Perspectives of Nanoemulsion Strategies in The Improvement of Oral, Parenteral and Transdermal Chemotherapy

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Abstract: Background: Targeting chemotherapeutic agents to the tumor tissues and achieving accumulation with ideal release behavior for desired therapy requires an ideal treatment strategy to inhibit division of rapid growing cancerous cells and as an outcome improve patient’s quality of life. However, majority of the available anticancer therapies are well known for their systemic toxicities and multidrug resistance.

Methods: Application of nanotechnology in medicine have perceived a great evolution during past few decades. Nanoemulsion, submicron sized thermodynamically stable distribution of two immiscible liquids, has gained extensive importance as a nanocarrier to improve chemotherapies seeking to overcome the limitations of drug solubilization, improving systemic delivery of the chemotherapeutics to the site of action to achieve a promising inhibitory in tumor growth profile with reduced systemic toxicity.

Results and Conclusion: This review has focused on potential application of nanoemulsion in the translational research and its role in chemotherapy using oral, parenteral and transdermal route to enhance systemic availability of poorly soluble drug. In summary, nanoemulsion is a multifunctional nanocarrier capable of enhancing drug delivery potential of cytotoxic agents, thereby, can improve the outcomes of cancer treatment by increasing the life-span of the patient and quality of life, however, further clinical research and characterization of interactive reactions should need to be explored.

Keywords: Chemotherapy, nanoemulsion, active targeting, oral chemotherapy, intravenous chemotherapy, transdermal chemotherapy.

1. INTRODUCTION

According to the GLOBOCAN 2012, cancer is the major cause of morbidity and mortality worldwide [1-3]. There were around 14 million new cases of cancer and 70% rise in new cases is expected in the next 2 decades. According to the World Health Organisation (WHO), it has been estimated in 2015 that approximately 8.8 million deaths globally occurred due to cancer [4]. It leads to about 1 in 6 deaths globally. Lung, liver, colorectal, stomach, and breast carcinomas are the most common causes of cancer death, with incidences of 1.69, 0.788, 0.774, 0.754, and 0.571 million deaths, respectively [4]. With respect to gender, lung cancer is the most common form of cancer in men (3240 cases) followed by colorectal, nasopharynx, prostate, and stomach cancer in Malaysia based on the WHO Cancer Country Profile 2014 [5]. On the other hand, breast cancer is the most commonly diagnosed cancer among women (5410 cases) followed by cervix uteri, colorectal, lung, and ovary cancer. Besides that, lung cancer and breast cancer also have the highest mortality rate per 100,000 with age standardisation in men and women, respectively [5].
To reduce the mortality rate due to cancer, there are several cancer therapies available in the market, such as chemotherapy, immunotherapy, radiotherapy, targeted therapy, and surgery [2, 3, 6-8]. The main goal of the treatment is to increase the life span and improve quality of life of the patient by curing cancer [4]. A specific regimen of treatment, which consists of one or more modalities, is required for different cancer types. Hence, it is important to obtain the correct cancer diagnosis in order to achieve effective treatment on time [2]. For instance, chemotherapy will be specified to patients who have small cell lung cancer [9, 10], but most of the patients with non-small cell lung cancers (NSCLC) in stage I and II receive surgery whereas those in stage III and IV receive chemotherapy alone or with radiation [3, 10]. Other than chemotherapy and surgery, immunotherapy against T cells’ programmed cell death receptor, and targeted therapy drugs, such as anaplastic lymphoma kinase are used in NSCLC [3]. Alternatively, androgen deprivation therapy, prostatectomy, radiation, or combination are used to treat prostate cancer [3, 7]. Abiraterone and enzalutamide are the newer form of hormone therapy that are approved recently to treat advanced prostate cancer [3].

Besides that, transdermal or systemic route of administration of cytotoxic drugs are also available in cancer treatment when oral administration is not favourable. For example, topical and injectable formulations of 5-Fluorouracil (5-FU) is available in current market to enhance its systemic bioavailability and prevent adverse effects caused by its oral administration [11]. Although there are advancements in current therapies, the success rate is very limited [6, 12], which may be due to some inherent factors, such as poor aqueous solubility, low oral bioavailability, short half-life, serious side effects, lack of specificity, etc. [2, 13-15]. Similarly, use of hormone therapy can increase the risk in cardiovascular disease and also death [16, 17]. Hence, long term treatment using chemotherapy drugs is not suitable [3].

Although oral route is the most common and preferable routes of drug administration, low oral bioavailability or drug-food interaction can lead to failure in chemotherapy [18]. For instance, toxoids (docetaxel (DTX) and paclitaxel (PTX)) have poor water solubility and undergo rapid efflux due to high affinity to P-glycoprotein (P-gp), which ultimately lead to poor bioavailability [19]. Simultaneously, use of lycobetaine (LBT) in treating lung cancer is limited due to its short half-life (only 30 seconds in blood) [12]. Besides oral administration, systemic administration also shows some limitation due to low bioavailability. To illustrate this, tocotrienols (T3) used in skin cancer treatment have lipophilic nature and hence low water miscibility and absorption from the site of administration [20]. Less than 10% of bioavailability was achieved after a single bolus dose of 10 mg/kg T3. Patients feel uncomfortable due to pain at injection site because of frequent dosing because of rapid fluctuation of drug plasma level [18]. In addition, serious autoimmune-related side effects or even death by immunotherapy drugs (such as ipilimumab used in melanoma) may occur [3, 21]. Immune mediated toxicities such as pneumonitis and nephritis can also be caused by immunotherapy drugs for lung cancer treatment [3]. Surgery, radiotherapy, and some chemotherapies can lead to infertility as they affect the reproductive organs in males and females. For example, radical prostatectomy and radioactive iodine in thyroid cancer treatment can lead to erectile dysfunction [3, 22]. As a result, a more selective delivery system is needed in order to transport hydrophobic chemotherapeutic drugs to achieve a more successful therapeutic effect and target specifically on cancerous cells [2, 18, 23]. Connecting section of the article has been emphasized with the modern nanotechnology based drug delivery systems incorporated in cancer therapy.

2. TRENDS OF RECENT NANOTECHNOLOGICAL RESEARCH IN CANCER THERAPY

2.1. Different Nanocarriers in Cancer

Chemotherapy is the mainstay treatment for cancer, but it has a few setbacks typically in the use of conventional therapeutic agents. These agents are often accompanied by unwanted side effects and systemic toxicity, which hampers the use of the chemotherapeutics safely by the patients. Further, non-specificity causes them to target both healthy and cancerous cells which is another reason for the setbacks [23]. In recent years, important outcomes with acceptable pharmacological properties of various nanocarriers, such as micelles, liposomes, solid-lipid carriers, carbon nanotubes, nanoemulsions, nanoparticles, solid lipid nanoparticles, polymeric micelle, nanoemulsion, and dendrimers, have been explored and developed to cater for several illnesses, including tumor, and targeting therapies [24-31]. Modifications of physicochemical properties of the chemotherapeutics with nanocarriers ensures therapeutic success of the treatment, amongst lipid base nanocarriers, which received greater attention by the researchers worldwide.

Liposomes are spherical vesicles containing an internal aqueous core that stores hydrophilic drugs and is surrounded by phospholipid bilayer that stores hydrophobic drugs [32]. Examples of chemotherapeutic drugs in which liposomes are used as carrier to get better efficacy and reduced toxicities include doxorubicin (DOX) and cisplatin. Cardiotoxicity and neurotoxicity are the reported side effects that comorbid these drugs respectively [33, 34]. In cancer therapy, DaunoXome and Doxil are two known clinically-approved marketed liposomal based delivery system for the treatment of either Kaposi’s Sarcoma or both ovarian and recurrent breast cancer [35, 36]. A lyophilized liposome-based PTX (LEP-ETU) has also been developed by Zhang et al. to increase the drug’s solubility without affecting the particle size or precipitation of drug. An in vitro study of LEP-ETU has showed a <6% release of entrapped PTX, proving that the formulation is stable at physiologic temperature [37]. There are many other potentially effective liposomal formulations of chemotherapeutic drugs still undergoing pre-clinical and clinical trials.

