancing the mechanical strength of solid lipid nanoparticles and Salinomycin [73], development of vitamin E-based redox-sensitive salinomycin and hyaluronic acid as prodrug nanoparticles and were fabricated with paclitaxel that increased the cellular uptake efficiency due to CD [74, 75]. Irmak *et al.*, developed salinomycin-encapsulated PLGA nanoparticles that released salinomycin in a regulated and extended manner. The Wnt/ß-catenin pathway (Wnt/ß-catenin route) and c-myc gene expressions were suppressed in osteosarcoma tumor cells by the researcher's attempted co-delivery of salinomycin and doxorubicin using Nano liposomes, which resulted in synergistic effects as well as the highest tumor inhibitory rate [76]. When developed nanoparticles containing PLGA for the co-delivery of salinomycin and docetaxel, they discovered that they had higher drug encapsulation efficiency, faster drug release, and a longer circulation time as a result. To deliver salinomycin and paxitaxel in the form of PLGA nanoparticles may have used an emulsion solvent diffusion method, with hyaluronic acid-coated over the surface of the nanoparticles, to target $CD44^{+}$breast cancer stem cells, which increased cytotoxicity and improved cellular uptake [77, 78]. A nanoparticle composed of salinomycin-loaded polymer–lipid hybrid anti-HER2 (Sali-NP-HER2) was also designed by J. Li and colleagues, and it was initiated to be successful in contrast to both tumor units and HER2-positive breast CSCs. Improved cytotoxic effects and a reduced rate of breast tumorsphere formation when compared to non-targeted nanoparticles or salinomycin alone were observed when comparing the two treatments [79]. In the following years, the development of novel iRGD-conjugated DSPE-PEG2000 Nano micelles with a size ranging of 10 nm and high penetration effectiveness, allowed salinomycin to be administered to both liver cancer cells and CSCs [80]. Because of the use of lipid-based Nanocarriers, it was considered to be a safe delivery technology. Aydin *et al.* prepared novel Herceptin-decorated salinomycin encapsulated PLGA nanoparticles (HER–SAL–PLGA) shown in table 3.

Table 3: **Various formulation strategies of salinomycin**

Type of formulation	Method of preparation	Components of delivery system	Purpose	Conclusions
Solid lipid nanoparticle [81]	Film-hydration methods	GTP, cytosol, Clathrin triskelia (extracted from rat liver tissue)	Slow premature drug release facilitated burst intracellular drug release.	Clathrin alteration increased intracellular uptake and inhibited cancer cells more effectively. It showed EE (93.50±1.93%), Particle diameter (312±33 nm), Zeta potentials (-36.63±0.23 mv), Drug loading (10.14±0.22%).
Nanoparticles [82]	Emulsion solvent by using an evaporator.	D-*α*-tocopheryl PEG 1000 succinate, Coumarin-6, Hyaluronic acid, cystamine	The synergistic effects of salinomycin-paclitaxel and Nanoparticles are cancer-affecting and glutathione-vulnerable.	Paclitaxel was delivered using a hyaluronic acid-coated vitamin E-established redox-sensitive salinomycin. Because of improved intracellular drug transport efficiency and cellular absorption efficiency, they displayed significant antitumor effectiveness. Particle size (238.5±21.82 nm), PDI (0.15±0.05), Zeta potential (-28.87±1.69mV), EE (87.64±0.44%), Drug loading (2.92±0.05 %).
lipid-polymer Nanoparticles labeled with CD133 and EGFR aptamers [83]	The solvent emulsion diffusion method	Acetonitrile, PLGA, DSPE-PEG-Mal, soybean lecithin, coumarin 6, CL4 aptamers, A15 aptamers	Increases cytotoxic effects, dual targeting nanoparticles were constructed, inorganic nanoparticles were not used, enhancing the focus on $CD133^{+}$osteosarcoma cells.	The majority of CSC-aiming drugs have the potential to harm hematopoietic stem cells. Here, dual-ligand lipid-polymer nanoparticles overcome that problem. Particle size (110.2±12.1 nm), PDI (0.15±0.06), Zeta potential (-17.7±8.2mV), EE (66.5±6.5%), Drug loading (9.4±0.9%)
PLGA nanoparticles [84]	Emulsion diffusion evaporation method	PLGA, ethyl acetate, DMAB	High encapsulation The efficacy for salinomycin was attained, Controlled and sustained release was seen.	The therapeutic anticancer impact of PLGA-Salinomycin nanoparticles was enhanced by activating and blocking various signaling pathways (They caused caspase-3 expression by curbing ß-catenin). Particle size (188 nm), PDI(≤0.1),Zeta potential(20 mV), EE(97%).
Nano liposomes [85]	lipid-based film method	Hydrogenated soybean phospholipids, Cholesterol, DSPE-PEG-2K, chloroform	Efficiently delivered drug to liver cancer cells along with CSCs. Quick drug release rate.	Co-delivering salinomycin along with doxorubicin using Nano liposomes bought synergistic effects and showed best tumor inhibitory rate. Particle size (115±1.53 nm), PDI(0.215±0.005),zeta potential(-41.1±2.00 mV), Drug loading (1.31±0.17 %),EE(68.34±9.02 %)
PLGA Nanoparticles [86]	Nano precipitation method	PLGA, Tocopheryl polyethylene glycol 1000 succinate, acetone	Increased cytotoxicity, prolongs circulation time, increased tumor	Co-Delivery of Docetaxel along with Salinomycin showed increased inhibition of breast cancer, elevated drug encapsulation

