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# Gambogic acid: A shining natural compound to nanomedicine for cancer therapeutics

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## Abstract

The United State Food and Drug Administration has permitted number of therapeutic agents for cancer treatment. Most of them are expensive and have some degree of systemic toxicity which makes overbearing in clinical settings. Although advanced research continuously applied in cancer therapeutics, but drug resistance, metastasis, and recurrence remain unanswerable These accounts to an urgent clinical need to discover natural compounds with precisely safe and highly efficient for the cancer prevention and cancer therapy. Gambogic acid (GA) is the principle bioactive and caged xanthone component, a brownish gamboge resin secreted from the of Garcinia hanburyi tree. This molecule showed a spectrum of biological and clinical benefits against various cancers. In this review, we document distinct biological characteristics of GA as a novel anti-cancer agent. This review also delineates specific molecular mechanism(s) of GA that are involved in anti-cancer, anti-metastasis, anti-angiogenesis, and chemo-/radiation sensitizer activities. Furthermore, recent evidence, development, and implementation of various nanoformulations of gambogic acid (nanomedicine) have been described.

### Keywords

Gambogic acid; cancer treatment; chemotherapy; chemosensitizer; adjuvant; nanoparticles; nanomedicine; drug resistance

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Declaration of Interest

The authors declare no competing financial interest.

## 1. Introduction

Cancer is the second-leading cause of deaths after cardiovascular diseases in the United States (US) and World-wide. In 2020, it was projected ~1,806,590 new cancer cases and 606,520 cancer deaths will occur in the US [1]. In the last two decades, major advances in the detection and management of cancers have changed early diagnosis, prevention, and treatment options. These efforts driven decline in death rates for the four leading cancers, namely, lung, colorectal, breast, prostate cancer [1]. Pancreatic cancer and glioma are highly challenging to treat, and its associated deaths are certain in these aggressive form of cancers within couple years. Still, lung cancer causes more deaths than breast, prostate, colorectal, and brain cancers combined. The US Food and Drug Administration (FDA) has approved number of therapeutic agents, including, small molecules, anti-angiogenics, viral therapy, and antibodies for cancer treatment. However, their expensive nature of treatments, systemic toxicities, and therapy resistance, limits their continuous use in the clinical setting. All these events advice an unmet clinical need to rediscover natural compounds which has precisely safe and highly efficient for the cancer prevention and cancer therapy.

Since ancient times, natural compounds have shown a broad spectrum of therapeutic potential for the treatment of various diseases. Therapeutic and preventive applications of natural compounds relate to anti-viral, anti-inflammatory, anti-cancer, chemo-preventive, and chemo-sensitization properties have been established [2, 3]. Most common categories of natural compounds that have shown to have anti-cancer properties, includes, polyphenols, phytochemicals, and xanthonoid. Gambogic acid (GA, chemical formula: $C_{38}H_{44}O_8$) is the principle bioactive and caged xanthone compound, isolated from the brownish/orange gamboge resin of the *Garcinia hanburyi* tree [4].

Due to abundance presence of *Garcinia hanburyi* in East Asia and India, and easy isolation process [5–7], GA availability is plentiful for medical applications. The implementation of GA in scientific reviewed studies began in 1966 (Figure 1).

There were only four scientific studies from 1966 to 2004 [1966 (1), 1984 (1), 1986 (1), and 1996 (1)]. Since 2004, there were about 375 reports on gambogic acid for in general medicinal applications out of which ~264 belongs to cancer-related studies (Figure 1B). These statistics advice that implication of gambogic acid in cancer therapeutic applications is widely studied. Detailed break-down of these cancer studies indicate majority of those studies belong to breast, lung, and liver (hepatocellular) cancers (Figure 1C). However, very limited work was done on cervical, renal, and ovarian cancers.

Previous studies reported that GA has anti-neoplastic activity and is a potent apoptosis inducer [8, 9]. Furthermore, unlike other natural anti-cancer agents, GA has shown to be effective at lower concentrations (nM range) [10, 11]. It also has a favorable safety profile and large therapeutic index [12–14]. There are also general pharmacological aspects and toxicity effects of GA on cardiovascular and respiratory, indicated minimal effect on blood pressure, heart rate, and respiratory system [13]. A high dosage of GA (>16 mg/kg) administration exhibited minor and insignificant side effects on central nervous system. In addition, GA showed high analgesic activity which could be connected to its anti-

inflammatory nature [13]. Furthermore, GA has been approved for phase II clinical trials [12] by the China Food and Drug Administration (CFDA) as an anti-cancer agent for lung cancer and other solid tumor therapy.