Micelles are colloidal dispersions made from amphiphilic block copolymers. Compared to liposomes, they have a smaller size, which permits them to escape from circulation through the vascular damage present at tumor sites to target the tumor tissues. In addition, the poor lymphatic drainage at these sites which is also known as enhanced permeability and retention (EPR) effect contributes to the effectiveness of the entrapped cytotoxic agents [33]. There are a rising number of micellar-based formulations currently in the different stages of clinical trials, such as, NK911, SP1049C, NK105, and NC6004 [33]. Zhu and partners designed nano-sized
micelles that when present in water will produce a positively charged surface through the formation of cationic micelle with PDMAEMA-PCL-PDMAEMA triblock copolymer. Their aim is to successfully deliver VEGF siRNA and PTX chemotherapeutic drugs [38]. Recently, the addition of targeting ligand into these micellar-based formulations are in the rise and have successfully shown to be more cytotoxic particularly to the cancer cells. For example, ovarian carcinoma cells were more susceptible to the cytotoxic effects of micelles containing folate than the non-targeted micelles [39].

Dendrimers are highly branched macromolecules [40]. They possess host-guest entrapment properties through encapsulation process, electrostatic interactions, and covalent conjugations [28, 38], Poly(amidoamine) (PAMAM) and poly(propyleneimine) dendrimers have been widely studied as carriers for cancer drug delivery [41]. Sanyakamthorn et al. suggested that the encapsulation process involves hydrogen bonding between the cancer drugs and the -NH groups in the interior of PAMAM. The encapsulation of chemotherapeutics is also due to the hydrogen bonding and electrostatic interactions with dendrimers’ surface amino groups [40]. Han and partners introduced peptide HAIYPRH (T7)-conjugated PEG-modified PAMAM dendrimer (PAMAMPEG-T7) for the co-delivery of pDNA and DOX. Between individual DOX or pDNA delivery system and the co-delivery system, the latter initiated tumor cells apoptosis in vitro and suppressed tumor growth in vivo more effectively [38].

Polymer-drug conjugates have a biodegradable linker to covalently connect low water soluble drugs to water-soluble polymer, which enables it to accumulate at tumor sites via the EPR effect. PEGylated drugs such as Oncaspar and PEG intron have been marketed to use for the treatment of acute lymphoblastic leukemia and other cancers [42, 43]. Besides that, Trastuzumab emtansine which is a monoclonal antibody-drug conjugate is also an approved delivery system used to treat breast cancer [37].

2.2. Advantages of Nanoemulsion from Others

Toxicological evaluation of most of the nanocarriers has not yet been completely revealed, hence preclinical and clinical studies are required to identify any underlying toxicity of these carriers. On the other hand, nanoemulsions are heterogeneous dispersions of two-phase mixtures of insoluble liquids (water-in-oil/oil-in-water) stabilized by surfactants to prevent the dispersed phase and continuous phase from coalescing [44, 45]. Usually in nanoemulsion, the continuous phase is aqueous and the hydrophobic drug often is transported in the oil phase of the emulsion with a mean droplet size of 50-200 nm (Fig. 1) [14]. Oils and surfactants used in nanoemulsions have acquired safety recognition through the various testing, and only the GRAS (generally recognized as safe) components are more widely and successfully utilized by the researchers [46]. Generally, nanoemulsions are more superior than the other conventional dosage forms in terms of specificity in delivering the drugs to the target site, greater bioavailability and stability, availability in different formulations and many others [14].

Nanoemulsions’ ability to protect chemically unstable compounds from oxidative degradation and degradation by UV light are additionally advantageous for the drug stability [47]. A special feature nanoemulsions possess is the defense against certain bacteria, fungi and viruses. Accumulation of these droplets at the epidermis and dermis of the wound allows them to directly distort the organisms [48]. Besides that, the size of therapeutic nanoemulsions enables the droplets to cross through pores and hair follicles in a manner without disrupting healthy tissues, therefore, they have a low report for skin irritation [47]. Comparing nanoemulsions with macroemulsions and coarse emulsions, the former has greater surface area and free energy as well as the absence of complications such as inherent creaming, sedimentation and coalescence [6]. Nanoemulsions and liposomes possess a few common properties when delivered topically such as diminished side effects, and enhanced physical stability. However, liposomes are inferior to nanoemulsions in terms of the lesser cost of preparation [49]. In vaccine delivery, nanoemulsions are considered a better carrier as it is able to stay in the circulation for a longer period, and are highly taken up by antigen presenting cells. Shi et al. reported a study on the effectiveness of nanoemulsion vaccine in co-delivering immunostimulatory CpG and a gastric cancer-specific antigen MG7 against MG7-expressing cancer cell. The results showed that mice which were pre-immunized with nanoemulsion vaccine co-encapsulating MG7 and CpG had their tumor growth significantly inhibited [23]. In another example involving heterotropic and lung metastatic tumor models, higher concentration of LBT was found in the tumor site when PEGylated LBT–OA–nanoemulsion was administered as compared to free LBT. The inhibitory effect and survival time of the LBT nanoemulsion was also greater in comparison to the free LBT [46]. These results clearly suggest that nanoemulsion could be a suitable carrier for vaccine delivery in the immunization against cancer and other pathogenic organisms. Additionally, in comparison to suspension to hydrophobic drug nanoemulsion have greater advantage as Tagne et al. demonstrated that nanoemulsion of tamoxifen (TAM) have higher anticancer properties towards breast cancer as compared to TAM suspension. This is due to the greater zeta potential and smaller particle size of the nanoemulsion of TAM that helps with the increased drug permeability [50]. Formulating lipophilic drugs in oil-in-water nanoemulsion are advantageous because of its self-assembled nature that is inherently sensitive to its surroundings. On top of that, the oil phase of the nanoemulsion system helps to solubilize the lipophilic drug to be stored in it. Since the lipophilic drug’s solubility is increased, therefore lesser administration volumes are required to that of aqueous solution [51]. In this context, Chen et al., incorporation of ethyl oleate, Tween 80, or PEG400 formulations into nanoemulsions further increases the solubility of low water-soluble drug like Resveratrol thereby increasing the drug loading [52]. An important feature of nanoemulsion is that it can protect the encapsulated drugs from enzymatic degradation and hydrolysis as well as from the detection of macrophages. An added advantage of oil-in-water nanoemulsion formulation is that the encapsulation of hydrophobic drug prevents the immediate contact with body fluids and tissues thereby minimizing tissue irritation caused by any pharmacokinetic incompatibility.

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**Keywords:** drug delivery, nanoemulsion, chemotherapeutic, dendrimer, polymer-drug conjugate, nanoemulsion vaccine.
3. TUMOR MICROENVIRONMENT

Tumor microenvironment (TME) refers to the complex tumor tissues which are composed of extracellular matrix (ECM), activated fibroblasts, immune cells, pericytes, adipocytes, epithelial cells, glial cells, vascular cells, lymphatic endothelial cells, and proteins [23, 53]. The components of the TME support the physiology, structure, and function as an environment that promotes the tumor to progress into a malignant phenotype especially the ECM which support the growth, migration, invasion, and metastasis of tumor [23, 53]. The different type of cells of the TME have different cell-special markers which expressed on the cells surface and these markers can act as the target site for the drug delivery systems [23].

There are significant differences between tumor tissues and normal tissues from the following aspects: angiogenesis, vascular abnormalities, oxygenation, perfusion, pH, and metabolic states [54]. Angiogenesis is the process of the development of new blood vessels from pre-existing blood vessels. This process occurs normally during fetal development, tissue regeneration, wound healing and in the female reproductive cycle. However, angiogenesis also involved in the development of various diseases like cancer [54]. When the tumor tissue reaches over 2 mm³, the core of the tumor will expose to hypoxic condition. Hence, the tumor tissue will stimulate angiogenesis to ensure the presence of oxygen and nutrients for further growth [23, 53]. Moreover, it also helps the tumor tissues to remove waste products, delivers immune cells, macrophages, and humoral factors [55]. This indicates that tumor tissues depend on the angiogenesis for their active growing and this could be the potential target therapy for treating cancer. Based on this hypothesis, angiogenesis inhibitors are developed to inhibit this process. For examples, VEGF-neutralizing antibody: Bevacizumab (Avastin), Ramucirumab (Cyramza) [53-56] and VEGF signaling pathways blockers: Sorafenib (Nexavar), Sunitinib (Sutent), Pazopanib (Votrient) are the currently clinical available angiogenesis inhibitors for the treatment of various types of cancers [23, 54, 56]. These inhibitors prolong the survival rate of the responsive patients in terms of months but the toxicity, drug resistance and delivery problems remain as the main challenges for cancer therapy. Nanoemulsion delivery systems are current technology used to encapsulate the angiogenesis inhibitors and this system helps in reducing toxicity and improving the therapeutic efficacy [23]. Dehelean et al. had shown that the betulinic acid nanoemulsion can efficiently inhibit the angiogenesis process as this system increase the delivery efficiency [57].