Type of formulation	Method of preparation	Components of delivery system	Purpose	Conclusions
			targeting, good biocompatibility	effectiveness, and fast release of the drug. Particle size (73.83±3.59 nm), PDI(0.193±0.021), zeta potential (−25.7±2.03mV), Drug loading (4.08±0.86/4.12±0.71%), EE(53.28±8.96/82.30±6.12%)
Drug loaded polymeric Nanoparticles [87]	Emulsion solvent diffusion method	PLGA, DMAB (Didodecyltrimethylammonium bromide)	Elevated the cytotoxicity against $CD44^{+}$cells, improved cellular uptake	Co-delivery of salinomycin and paclitaxel in the form of PLGA nanoparticles showed the highest cytotoxicity to CD44+cells. Hyaluronic acid was coated over the surface of nanoparticles to target the CD44 receptor. Particle size(153.41±4.78), PDI (0.258±0.12), zeta potential(49.1±1.2), Drug loading(10%), EE(71.2±3.4)
Polymer–lipid hybrid nanoparticles [88]	Nanoprecipitation method	soybean lecithin, PLGA, DSPE-PEG2000, anti-HER2 antibodies	higher efficacy in blocking cancerous growth.	Salinomycin-loaded polymer–lipid hybrid anti-HER2 nanoparticles improve drug distribution, targeting efficacy for cancer cells, and enhanced cytotoxic effect. Particle size (135.6±17.6 nm), zeta potential (-28.3±5.8mV), PDI (0.15±0.03), Drug loading (8.0±3.9%), EE (55.4±8.5%)
Nano micelles [89]	lipid-based film method	Methanol, chloroform, DSPE-PEG2000	Possessed a smaller size near to 10 nm	iRGD-conjugated DSPE-PEG2000 Nano micelles possessed smaller size and high permeation efficacy, enhancing antitumor activity. zeta potential (-17.1±3.1), Particle size (13.7±0.4 nm), drug encapsulation efficiencies (96.6±5.8), PDI**(**0.31±0.13), EE **(**93.4±4.2)
Nanoparticles [90]	Homogenization method	PLGA, ethyl acetate, dodecyl dimethyl ammonium bromide, N-hydroxysuccinimide	controlled release of drug was achieved	Herceptin-decorated Salinomycin-loaded nanoparticles showed promising cellular uptake of salinomycin which proposes novel drug-delivery system. Particle size (257.5 6±10.1 nm), drug loading(18.1 6 0.12), PDI(0.297 6 0.053), EE(61.3 6 3.3%)
Nanoparticles Polymer [91]	Single emulsion method. Gelatinase-responsive copolymer-carboxylation and double amination method	MPEG-NHS, PVGLIG, DMF, succinic anhydride, dimethylamiopryidine, pyridine, EDC, PCL-NH2,	Increased *in vitro* release profiles, stability, drug loading content, prolonged circulating time,	Since gelatinases are present in most tumors PEG-Pep-PCL nanoparticles can circumvent the cancerous location by EPR.

Pharmacokinetic parameters of salinomycin

Salinomycin's pharmacokinetic criteria have been thoroughly explored in a variety of animal models. It is readily digested in the GIT and distributed throughout the serum and tissues due to its lipid solubility. Fat, liver, and muscle tissues in chickens had the highest affinity for salinomycin, according to tissue distribution [92]. Salinomycin can permeate through the BBB. The liver is the primary organ for metabolization and its elimination is moderately fast. The adequate elimination time of salinomycin in chicken was suggested at 24 h. In broiler chicken values were 108 and laying hens the oral LD50 the values was 104 mg/kg body mass [93], while the LD50 amount of salinomycin in the horse is 0.6 μm/kg [94, 95]. A patient of progressive metastatic squamous cell carcinoma of the vulva acknowledged intravenous management of 200–250 m/kg salinomycin every second day in grouping with another chemotherapeutic drug, erlotinib, which resulted in advanced clinical effects, initiating a pharmacodynamic outcome in next-generation human studies [96].