Accumulated pre-clinical and clinical evidence indicates that GA has a significant anti-cancer effect on various types of cancers, including, breast cancer (BC), lung cancer (LC), hepatocellular carcinoma (HCC), prostate cancer (PrCa), colorectal cancer (CRC), pancreatic cancer (PC), ovarian cancer (OC), glioma, melanoma, head and neck cancer, and cervical cancer (CxCa), by targeting different molecular pathways (Figure 2).

Such broad range of mechanisms of action evident for its excellent potency. The anti-tumor effects of GA include, but are not limited to, induction of apoptosis, accumulation of reactive oxygen species (ROS), autophagy, anti-proliferation, inhibition of enzymes (e.g. telomerase), inhibition of growth factors (e.g. Vascular Endothelial Growth Factor, VEGF), and interception of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways. A high-throughput screening of natural product libraries identified GA as a potential heat shock protein 90 (Hsp90) inhibitor (similar potency as celastrol, a known Hsp90 inhibitor) [16]. It binds Hsp90 at a distinct site than Adenosine triphosphate (ATP) binding pocket. In addition, Duan *et al.,* [17] have reported that healthy cells exhibit less sensitivity to GA compared to cancer cells, presumably due to redox homeostasis. Moreover, GA has shown chemotherapy/radiation sensitization properties in different types of cancers [18, 19]. In general, chemo-sensitizers are small molecules that make cancer cells more sensitive to chemotherapeutic agents or radiation, thus improving the therapeutic index and anti-tumor effects of these agents. These compounds are also known as resistance modulators, which inhibit or block drug efflux from cancer cells *via* the P-glycoprotein (P-gp) pump and elevate the concentrations of therapeutic agents inside MDR cells [20].

Unlike other chemotherapy agents, GA does not cause bone marrow suppression [12, 15, 21]. Unfortunately, the aqueous solubility of GA (0.013 mg/mL) is very low, thus limiting its clinical application. Hua *et al.,* [22] developed an accurate quality control analytes that can determine hydrophobic GA levels in serum by liquid chromatography-mass spectra/mass spectra. Therefore, the design and development of water-soluble derivatives of GA will be crucial for the application of GA-based anti-tumor agents in the clinic. The structure–activity relationships of GA have unveiled it and can be amicable for number of modifications without altering its biological and pharmacological activities. For example, GA can be coupled with alkanolamines to improve aqueous solubility and achieve equivalent anti-proliferation effects [23]. Another method of improving aqueous solubility and stability is dispersing in polymer matrices. However, these approaches may improve solubility but not tumor specific delivery where a nanotechnology can be a suitable approach.

Table 1–2 documents a number of valuable recent review articles that often covered GA and its analogues in medical applications. Table 1, documents pertaining GA to delineate its potential targets and roles in cancer and other chronic diseases. Table 2, most of the review literature identified to be underlying broadly for xanthones, photo chemicals, neogambogic acid, spice, and other naturally occurring molecules that focuses on cancer therapies.

Nevertheless, until now there is no research review article that can provide information on the use of GA in various cancers and no emphasis to improve its inherent delivery and therapeutic issues for effective use in cancer treatment. In addition, there is no review that refer to nanotechnology mediated delivery of GA for cancer therapeutics. Thus, the overall focus of this review is to describe molecular actions of GA, its useful chemo-sensitization pathways, and to discuss the possible use of nanoparticle mediated delivery for a successful translation for cancer therapeutics.

## 2. Role of GA in multiple cancers

As mentioned above, multiple comprehensive reports have confirmed the anti-cancer ability and involvement of GA in reducing the growth, invasion, and migration of tumor cells. Pre-clinical and clinical studies are also evident that GA can efficiently reduce tumor burden without introducing severe systemic toxicities. The efficacy of GA is associated due to the binding of the transferrin receptor and Hsp90 and suppressing major oncogenic signaling pathways. This section provides a detailed overview of biological effects of GA in various types of cancers.