Apart from that, the TME has pH of around 6 to 7, which is slightly acidic compared to healthy tissues and blood (pH 7.4). This difference provides a design internally controlled drug delivery system for cancer therapy. The drug in this system has been designed in order to stabilize at normal physiological pH however, rapidly destabilized in the acidic environment in the TME. The pH mediated cellular uptake of perfluorocarbon (PFC) nanoemulsions has studied by Patrick et al. in rat glioma cells with real-time pH measurement. They found out that intracellular pH changed from 6.7 to 5.5 over three hours’ uptake experiment and this indicated that the nanoemulsion migration from neutral cytosomes to acidic lysosomal compartments over this time period [58]. Nanoemulsion delivery system that used in the treatment for pH sensitive tumor have shown improvement in anticancer efficacy and reduced chemotherapy related toxicity [23].

4. MECHANISM FOR IMPROVED BIOAVAILABILITY WITH NANOEMULSION

The conventional chemotherapeutic agents exert its action by killing rapidly proliferating cells as well as divisible normal cells. This leads to systemic toxicity(ies) which are unwanted and unacceptable [23]. As discussed earlier, nanoemulsion nanocarrier can deliver either small hydrophobic drug molecules or other macromolecules like antigenic proteins and peptides. It can deliver locally or systemically to tumor resulting in potent humoral and cellular antigens-specific immune responses [59]. For instant, the delivery of MAGE-1 HSP70/SEA encapsulated within a nanoemulsion has shown an increase in the tumor-specific responses and protection when compared to the non-encapsulated delivery [60].

Moreover, nanoemulsion delivery system also increases the intratumoral accumulation of chemotherapeutics drugs which then reduce the therapeutic dose [23]. Cao et al. had proven that the co-encapsulated DOX and bromotetrandrine lipid nanoemulsion showed improved cytotoxicity due to increased intracellular uptake in DOX-resistance human breast cancer cells when compared to DOX lipid nanoemulsion [61]. Melphalan incorporated in nanoemulsion had shown higher values of bioavailability in treatment of ovarian cancer compared to the free drug as they enhance the retention time in the tumor [62].
4.1. Solubility Enhancement and Permeability Improvement

The unique structure of the nanoemulsion system is advantageous with improved solubility of drugs, because it provides both, lipophilic and hydrophilic environments. This delivery system also increases mucosal permeation, prevents P-gp efflux, prevention of premature degradation of drugs, decreases liver by-pass and thus increases availability of drugs in systemic circulation [29]. Further, improvement of bioavailability of incorporated drugs in nanoemulsion can be explained by improved solubility within the gastro-intestinal fluid, and decreased rate of gastric emptying [63]. Huge increase in the interfacial area due to the formation of nano structured dispersed droplets, and integration of permeation enhancers (e.g., D-α-tocopheryl polyethylene glycol 1000 succinate, hydroxypropyl-β-cyclodextrin, Lipoid E 80, sodium taurocholate, etc.) also contributes towards enhancement of drug bioavailability. These permeation enhancers aid to open the tight cellular junctions temporarily, and thereby promote permeation of the drug molecule [63, 64].

Delivery of drugs through transdermal route need to cross the outermost horny epidermal layer, stratum corneum. Percutaneous absorption of drugs is important to cross the dense impermeable layer. The interesting fact is that this stratum corneum swells several folds in the water than in dry condition. Thus, when delivered nanoemulsion formulation, the dispersed phase act as reservoir for the drug whereas the continuous phase ensures wetting of the skin layer [65]. All these attractive properties allow nanoemulsion to explore as potential delivery tool through different routes of drug administration via enhancement of solubility and permeability.

4.2. Lymphatic Transport

Due to formation of lipoproteins, several researchers have suggested facilitation of lymphatic transport of the lipophilic agents. Administration of nanoemulsion formulation into gastro-intestinal route allows formation of mixed micelles of the oil droplets with bile salts, and internalize into the enterocytes through clathrin mediated enterocytosis with the help of aqueous layer and mucin, and thus reach to the systemic circulation via portal veins or through the lymphatic system [66]. Correspondingly, other studies suggested that oil in water nanoemulsion will be better absorbed than the oil solution of corresponding lipophilic drugs [67]. Cho et al. had reported that dispersed oil droplets facilitate transportation of incorporated drugs including long chain fatty acids by digestion of the lipids. Thereby, digestion process aids in solubilisation and transportation of the drugs to the enterocytes and facilitate formation of chylomicrons within the enterocytes, which in turn facilitate transportation of lipophilic components into lymphatic channel. Therefore, the authors concluded that smaller droplet size in nanoemulsion facilitate absorption of lipophilic components into systemic circulation [68].

4.3. Passive Targeting

Since tumor tissues depend on angiogenesis for survival and metastasis, therefore tumor sites have poorly developed tumor vasculature. This leads to pericyte deficiency, abnormal basement membrane and fenestration that accounts for the increased in vascular permeability [53]. This causes raise in interstitial fluid pressure and unevenness of blood flow, oxygenation, nutrient, and distribution of drug in the TME, which lead to increase hypoxia and thus, facilitates metastasis. The tumor cells are lack of lymphatic system, which leads to increase in the retention time of drug as their growth compresses the lymph vessels especially in the central portion of the tumor and causing collapse. This leads to the poor lymphatic drainage from tumor cells and causes delays in the clearance of macromolecules, which accumulated in the solid tumor tissues. The combination of increase vascular permeability and poor lymphatic drainage is known as the EPR effect. This effect serves to deliver several nanotechnological delivery effectively to the tumor tissues (Fig. 2) [23]. However, EPR effect can be achieved via intravenous delivery of the nanoemulsion, where the nanoemulsions accumulate within the tumor microenvironment by this effect [53].

The EPR effect is mainly observed for biocompatible

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Fig. (2). Nanoemulsion–based drug delivery exploiting tumor microenvironment by passive targeting mechanism.
macromolecules such as macromolecular drugs and lipids. The mechanism of EPR effect for macromolecules are retention whereas for low molecular weight is the returning to blood circulation by diffusion [29]. Generally, the ideal size of nanoemulsion is to control within the diameter range of 20-100 nm in order to ensure the accumulation of the drugs in tumor tissues but not penetrate to normal vessel walls to decrease side effects towards normal cells (Fig. 2) [14]. Their small size helps in avoiding them from mononuclear phagocytic system (MPS) in the liver but their size is also large enough for them to preclude fast renal clearance. Hence, this indicates that the particle with this size are able to circulate in the blood for longer period. For increasing the circulation half-life and therapeutic effect, the surface of the nanoemulsion can be modified with the hydrophilic polymers, like polyethylene glycol (PEG), poloxamer, etc. It can suppress the blood protein adsorption and recognition by MPS cells, thereby facilitate EPR [23].

Furthermore, other researches revealed that the positively charged particles are more easily taken up by tumor cells and retained for a longer time as compared to negatively charged or neutral particles. This is because the surface of tumor cells is translocated with a negatively charged residue which is known as phosphatidyl serine [23]. The encapsulated daunorubicin with dextran and chitosan directly on nanoemulsion liquid core formed positively charged particles and localized delivery showed improved antitumor efficacy against colon MC38 cancer cells. This is due to the electrostatic interactions between the positively charged nanocarrier with the negative charged cancer cells [69].

However, passive targeting also has various limitations like inability to actively distinguish between normal tissues from tumor tissues. This is because the passive targeting is not feasible in all tumors as different tumor types have different degree of tumor vascularization and fenestration of blood vessels [23].