Toxicity of salinomycin

Salinomycin, is an antibiotic with K^{+}-ionophore characteristics that have been used in animal farming for decades for both enhancing nutrient absorption and treating parasite infections. Salinomycin has never been established as a drug for human diseases until now [97]. The various research studies published in the last three decades, particularly, showed salinomycin's significant toxicity in animals such as horses, pigs, cats, and alpacas following accidental oral or inhalation ingestion. A 35-year-old male accidentally inhaled about 1 $mg{\cdot}kg^{-1}$ salinomycin, resulting in severe acute and chronic salinomycin toxicity, including acute nausea, photophobia, leg weakness, tachycardia, and blood pressure elevation, as well as chronic creatine kinase elevation, myoglobinuria, limb weakness, muscle pain, and mild rhabdomyolysis [98]. The daily consumption of salinomycin more than 500 $\mu g{\cdot}kg^{-1}$ by dogs causes neurotoxic consequences such as myelin loss and axonal degeneration, so risk analysis data recently released by the European Food Safety Authority declares an acceptable daily intake (ADI) of 5 $\mu g{\cdot}kg^{-1}$ salinomycin for humans [52, 99–101]. (Salinomycin kills tumor cells by inducing both apoptotic and autophagic cell death and also acting as a protective mechanism initially. Jaganmohan *et al.*, discuss the toxicity of salinomycin in the presence of glucose deficiency or competitive inhibition of the glycolytic pathway (pharmacologically induced starvation-like circumstances), as well as in the presence of hypoxia (natural inhibition of phosphorylative oxidation) [102]. *In vitro* studies also show that using 2-Fluoro 2-deoxy D-glucose or 2-deoxy D-glucose with Salinomycin is fatal in cancer cells, whereas using oxamate does not increase cell death by inducing characteristics of Salinomycin. Additionally, they show that starvation-induced treatment of cancer cells with Salinomycin not only enhances apoptotic caspase activity but also reduces the protective autophagy typically induced by Salinomycin alone. As a result, this research study explains the potential for Salinomycin to be used as a cancer therapy, maybe in conjunction with short-term hunger or pharmacologic intervention that simulates starvation [103].

Salinomycin is toxic to normal neuronal cells (murine dorsal root ganglion neurons, toxicity at 1M, cell viability 25%, *in vitro*-experiment), according to Boehmerle *et al.*, induce mild to severe neuropathies [104]. Further by using animal models, the same researcher found that a combination of Salinomycin (5 mg/kg daily injection) and suppression of the mitochondrial Na+/K+ exchanger had no neuronal toxicity while not affecting cancer cell cytotoxicity. Furthermore, partially successful pilot research in people revealed modest side effects while promoting metastatic tumor remission. As a result, the efficacy of Salinomycin will most certainly be studied in a larger number of cancer patients [48]. Recent research studies about gastrointestinal sarcoma, osteosarcoma, colorectal and breast malignancies have also shown salinomycin's toxicity to cancer stem cells. Despite cell death generated by Salinomycin being still unknown, a recent study by Lu *et al.* found that Salinomycin targets cancer stem cells by inhibiting the Wnt/-catenin pathway, which is crucial for stem cell self-renewal. Salinomycin was shown to promote an increase in intracellular calcium levels by interrupting $Na+/Ca^{2+}$exchange in their previous investigations. So the drug-induced deposition of intracellular calcium has been implicated as salinomycin toxicity. Steinhart *et al.*, explain the intravenous injection of 200–250 $\mu g \cdot kg^{-1}$salinomycin every other day for three weeks resulted in partial tumor regression with relatively minimal acute and long-term side effects, compared to no significant acute and long-term negative effects with standard chemotherapeutic medicines. As a result, a phase I/II clinical trial in patients with triple-negative breast cancer with VS-507 (a proprietary formulation of salinomycin made by Verastem Inc., Cambridge, MA, USA) [105].

Salinomycin's exhibit tumor cell toxicity is caused by mitochondrial hyperpolarization, which is seen more frequently in cancer cells. Salinomycin targeted and shows toxicity against cancer and cancer stem cells, with less toxicity toward normal cells, so it can use as a chemotherapeutic drug in combination with autophagy inhibitors [106].