### 2.1. Breast cancer

Breast cancer remains the most commonly diagnosed cancer among women and is the fourth leading cause of death after lung cancer in the US [1, 42]. In 2020, it is estimated that 276,480 new cases of invasive BC will be diagnosed among women, 42,170 women are expected to die. There are many different types of BC and common ones include *in situ* ductal carcinoma and invasive carcinoma. Others, like phyllodes tumors and angiosarcoma are less known. BC is classified by some proteins, estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor 2) (HER2). However, there are some cancers that may not express these proteins termed as triple negative breast cancer (TNBC). Based on this information and tumor grade helps to choose a treatment option for BCs. Common detection tests include chest x-ray, computed tomography (CT) scans, bone scans, positron emission tomography (PET) scans, or magnetic resonance imaging (MRI) scans. There are many FDA-approved chemotherapies available to treat BCs, however, natural compounds may offer unharmed treatment option.

Gambogic acid is a highly used compound for BCs in the literature. Earlier investigations showed GA promptly inhibited growth of human breast cancer cells (Michigan Cancer Foundation-7, MCF-7) in a time-dependent way. This inhibition was co-related with increase of p53 levels and reduced bcl-2 levels, thus, promotes apoptosis [43]. It exhibited intracellular microtubular cytoskeleton disruption and microtubule depolymerization activity at 2.5 μM concentration in MCF-7 cells. This activity reduced the amount of microtubular polymer bundles and increased monomeric tubulin forms. This was further strengthened by the expression of depolymerized microtubule associated protein (MAP)-free microtubules and MAP-rich microtubules. These effects are also influenced by the elevated the phosphorylation levels of JNK-1 and p38, and cause in G2/M cell cycle arrest and apoptosis [44]. GA inhibited the proliferation of MCF-7 cells and indicated IC50 values of 1.46 μM. Such effect was received for GA due to production of ROS which can be removed by *N*-

acetyl-*L*-cysteine (NAC, a ROS inhibitor). These effects were modulated *via* inhibition of the expression of SIRT1, increased phosphorylation of FOXO3a, and the expression of p27Kip1, while enhanced the cleavage of caspase-3 [45]. A following study proved its anti-tumor and anti-invasive effects in human breast carcinoma cells (MDA-MB-231, triple negative breast cancer representative cell line) both *in vitro* and *in vivo* [46]. These results were confirmed through multiple heterotypic assays of adhesion, wound migration, and invasion assays. GA also suppress the expressions of matrix metalloproteinase 2/9 (MMP-2 and MMP-9). The inhibitor effects of gambogic acid were demonstrated by the phosphorylation of ERK1/2 and JNK.

Transferrin receptor (TfR) is known to play crucial roles in cancer cell signaling and permits greater extent of cell survival. Transferrin receptor has been widely employed as a potential target to deliver drugs. Kasibhatla *et al.,* [47] revealed GA has an independent of transferrin binding site on TfR1 and hinders transferrin internalization. GA demonstrated a distinctive apoptosis potential in cancer cells compared to normal cells. A superior apoptosis action was noticed in cancer cells with higher TfR on cell surface expression, i.e., GA demonstrated apoptosis potential in the order: T47D (++++) > 293T (+++) > human umbilical vein endothelial cells (HUVEC, ±) > human mammary epithelial cells (HMEC, –). GA activates three major signaling pathways of the MAPK family, along with caspase-3, −8, and Poly(ADP-ribose)-polymerase (PARP) apoptotic pathways [48]. However, Src inhibition significantly contributed the cancer cells for GA-enhanced cellular apoptosis [49]. The possible network of GA was established using proteomic technology and bioinformatic analysis revealed 23 possible target proteins that were related to: protein transport and modification, ubiquitin-proteasome system, transcription and translation, regulation of redox state, metabolism, cytoskeleton & transport, and cytokine.

In a three-dimensional multicellular inflammatory breast cancer patient-derived xenograft (MARY-X), cancer spheroid model, GA indicated dose-responsivity and showed the half maximal inhibitory concentration (IC50) value, 0.42 μM which is superior than paclitaxel treatment (IC50, 7.8 μM) [50]. Additionally, hydroxyl or fluorine group introduction(s) at discrete positions of the A-ring in caged xanthones (GA) promotes cytotoxicity of molecule even at sub-micromolar concentrations [51]. Interestingly, these modified compounds were capable of penetration inside tumor spheroids which can induce apoptosis both at the peripheral and centrally located cells (hypoxic region where proliferative and quiescent-prone). Screening studies demonstrate that chiral resolution of a caged xanthone show a broad spectrum activity against of breast cancer cell lines of basal-like subtype and triple negative receptor status [52]. These studies also provide feasibility of promoting GA as a therapeutic lead molecule for chemoresistant and metastatic BC.