4.4. Active Targeting

Attaching various ligands on the surface of the nanoemulsion can help to recognize active target within the tumor and/or affected organ, tissue, cells or intracellular organelles and this mechanism is known as active targeting [23]. The ligands commonly used are monoclonal antibodies or antibody fragments, antigen binding fragments, and single chain variable fragments [70]. The active targeting delivers the drug to the tumor site by binding to the receptors or substrates that express on the target cancer cells such as engineered antibodies, folic acid, transferrin, enzymes like hyaluronic acid (HA) and aptamer which is a short DNA sequence (Fig. 3) [23, 70, 71]. However, this mechanism needs the exclusive expression of receptors on the target cancer cells and the expression should be homogenous across all targeted cells. The active targeting mechanism increases the cytotoxicity to tumor tissues and reduce the delivery of toxic drugs to normal tissues [23]. Like passive targeting mechanism, this active targeting is also limited to parenteral delivery of the adorned nanoemulsions [29]. Ganta et al. had shown that the folate-targeted nanoemulsion improved its cytotoxicity by decreasing 270-folds in the concentration of DTX needed for inhibiting growth of cells by 50% (IC50) when compared to DTX alone. This proved that the folate-targeted nanoemulsion can be designed to target the folate receptor-positive ovarian tumors [72]. The PTX loaded polyurethane nanocarrier decorated with folic acid (PTX-PU-NP-FA) had shown about 90% of fraction of apoptotic cells in tumors and this indicates the effectiveness in therapeutic effect of folate-targeted nanoemulsion [73]. Afizal et al. had proven that the transferrin targeted-DTX lipid nanoemulsion reduce the systemic toxicity but improve the antitumor activity when compared to free drug [74].

Therefore, combination of surface charge modification and stimuli sensitivity like sensitivity towards pH, enzyme, magnetic field, temperature, ultrasound, and UV light can also be a part of active targeting [23]. Jiang et al. incorporated the cationic polymer chitosan and target ligand biotin on the surface of PLGA nanocarrier. They evaluated the effect of surface charge of nanoparticle along with targeting ligand on the cellular uptake and found in increased cellular uptake by the synergistic effects of electrostatic interaction and receptor-mediated internalization [75]. Hence, it can be concluded that the increased cellular uptake is not affected by the size of nanocarrier, besides this is able to facilitate the tumor cell adsorption and tissue retention of drug as the acidic TME increases its zeta potential.

5. OUTCOMES OF RECENT NANOEMULSION BASED CHEMOTHERAPY

Delivery of nanoemulsion can be approached by different routes of administration to obtain better efficacy and bioavailability with decreased toxicities of cytotoxic drugs. Such routes involve oral, parenteral, and transdermal. The following section will provide evidences of improved delivery of nanoemulsion in different delivery routes.

5.1. Delivery of Nanoemulsion Using Most Preferred Oral Route

In the recent years, the development of nanoemulsions-based delivery system via oral route has gained attentions in the treatment of cancer. Nanoemulsions aid in drug delivery system by protecting and delivering lipophilic drugs by coating and preventing them from being damaged by several environmental factors like pH and hydrolytics enzymes [47]. Oral route administration become the most commonly used route for systemic delivery because it is convenient, cheap, easy to use, and preferentially preferred by patients. However, oral administration of anticancer drugs becomes a challenge due to its low oral bioavailability, poor solubility, stability, and high drug efflux through P-gp transporters in the lumen [14, 76]. This can overcome by introducing nanoemulsion delivery system in order to enhance solubility, stability, absorption, and bioavailability of drug. There is a wide variety of anticancer drug used to deliver using nanoemulsion delivery system to increase oral bioavailability. Pangeni et al. used oxalaplatin (OXA) in combination with 5-fluorouracil (5-FU) for the treatment of resectable and advanced colorectal cancer. Due to poor membrane permeability of OXA, deoxycholic acid derivative (DCK) is used as a permeation enhancer in order to improve oral bioavailability. The results of membrane permeability test in Caco-2 cell monolayer showed 4.80- and 4.30-fold increase in mem-
brane permeability of OXA/DCK and 5-FU in comparison to free OXA and 5-FU. Simultaneously, the oral bioavailability of OXA/DCK and 5-FU in the nanoemulsion system were 9.19- and 1.39- folds greater than those OXA and 5-FU, respectively. Thus, using of oral nanoemulsification technique the absorption of OXA/DCK and 5-FU has been increased, resulting in inhibition of growth of tumor in the colorectal cancer in mice in in vivo model [77].

Li et al. developed oil-in-water nanoemulsion system of berberine hydrochloride (BBH) to enhance its permeability, stability and oral bioavailability. In bioavailability study, the BBH nanoemulsion showed 440.40% relative bioavailability in rat as compared to unencapsulated BBH. In addition, the intestinal permeability of BBH increased when tested with Caco-2 cell monolayers. The efflux of BBH through P-gp transporters in the lumen also decreased which indicates that oil droplets of the nanoemulsion aids in improving absorption of BBH [78]. Besides that, Zhang et al. used low-energy emulsification method to develop nanoemulsion for pterostilbene due to its low solubility and stability. Based on the solubility and stability test, the nanoemulsion delivery system enhanced the solubility and stability as well as in vitro release of pterostilbene also improved (96.5% in pH 3.6 buffer; 13.2% in pH 7.4 buffer) compared to pterostilbene suspension (<21.4% in pH 3.6 buffer; 2.6% in pH 7.4 buffer) [79].

Due to some undesirable side effects and poor oral bioavailability DTX, Verma et al. developed oral nanoemulsion delivery system of DTX to improve the chemotherapy. Developed nanoemulsion showed 2.83 times higher cellular uptake than control. According to the results, there is a strong cytotoxic activity against MCF-7 cancer cell line, which confirmed DTX to be more effective in this formulation. When single high dose of DTX nanoemulsions (520 mg/kg) is administered, there was no specific toxicity or necrosis in the liver and kidney tissues observed in mice, indicating it as safe for further action [19]. In another study by Pandey et al., DTX loaded nanoemulsion was formulated with additional loading of P-gp inhibitor in order to increase the bioavailability of the drug. The formulation exhibited higher uptake in Caco-2 cells and inhibited P-gp transporter significantly. In MDA-MB-231 cells, it shows less IC50, arrested the cells in G2-M phase and showed more cell apoptosis than marketed formulation (Taxotere®). Based on the bioavailability test, there was an increment in oral bioavailability, resulting in higher in vivo anti-proliferative activity manifesting 19% more inhibition compared to Taxofere® [80].

Similarly, hydrophobic drug- PTX always face challenges in achieving effective delivery due to its low solubility nature and high hemolytic toxicity. Thus, nanoemulsion is used to enhance the condition as well as to achieve safe and better delivery. Pawar et al. incorporated PTX into a vitamin E nanoemulsion using high-pressure homogenization. When tested in MCF-7 breast cancer cell line, PTX loaded nanoemulsion showed greater cytotoxicity in comparison with free PTX and marketed formulation (Taxol®). There was also significant improvement in efficacy of PTX loaded nanoemulsion in in vivo anticancer activity compared to pure PTX and Taxol® [81]. Similar results have also been accomplished in our previous experiment on PTX nanoemulsion [14]. In another study, Ahmad et al. used solid-nanoemulsion pre-concentrate system to improve the bioavailability of PTX. Results appeared to show an increase in in vitro cumulative drug release as well as permeability coefficient through everted gut sac in compared to pure drug suspension and commercial intravenous product (Intuxel®). The spherical globule size of nanocarrier provides high membrane permeability. Solid nanoemulsion preconcentrate of PTX also showed strong inhibitory effect on proliferation of MCF-7 cells. Hence, solid nanoemulsion pre-concentrate can be used as an oral dosage form as it can enhance dissolution and oral bioavailability of PTX [82]. To sum up, nanoemulsion and/or solid-nanoemulsion pre-concentrate system are
successful in improving oral bioavailability of hydrophobic drugs. Ganta et al. evaluated the efficacy of co-administration of PTX and CUR, an inhibitor of nuclear factor kappa B (NFkappaB) and P-gp in nanoemulsion formulation to SKOV3 tumor-bearing mice. The mice pretreated with CUR showed a 4.1-fold increase in PTX plasma concentration, which suggest increased PTX cytotoxicity in ovarian adenocarcinoma [83].