Patents related to salinomycin based drug delivery system

The records were found using a variety of databases, including Google Patents, Espacenet, WIPO, and the USPTO search engines. Terminologies like Salinomycin formulations for Breast cancer were used to perform a search in different databases. Considering patents written in English, we concentrated on the relevant material, title, abstract, and study status. There are some patents on the salinomycin-based drug delivery system that were considered for this review and are listed in table 4**.**

Table 4: Patents related to salinomycin based drug delivery system

Topic	Nanocarrier system	Patent application number	Patient proprietor	Outcome
Salinomycin-loaded PEG-ceramide micelle, preparation method, and application	Solid lipid nanoparticles (SLN)	CN105250238B	Univ pla 2nd military medical	Salinomycin-loaded PEG-ceramide micelle reduced the toxic and side effects[107].
Tumor stem cell-targeted lipid nanospheres and preparing method	Solid lipid nanoparticles (S LN)	CN107137377B	Zhejiang University	Lipid nanoparticles comprising of A15-polyethylene glycol-octadecyl alcohol graft, glyceryl monostearate, and salinomycin had developed tumor stem cell targeting and a strong affinity with CD133 molecules[108].
Salinomycin sodium and doxorubicin co-loaded Nano-liposome preparation method and application	Nano liposomes	CN107137377B	Univ pla 2nd military medical	Nano liposomes co-loaded with salinomycin and doxorubicin target liver cancer cells in a synergistic effect[108].
Salinomycin-loaded micelle preparation method and application	Micelle	CN104257628B	Univ pla 2nd military medical	Salinomycin is loaded on DSPE-PEG2000 copolymer which develops efficiency in targeting and infiltration capacity[107]

Future prospective

Salinomycin's specific effects on apoptosis and autophagy, as well as the interaction between mitoptosis, mitophagy, ferroptosis, and necrosis, required further investigation due to the lack of research on its modes of action and its pathways. Furthermore, despite their relevance in investigations of the effects of salinomycin and the development of CSC-targeted treatment, adequate procedures for the isolation and identification of CSCs are lacking. Furthermore, for the development of new CSC-targeting strategies, a better understanding of the different characteristics of breast cancer stem cells and CSCs, such as the signaling pathways that regulate self-renewal and cell fate, is required. As previously stated, the complete eradication of breast cancer following therapy using hyaluronic acid-based drug delivery systems lends credence to the notion that medications targeting $CD44^{+}$will lead to more successful therapeutic techniques and may even completely eradicate cancer cells.

CONCLUSION

The intensity of tumor formation and drug resistance of BCSCs are important variables in BC metastasis and recurrence, as well as higher mortality. Salinomycin appears to reduce cell proliferation, invasion, and migration in BC, as well as reverse the immune-suppressive microenvironment, preventing tumor development and metastasis. Salinomycin is used either alone or in combination with natural substances, and it has an anti-proliferative impact on breast cancer malignancies through various pathways. The Stat3, autophagy, NF-kB, P-glycoprotein, EMT, Wnt signaling, ER stress, oxidative stress, altering membrane potential, and initiating caspase-mediated apoptosis, have all been demonstrated to be affected by SAL. The reviewed literature in this article emphasizes the recent advances of salinomycin-containing Nano-drug delivery systems such as SLNs, LPNPs, PLGA NPs, liposomes, PLHNPs, Nano micelles, etc, as a better option for increasing the effectiveness of DDS in tumor treatment. However, to date, these Nano formulations containing MT are used only in *in vitro* and *in vivo* cell line studies.

ABBREVIATIONS

CSC-Cancer stem cells, MDR-Multidrug resistance,TNBC-Triple-negative breast cancer, ER-Estrogen receptors, PR-Progesterone receptors, HEG-Human epidermal growth, IHC-Immunohistochemistry, MDA-MB-Metastatic mammary adenocarcinoma, BCS-Biopharmaceutics classification system, NP-Nanoparticles, HGC-Human chorionic gonadotropin, MRI-Magnetic resonance imaging, HT-Hormone therapy, EMT-Epithelial-mesenchymal transition, ABC transporter-ATP-binding cassette transporter, ALDH-Aldehyde dehydrogenase, ROS-reactive oxygen species, LEF-Lymphoid enhancer-binding factor, PI-Propidium iodide, LRP-Lipoprotein receptor, PARP-Poly adenosine diphosphate-ribose polymerase, PLGA-Poly D, L-lactic-co-glycolic acid, GTP-Guanosine-5′-triphosphate, PEG-Poly ethylene glycol, PDI-Polydispersity index, GIT-Gastrointestinal tract, HBMSC-Human bone marrow-derived mesenchymal stem cells, ADI-Acceptable daily intake, BCSC-Breast cancer stem cells

ACKNOWLEDGMENT

All the authors have contributed equally to the work done.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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