Sunitinib is an anticancer drug where it works by inhibiting proliferation of tumor cells and angiogenesis. In one study, Nazari-Vanani et al. developed self-nanoemulsifying drug delivery system (SNEDDS) as a carrier for sunitinib. Sunitinib released from SNEDDS was found to be enhanced accompanied by controlled dissolution of the drug. According to the cytotoxicity studies, the results showed an improved toxicity of sunitinib by SNEDDS when tested in 4T1 and MCF-7 cell lines. In bioavailability study, the maximum plasma concentration and the mean under the plasma concentration-time curve had increased by 1.45- and 1.24-times respectively, when compared to the drug suspension [84]. Tamoxifen (TAM) is also another lipid-soluble drug with low aqueous solubility; hence, Tagne et al. prepared a water-soluble nanoemulsion of TAM. When compared between nanoemulsion of TAM and pure TAM, nanoemulsion can inhibit the proliferation of tumor cells 20-fold greater as well as increased cell apoptosis 4-folds greater in the HTB-20 breast cancer cell line. In conclusion, the nanoemulsion of TAM increases anticancer properties of TAM in treatment of breast cancer [50].

Curcumin (CUR) from Curcuma longa has low aqueous solubility and low oral bioavailability. Therefore, in order to increase the oral absorption of CUR, Guan et al. prepared CUR nanoemulsion to target prostate cancer. The results showed that CUR nanoemulsion increased cytotoxicity and cell uptake as well as exhibited prolonged biological activity, showed better therapeutic efficacy compared to pure CUR. In in situ single-pass perfusion studies, CUR nanoemulsion has higher effective permeability coefficient and absorption rate constant than free CUR. Therefore, this study proved that CUR nanoemulsion is useful as an effective drug delivery system to improve oral bioavailability and anticancer effect of CUR [85]. In another study, Sun et al. developed functional nanoemulsion-hybrid lipid nanocarriers containing diferuloylmethane (DNHLNs) to treat lung adenocarcinoma. The absorptive constants and penetrability of DNHLNs in four gastrointestinal sections increased by 1.43-3.23 times and 3.10-7.76 times compared to free diferuloylmethane (DIF), respectively. Thus, the relative oral bioavailability of DNHLNs to free DIF was 855.02% and it has stronger inhibitory effects on viability of lung adenocarcinoma A549 cells than free DIF [86].

Fofaria et al. have developed two nanoemulsion formulations of pipplarine (PL) for oral delivery and then assess its toxicity, pharmacokinetics and therapeutic efficacy. Both nanoemulsions improved dissolution, cellular permeability and cytotoxic effects in comparison with plain PL. Upon long-term administration through oral route, the developed formulations did not show toxicity when tested in mice. Pharmacokinetics study of PL was conducted, which followed two-compartment model following intravenous administration. PL loaded nanoemulsions showed 1.5-fold raise in oral bioavailability as well as increased in anti-tumor activity and hence suppressed melanoma tumor growth in experimental mice [87].

Genistein (Gen) has anticaner activities in oral cavity and oropharyngeal cancer. However, its usage is limited by low aqueous solubility and extensive metabolism, causing it to have low oral bioavailability and pharmacokinetics profiles. Therefore, Gavin et al. developed nanoemulsion loaded with proapoptotic lipophilic Gen as a mucoadhesive buccal tablet to target proliferating oropharyngeal cancer. Chitosan was added as a layering to overcoat Gen-containing nanoemulsion in order to enhance mucoadhesion properties. The chitosan polyelectrolyte solution overcoat rendered nanoemulsion droplets cationic, by acting as a mucoadhesive nanoemulsion layer. Biocompatibility screening of prototype chitosan-layered nanoemulsions showed better anticancer activity against oropharyngeal carcinomas [88]. Above discussions on oral delivery of nanoemulsion suggest that nanoemulsion can improve solubility of poorly water soluble drugs by dissolving it in the oil core and can improve oral bioavailability by delivering the drug entrapment within the oil core.

5.2. Parenteral Approach to Deliver Nanoemulsion for Cancer Therapy

Nanoemulsion is an ideal for loading active pharmaceutical ingredients for parenteral drug delivery as they have the potential to achieve targeted drug delivery through the addition of specific ligands. We have seen that nanoemulsion can increase the solubility of hydrophobic drugs, and provide protection from enzymatic degradation and hydrolysis. Furthermore, their small, uniform droplet size provides a large surface distribution, leading to an increase in API concentration in the human body [53, 89]. An extensive research has been performed on nanoemulsion for cancer chemotherapy. A study conducted by Chen et al. to compare the efficiency of LBT when formulated with oleic acid (OA) as a nanoemulsion (LBT-OA-NE) and a liposome (LBT-OA-Lipo) for lung cancer therapy [12]. The formulations were also investigated in the presence of PEGylated lecithin; PEGylated nanoemulsion (LBT-OA-PEG-NE) and PEGylated Lipo (LBT-OA-PEG-Lipo). A therapeutic adjuvant, nRGD was also used in PEGylated Lipo (LBT-OA-PEG-Lipo-nRGD). The study demonstrated that LBT-OA-PEG-Lipo had the highest cytotoxicity actions towards cancer cells. OA, in the presence of sodium bicarbonate, forms an ion-pair complex with enhanced lipid solubility, as the ammonium group on the LBT ionizes the carboxyl group on OA. This allows the nanoemulsion to entrap larger amounts of LBT [12, 90]. However, liposomes were shown to entrap more LBT-OA compared to NE, as shown by the oxygen content of LBT-OA-Lipo (31.10%) and LBT-OA-NE (25.47%). Further, PEGylated lecithin acts as a protective measure for drug carriers, reducing recognizing them as foreign bodies by MPS. This decreased rate of removal of the compound from the systemic circulation [12]. The result at the 8th hour had depicted the lowest cumulative amount of LBT release (56.2%) from LBT-OA-PEG-Lipo compared to LBT-OA-Lipo (61.3%), LBT-OA-PEG-NE (77.8%), and LBT-OA-NE (86.5%). Lastly, the addition of nRGD further en-
hances the cytotoxicity effect of LBT-OA-PEG-Lipo. nRGD acts on integrin receptors and neuropilin-1, improving the build-up of the drug in tumors [91].

Natesan et al. developed a nanoemulsion formulation of CPT, by stabilizing with chitosan [92]. The resulting stabilized nanoemulsion produced a uniform droplet size (46-54 nm vs. 39-58 nm), a prolonged period of drug release (61.65% vs. 58.87%) and lower haemolytic activity (16.4 ± 1.4% vs. 17.6 ± 1.2%). The stabilized nanoemulsion also showed higher cytotoxicity (285 ± 11ng/ml vs. 178 ± 4.3 ng/ml) towards human breast cancer cells (MCF-7). When administered intravenously to BALB/c mice with 4T1-breast tumor, the stabilized nanoemulsion showed improved levels of passive targeting and bio-distribution, at 2495.22 ± 174.66 ng/gm compared to non-stabilized nanoemulsion (1677.58 ± 134.21 ng/gm). This occurs as the stabilized nanoemulsion experience and increase in circulation time, improving the uptake of CPT into cancer cells due to EPR [51]. Chitosan, having a pKa value of about 6.5, will only swell and enlarge in the acidic tumor environment, thereby accelerating the rate of CPT release [93]. This shows that the stabilized nanoemulsion improved the solubility and targeting capacity of CPT, thereby improving its clinical use for breast cancer.

When PTX was co-encapsulated with baicalein (BA) in nanoemulsion, a reduction in PTX resistance was observed, as BA possibly suppresses the activity of P-gp and induce oxidative stress. The nanoemulsion exhibited increased cytotoxicity towards MCF-7/Tax cells compared to free PTX due to the combined effect of PTX and BA. It is also shown that the cellular uptake of the nanoemulsion was higher on MCF-7/Tax cells compared to free PTX due to nanoemulsion-mediated endocytosis. The observed increase in uptake could be due to the inhibition of P-gp by BA. A significant increase in intracellular reactive oxygen species (ROS) was also observed in MCF-7/Tax cells when treated with the nanoemulsion, as BA increases the production of intracellular ROS. This overproduction of intracellular ROS contributes towards the synergism of PTX and BA. The administration of the nanoemulsion also greatly reduces the intracellular glutathione levels and elevates caspase-3 activity, which attributed to increased apoptosis (Fig. 4) [94]. The results of the study suggest that the PTX/BA nanoemulsion could function as a reversal agent for tackling drug resistance. Besides that, PTX is having low water solubility that restricts its use in treatment. Kim et al. formulated a novel nanoemulsion, PTX-loaded hyaluronic solid nanoemulsion (PTX-HSNs) to investigate on the efficacy by enhancing tumor targeting. Cytotoxic effect of PTX-HSN is enhanced due to prolonged level of PTX in blood leading to PTX accumulation in ovarian cancerous cells, thereby, suppresses tumor growth [95].

The non-steroidal anti-inflammatory drug, ketoprofen (KP) when developed as a nanoemulsion showed higher photo-stability and increased solubility. The nanoemulsion consists of KP and pullulan (as a stabilizer) encapsulated in pomegranate seed oil (PSO), protected against photodegradation. This is due to the ability of PSO to confine KP in oil droplets, resulting in the dispersion of light, hence increasing photo-stability. When the nanoemulsion was incubated with C6 cells, demonstrated increased anti-tumor activity by inhibiting 40% of cell growth, which is significantly more than blank nanoemulsion. The increased activity can be attributed to the smaller size of the nanoemulsion, leading to an increased contact area with the C6 cells, which allows an increase in the inflow of KP. Therefore, the KP and pullulan nanoemulsion encapsulated with PSO has shown to be favorable for the treatment of glioma [96].

In 2016, Monge-Fuentes et al. investigated the potential of acai oil as a nanoemulsion (NanoA) for the photodynamic therapy (PTD) of melanoma [97]. The study utilized acai oil as a photosensitizer (PS) to induce the death of melanoma cells. The results showed an 85% melanoma cell death and a decrease in tumor size by 82% in tumor bearing experimental mice as compared to the control. It has been explained that the presence of polyphenols in acai oil generates ROS and producing irreversible photo-damage to melanoma cells. Small size of the acai oil droplets enables increased permeability into tissues and subsequently this increases cellular uptake [98].

Based on a study conducted by Ahmad et al. the encapsulation of omega-3 fatty acid conjugated with taxoid produg (NE-DHA-SBT-1214), a hydrophobic drug to treat prostate cancer, in nanoemulsion shows increased cytotoxic effect in nanoemulsion compared with aqueous solution of DHA-SBT-1214 in vitro in human prostate cancer cell line, PTT2 cells [99]. The increase in effect is correlated with pharmacokinetic and biodistribution which is found to be caused by the reduction in particle size rather than the characteristic of the encapsulated drug. Besides that, tumor growth is suppressed extensively when NE-DHA-SBT-1213 is administrated intravenously into NOD/SCID mice with PTT2 tumor xenografts weekly when compared with Abraxane as well as placebo nanoemulsion formulation [99]. On the other hand, PEG-modified nanoemulsion improved the duration of drug present in the circulation. Consequently, this allows accumulation of the drug at the tumor site due to EPR effect [100]. Therefore, nanoemulsion based delivery system is having higher efficacy in treating prostate cancer by enhancing superior regression and inhibition of tumor growth.

The compound α-tocopherol succinate (α-TOS), derived from Vitamin E and succinic acid contains anti-tumor properties but has low bioavailability. Gao et al. has shown that when α-TOS is formulated as a nanoemulsion (α-TOS-NE), the bioavailability increases [101]. This is seen due to higher AUC_{0→∞} of α-TOS-NE compared to free α-TOS. The bioavailability was further improved when administered intravenously. The increased bioavailability is reflected by the presence of small, uniform size of the nanoemulsion, and the capability of the nanoemulsion to deliver α-TOS to serum lipoproteins, the binding protein responsible for the transport of α-TOS into tumor tissues [102]. The α-TOS-NE also demonstrated higher cytotoxicity towards tumor cells compared to free α-TOS. As the method of cell apoptosis induction is mediated by the mitochondria, when the mitochondria is damaged, depolarization occurs, lowering the mitochondrial membrane potential (ΔΨ) [103]. Simultaneously, a lower ΔΨ was seen when α-TOS-NE was administered.

Similarly, Loureiro et al. showed that carbon monoxide releasing molecule-2 (CORM-2), when formulated as a
nanoemulsion tagged with folic acid showed higher anti-tumor activity and prolonged survival lymphatic cancer induced experimental animal [104]. CORM-2 can induce apoptosis and block the proliferation of tumors. The increased anti-tumor activity of CORM-2 is due to the increased uptake of the nanoemulsion into the tumor bearing cells, as folic acid on the nanoemulsion recognizes the folate receptor present on the cells. Besides that, the increased uptake increases the ability of CORM-2 to induce the anti-Warburg effect, prolonging the survival of mice with A20 lymphoma tumors. The presence of carbon monoxide compels cancer cells to use more oxygen, leading to cellular exhaustion and death [105].

Nanoemulsions have been shown to be promising alternatives to conventional chemotherapy. The increasing amount of studies being carried out have shown an overall increase in anti-tumor activity, extended drug release and circulation, and could also be the key in tackling MDR.

5.3. Transdermal Delivery of Nanoemulsion in Cancer Therapy

Researches have now focused more on areas of transdermal delivery of nanoemulsion in chemotherapy as it is well established and accepted as alternative route of administration. Taking in consideration of the bioavailability of drug via oral route, the first pass metabolism and gastrointestinal P-gp efflux reduces the amount of drug in systemic circulation and thereby lacking its desired goal. Patient receiving chemotherapy has substantial risk of nausea and vomiting, making them feeling extreme discomfort and inconvenience. Hence, transdermal application can help increase tolerability towards GI irritation. Introduction of drug delivery through the skin is appreciably important, as it can possibly avoid first pass metabolism and to alleviate the discomfort when injected. As compared to other route, transdermal application is beneficial for long-term treatment of chronic pain as the skin provides a larger surface area for absorption [106].

The current marketed formulation of 5-FU is in topical and injectable route. This is because oral administration exhibits serious adverse effect and low bioavailability. However, the permeability through lipophilic human stratum corneum has risen issue for dermal/transdermal administration due to the hydrophilic characteristics of 5-FU. Therefore, many trial and error were carried out to find the suitable carriers of hydrophilic drugs [11]. Recent studies show the dispersion of oil based nanocarriers like nanoemulsions and microemulsions are rather stable for transdermal/topical delivery of many lipophilic drugs compared to other preparation compound.

A study conducted by Shakeel et al. is to develop w/o nanoemulsions of hydrophilic drug 5-FU for topical chemoprevention of skin cancer using low HLB surfactants [11]. Lauroglycol-90 is used as an oil phase while Transcutol-HP and IPA are selected as the surfactant and co-surfactant due to low HLB value the favors the formation of w/o nanoemulsions [107]. The permeation data analysis performed concluded that the formulated nanoemulsions were extensively superior when compared to the controlled aqueous solution of 5-FU [11]. In vitro cytotoxicity study on SK-MEL-5 melanoma cell line was conducted to compare the therapeutic effect of the formulated nanoemulsion. As expected, melanoma cells were resistant toward the free 5-FU solution due to the absence of cell inhibition. However, free 5-FU shows 5.40±0.32% of cytotoxic effect at molar concentration of 120 μm. With the same concentration of 5-FU in nanoemulsion, the cytotoxicity increases by approximately 11 folds which indicates the potency and efficacy of the nanoemulsion in chemotherapy against skin cancer. Based on the obtaining results, the nanoemulsion formulation is more effective in chemopreventive of skin cancer than free 5-FU [11].
To evaluate the effectiveness of Tocomin nanoemulsion as an anti-proliferative agent, a time-dependent profile was plotted against both human keratinocyte cancer cells, A431 and SCC-4. A positive result was obtained for both controls while Tocomin hybrid nanoemulsion showed a prominent cell inhibition effect over a period of incubation (up to 96 hours). Cell inhibition was superior when using Tocomin hybrid nanoemulsion (p≤0.05) and the IC50 values in A431 and SCC-4 were 42.6±3.8 mM and 47.3±3.2 mM, respectively. Hence, with all the data obtained, the hybrid-nanoemulsified delivery system has substantiated a great potential therapeutic benefits for such topical delivery [20].

The following study is regarding the use of carbopol-based nanoemulsion gel of apigenin for UV-induced skin carcinoma. Apigenin is a naturally occurring plant belonged to the flavone class. It selectively inhibits the cell growth, induces apoptosis in cancer cells, and most importantly, impede the invasion process [108]. In the cytotoxicity experiment, MTT assay was carried out on HaCaT cells and A431 cells. After that, 25μL of apigenin, plain drug loaded gel and apigenin-loaded nanoemulsion gel are incubated for 24 hours. The ex-vivo skin permeation test analysis reveals that the amount of apigenin permeated through skin is from nanoemulsion gel was at value of 6.68±0.46 μg/cm² h⁻¹, significantly higher than the pure apigenin and plain drug loaded gel 5.43±1.02 μg/cm² h⁻¹ and 5.60±0.63 μg/cm² h⁻¹ respectively [109].

Based on a research conducted by Pathan et al., transdermal permeation and bioavailability of TAM increased remarkably in vitro and in vivo, respectively. Increased permeation might occur due to droplet size and viscosity which facilitate percutaneous TAM uptake while increased bioavailability is due to increased skin permeation and by-pass first pass metabolism [110]. Nanoemulsion enhance cytotoxicity of TAM by avoiding first pass metabolism and increase membrane permeation. Another anticancer drug, caffeine in nanoemulsion, has caught Shakeel and Ramadan’s research attention. The hypothesis developed in this study is efficacy of transdermal application of nanoemulsion of caffeine in preventing ultraviolet light induced skin cancer. The investigation performed through in vitro studies on Franz diffusion using animal rat skin as permeation membrane. A histopathological examination of a normal skin versus nanoemulsion treated skin specimens were evaluated and the photomicrograph indicates nanoemulsion is safe for transdermal delivery of caffeine as there is no signs of inflammation or edema on the stratum corneum [111].

Neuroblastoma is a cancer commonly found in infants and young children that affects the immature or developing of cells [112]. According to the reported neuroblastoma cell culture studies, anti-oxidant synergy formulation can induce differentiation and buffer neuronal degeneration and oxidative stress in cultured cortical neurons and in central nervous system tissue of apolipoprotein E-deficient mice. The main goal of the study by Kuo et al. was to investigate the comparative efficacy between subcutaneous injection and transdermal application of a nanoemulsion preparation of ASF in reducing tumor growth rate using a neuroblastoma xenograft mouse model. The data proposed that ASF suspension is fairly ineffective when compared to nanoemulsion in the treatment of neuroblastoma mouse. However, the conclusive result shows that both subcutaneous and transdermal route of nanoemulsion are as effective with an average reduction of 65% in tumor growth rate in this neuroblastoma mouse model [113].

Therefore, the delivery of chemotherapeutic agents through skin via nanoemulsion could be advantageous for local as well as systemic delivery of drugs and further effectively treat the cancers with increased safety profile. In summary, nanoemulsion is employed to overcome physiological and anatomical barriers as well as engineered to prolong anti-cancer drug in blood circulation and improves target binding ability. As a result, the cytotoxic effect of poorly soluble anti-cancer drugs is improved by enhancing solubility of drugs, providing physicochemical improvement and protection against enzymatic degradation and P-gp efflux, thereby facilitate cellular uptake and promoting controlled-release chemotherapy. Various nanoemulsion approaches against different cancers have been summarized in Table 1.

CONCLUSION AND FUTURE PERSPECTIVES

Conventional cytotoxic agents are often hydrophobic in nature, which contributes to low aqueous solubility and serious adverse effects, thereby, reducing its use as well as its efficacy. Therefore, utilization of nanotechnology in cancer treatment is significantly important. Among various types of nanocarriers, nanoemulsion is prominent due to its capabilities in improving stability of unstable chemicals by protecting them against oxidative, UV light degradation, distorting organisms such as bacteria, fungi and viruses directly, reducing incidence of skin irritations and adverse effects as well as prolonging the agents in the body by controlling drug-release pattern. There are several routes of administrating cytotoxic agents, which include oral, parental, and transdermal. The incorporation of nanoemulsion formulation possess several advantages in drug formulation. Enhancement of solubility further pharmacokinetic and pharmacodynamics properties of poorly water soluble drugs by means of nanoemulsion formulation through different routes of administration, targeting properties in chemotherapy, desirable release profile, drug uptake control, avoidance of drug resistance, prolonged efficacy with improved safety profile make the nanoemulsion unique in recent formulation development field. Availability several of preparation methods, appreciable thermodynamic stability and accessibility diversified safe oil, surfactants, co-surfactants and combinations for nanoemulsion make it more flexible with wide valued application. As a result, the efficacy of the chemotherapeutic agents’ increases via active and passive targeting of the nanocarrier to the site of action, which further provide promising decrease in systemic toxicity. In conclusion, nanoemulsion is a good candidate in enhancing drug delivery of cytotoxic agents, thereby, improving the outcomes of cancer treatment by increasing life span of the patient, and quality of life.

Nanotechnology is a potential drug-delivery system to be employed in our daily life especially in healthcare setting [46]. Cost effective well-designed targeted nanoemulsion with improved safety profile are developing to address several
Table 1. Representation of nanoemulsion based approaches for parenteral, oral and transdermal treatment of various cancers.

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Drug Name</th>
<th>Method Used for Preparation</th>
<th>Cancer Type</th>
<th>Targeting Ligand</th>
<th>Cell Line/Animal</th>
<th>Outcome of the Study</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenteral</td>
<td>LBT</td>
<td>Homogenization</td>
<td>Lung cancer</td>
<td>nRGD and PEG</td>
<td>LLC cells, A549 cells and B16 cells</td>
<td>Increased LBT entrapment capacity with extended drug release and circulation time. Nanoeulsion showed enhanced anti-tumour effect on cells.</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>CPT</td>
<td>Sonication</td>
<td>Breast cancer</td>
<td>-</td>
<td>MCF-7 and 4T1 cell lines</td>
<td>Stablized nanooemulsion showed uniform droplet size and extended drug release with lower haemolytic activity and higher cytotoxicity along with higher passive targeting.</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td>PTX and baicalein (BA)</td>
<td>High-pressure homogenization</td>
<td>Breast cancer</td>
<td>-</td>
<td>MCF-7 and MCF-7/Tax</td>
<td>Loaded nanoeulsion enhanced cellular uptake, cytotoxicity and apoptosis due to inhibition of P-glycoprotein and increased oxidative stress.</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>KP</td>
<td>Solvent diffusion</td>
<td>Brain cancer</td>
<td>-</td>
<td>C6 and 3T3 cells</td>
<td>Nanoeulsion increased drug photo-stability as well as anti-tumour effect.</td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>Acai oil</td>
<td>-</td>
<td>Skin cancer</td>
<td>-</td>
<td>NIH/3T3 normal cells and B16F10 melanoma cell lines, C57BL/6 mice</td>
<td>Nanoeumol of Acai oil killed 85% of melanoma cells, while maintaining high viability in normal cells. It also showed 82% reduction in tumor growth.</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>α-TOS</td>
<td>-</td>
<td>Breast cancer</td>
<td>RGD peptide</td>
<td>MCF-7 cells, rat</td>
<td>Nanoeumol of α-TOS increased cytotoxicity and enhanced bioavailability compared to free drug.</td>
<td>[114]</td>
</tr>
<tr>
<td></td>
<td>CORM-2</td>
<td>-</td>
<td>Lymphatic cancer</td>
<td>Folic acid</td>
<td>A-20 cell line, BALB/c mice</td>
<td>Nanoeumol increased uptake of (CORM-2) into the tumour bearing cells. Besides that, the increased uptake increases the ability of CORM-2 to induce the anti-Warburg effect, prolonging the survival of mice with A20 lymphoma tumours.</td>
<td>[104]</td>
</tr>
<tr>
<td>Oral</td>
<td>OXA and 5-FU</td>
<td>Ion-pairing complex</td>
<td>Colorectal cancer</td>
<td>-</td>
<td>Caco-2 cell, mouse</td>
<td>The permeability of OXA and 5-FU increased 4-5 folds, which is higher than free OXA and 5-FU. Nanoeumol also enhanced cytotoxicity compared to free drug.</td>
<td>[77]</td>
</tr>
<tr>
<td></td>
<td>BBH</td>
<td>Oil-in-water nanoeumulsion</td>
<td>Cancer (anti-tumor)</td>
<td>-</td>
<td>Caco-2 cell mono-layer, rat</td>
<td>The BBH nanoeumulsion showed 440.40% relative bioavailability which enhanced absorption of BBH. The intestinal permeability of BBH increased while there is a decrease in BBH efflux through P-glycoprotein.</td>
<td>[78]</td>
</tr>
<tr>
<td></td>
<td>Pterostilbene</td>
<td>Low-energy emulsification method</td>
<td>Cancer</td>
<td>-</td>
<td>-</td>
<td>The nanoeumol delivery enhanced the solubility, stability and in vitro release of pterostilbene.</td>
<td>[79]</td>
</tr>
<tr>
<td></td>
<td>DTX</td>
<td>Hot homogenization followed by ultra-sonication</td>
<td>Breast cancer</td>
<td>-</td>
<td>MCF-7 cell line</td>
<td>Nanoeumol of docetaxel is stable, effective and safe for use as the nanoeumol exhibited higher cell uptake, strong cytotoxicity activity against MCF-7 cancer cell and no any toxicity found in liver or kidney tissues of mice.</td>
<td>[19].</td>
</tr>
<tr>
<td></td>
<td>DTX</td>
<td>-</td>
<td>Breast cancer</td>
<td>-</td>
<td>Caco-2 cells, MDA-MB-231 cell lines</td>
<td>The formulation showed more cell apoptosis and increase oral bioavailability, hence anti-proliferative activity was enhanced, manifesting 19% more inhibition compared to Taxofere.</td>
<td>[80]</td>
</tr>
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</table>

(Table 1) Contd....
<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Drug Name</th>
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<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTX</td>
<td>Emulsification method</td>
<td>Breast cancer</td>
<td>-</td>
<td>MDA-MB-231 cell lines</td>
<td>Docetaxel loaded chitosan nanoparticles exhibited 65-76% of drug entrapment and 8-12% loading capacity in which drug released about 68-83% and also an increase of 20% in cell growth inhibition in breast cancer cell line.</td>
<td>[115]</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>Emulsification method</td>
<td>Breast cancer (particular)</td>
<td>-</td>
<td>MDA-MB-231 cell lines</td>
<td>IC50 value of PTX-CS-NP-10 for anticancer activity was 1.6 folds less than pure drug while haemolytic toxicity was 4 folds less than naive drug. PTX-CS-NP-10 treatment showed enhancement in overall toxicity and anticancer efficacy of PTX.</td>
<td>[116]</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>High pressure homogenization</td>
<td>Metastatic breast cancer</td>
<td>-</td>
<td>MCF-7 cell line</td>
<td>PTX loaded nanoemulsion showed greater cytotoxicity as well as improved efficacy in anticancer activity in comparison with free PTX and marketed formulation (Taxol).</td>
<td>[81]</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>-</td>
<td>Breast cancer, ovarian carcinoma, non-small cell lung cancer, and AIDS-related Kaposi’s sarcoma</td>
<td>-</td>
<td>Animal tumor cell lines, rabbit</td>
<td>EmPAC exhibited higher anti-tumor efficacy and better safety than Taxol.</td>
<td>[117]</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>Modified solvent injection method</td>
<td>Breast cancer, ovarian cancer, head/neck cancer, small and non-small cell lung cancers.</td>
<td>-</td>
<td>Male Swiss albino mice</td>
<td>PTX-SLNs showed 10- and 2- folds higher improvement in oral bioavailability of PTX compared to free PTX solution.</td>
<td>[118]</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>Fusion method</td>
<td>Breast cancer, ovarian cancer, head/neck cancer, small and non-small cell lung cancers.</td>
<td>-</td>
<td>MCF-7 cell line</td>
<td>Solid nanoemulsion preconcentrate of paclitaxel exhibited strong inhibitory effect on proliferation of MCF-7 cells.</td>
<td>[82]</td>
<td></td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Self-emulsification</td>
<td>Breast cancer</td>
<td>-</td>
<td>4T1 and MCF-7 cell lines</td>
<td>Cytotoxicity studies showed toxicity enhancement in sunitinib by SNEDDS. In bioavailability study, maximum plasma concentration and mean under the curve were increased 1.45- and 1.24- times respectively compared to a drug suspension.</td>
<td>[84]</td>
<td></td>
</tr>
<tr>
<td>TAM</td>
<td>Water-soluble nanoemulsion</td>
<td>Breast cancer</td>
<td>-</td>
<td>HTB-20 cell line.</td>
<td>Nanoemulsion of tamoxifen can inhibit cell proliferation 20-fold greater as well as increased cell apoptosis 4- folds greater in comparison with pure tamoxifen.</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>CUR</td>
<td>Self-microemulsifying method</td>
<td>Prostate cancer</td>
<td>-</td>
<td>PC-3 cells</td>
<td>Curcumin nanoemulsions increased the cytotoxicity as well as exhibited prolonged biological activity and showed better therapeutic efficacy. Cur nanoemulsion also has higher effective permeability coefficient and absorption rate constant than free curcumin.</td>
<td>[85]</td>
<td></td>
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</table>

(Table 1) Contd…
formulation issues in chemotherapy through different route of administration. Moreover, there are still lack of research in this field such as the characteristic of the system and its establishment of clinical benefits in human. Understanding of detailed mechanism, which make nanoemulsion targeted and more efficient chemotherapy compared to conventional chemotherapy is in demand. Further investigations are in need for detailed pre-formulation interaction studies, exclusive studies on preparation method to make more stable nanoemulsion, influence parameters behind targeted nanoemulsion as well as drug uptake through different routes of administration and finally in cancer cells, and the reason behind improved safety profile should be explored in search of mechanistic details. These issues should be addressed and evaluated particularly safety assessment prior to marketing to prevent unforeseen complications.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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<table>
<thead>
<tr>
<th>Formulation</th>
<th>Preparation</th>
<th>Ligand</th>
<th>Microval</th>
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<tr>
<td>Diferuloylmethane (CUR)</td>
<td>Thin film-sonication dispersion technology</td>
<td>Lung adenocarcinoma</td>
<td>A549 cells</td>
<td>The absorptive constants and permeabilities of DNHLNs increased by 1.43-3.23 times and 3.10-7.76 times compared to diferuloylmethane (DIF). DNHLNs enhanced the absorption and bioavailability of DIF and also had stronger inhibitory effects.</td>
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<tr>
<td>Pipartine (PL)</td>
<td>Self-emulsification and homogenization-sonication method</td>
<td>Melanoma tumor</td>
<td>Mice</td>
<td>PL loaded nanoemulsions showed 1.5-fold increase in oral bioavailability and increased anti-tumor efficacy.</td>
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<tr>
<td>Genistein (Gen)</td>
<td>Coarse homogenization followed by low-amplitude ultrasonication</td>
<td>Oral cavity and oropharyngeal cancer</td>
<td>Oral carcinoma cell line</td>
<td>Cationic Genistein-loaded nanoemulsions have anticancer activity against oropharyngeal carcinomas.</td>
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<tr>
<td>Transdermal 5-FU</td>
<td>Oil phase titration method</td>
<td>Skin cancer</td>
<td>MEL-5 cancer cells lines</td>
<td>Nanoemulsion of 5-FU was found to be physically stable with enhanced skin penetration and more efficacious then free drug.</td>
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<tr>
<td>Tocotrienols</td>
<td>Homogenization</td>
<td>Skin cancer</td>
<td>Propylene glycol</td>
<td>Nano emulsion of tocotrienols demonstrated significantly stronger cytotoxic profiles along with low IC50 value compared to free drug.</td>
</tr>
<tr>
<td>Apigenin</td>
<td>High speed homogenization</td>
<td>UV induce skin cancer</td>
<td>HaCaT Cells and A431 cells</td>
<td>Nanoemulsion showed high penetrability through stratum corneum and high toxicity on A341 cells, However less toxicity toward HaCaT cells.</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Oil phase titration method</td>
<td>Skin cancer</td>
<td>-</td>
<td>Significant increase in permeability parameters was observed in nanoemulsion formulations as compared to aqueous solution of caffeine.</td>
</tr>
</tbody>
</table>

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REFERENCES


Perspectives of Nanoemulsion Strategies in The Improvement of Oral Drug Delivery Systems for Cancer Therapeutics: Towards Precision Medicine Overcoming Drug Resistance