### 2.2. Lung cancer

Lung cancer is the leading cause of cancer-related death in the US [1]. By the time of the diagnosis, about 60–65% patients have reached advanced stage or distant metastases. Various cytotoxic and chemotherapy agents (docetaxel, gemcitabine, irinotecan, paclitaxel, carboplatin, ifosfamide, cyclophosphamide, etoposide, mitomycin, vindesine, etc.,) are the most effective therapeutic regimen for lung cancer. Nevertheless, these regimens offer

successful initial therapeutic response and benefit but no significant increment in overall survival due to emergence of drug resistance. Natural compound to tackle these events could be a helpful new therapeutic option.

Gambogic acid was a proven ligand for a transferrin receptor [53]. Its anti-cancer potential is originated by Tumor Necrosis Factor-alpha (TNF-α)-induced apoptosis through modulation of the NF-κB signaling. Zhu *et al.,* [54] further proved that GA-induced apoptosis in Non-small cell lung cancer cells (NSCLC, up to 90% of lung cancers belongs to NSCLC) is in relation to the level of transferrin receptors. GA displayed enhanced uptake/target human lung SpC-A1 (higher TfR) compared to SK-meS-1 (lower TfR) in 125I tracker assay. Further, activity of GA is greater on SpC-A1 cells (1.5 μM, ~47.9% apoptosis) than SK-meS-1 cells (1.5 μM, ~21% apoptosis). Transferrin receptor 1 found to be overexpressed in multiple cancers and a predictor of the sensitivity of patients' tumor for GA treatment. GA demonstrated to have cell invasion and migration inhibition capacity even at 1.5 μM concentration [55]. Reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a membrane anchored glycoprotein negatively regulating matrix metalloproteinases (MMPs) was involved in GA-induced anti-invasive property. This study also proved that 8 mg/kg dose restrict tumor growth volume (A549 cells xenografts) to ~420 $mm^3$ compared to control tumor volume, ~1100 $mm^3$. The immunohistochemistry analysis of the tumor tissues represents significant lower expression of CD31 in the treatment group supporting its anti-invasive characteristic of GA.

A study by Ye *et al.,* [56] elucidates a significant growth inhibitory effect of GA in NCI-H441 cells. This is true in the *in vivo* evaluation at a concentration of 25 or 50 mg/kg GA treatment. The possible molecular mechanism of GA's action reported to be autophagial through a ROS pathway. Similar way, GA proven to be efficient in achieving ROS-induced Endoplasmic Reticulum (ER) stress-mediated apoptosis in A549 cells [57]. This treatment lead expression levels of glucose-regulated protein (GRP 78), C/EBP-homologous protein (CHOP), activating transcription factor (ATF 6), and the phosphorylation levels of protein kinase R-like ER kinase (p-PERK), and inositol-requiring enzyme (IRE-1α). GA can strongly inhibit the growth of A549 and NCI-H460 cells by overcoming multidrug resistance phenomenon through modulating autophagy and suppressing the activation of Akt, mTOR, and ribosomal S6 kinase [58]. A series of *in vitro* and *in vivo* experiments confirms that Liver kinase B1 (LKB1, is a tumor suppressor that functions as a master regulator of cell growth, metabolism, and survival) enhances the sensitivity of NSCLC cells to GA [59]. The mechanistic investigations provide persuasive evidence that LKB1 triggers cancer cell to GA treatment which is due to up-regulation of E-cadherin → p-AMPK → blockade of mTOR.

### 2.3. Hepatocellular carcinoma

Liver cancer is the six most common cause of cancer-related mortality in the US [1]. An estimated 42,810 people (30,170 men and 12,640 women) will be diagnosed with liver cancer and 30,160 deaths (20,020 men and 10,140 women) from this disease will occur this year [1]. Hepatocellular carcinoma (HCC) is the most common type of liver malignancy. The molecular alterations in HCC promotes an increased level of difficulty in finding a

promising treatment. Therefore, utilizing a natural molecule for its adjuvant therapy or as therapy sensitizer can shed new therapeutic option for HCC.

In an earlier study, it was detected that a selective uptake and action of GA takes place on human hepatoma (SMMC-7721) cells than on human normal embryo hepatic L02 cells [60]. 10 μM GA treatment exhibited ~95% growth inhibition in SMMC-7721 *vs* ~40% in L02 cells. At the same time, a neglectable survival change was noticed in primary rat hepatocytes even at higher GA concentrations (up to 50 μM). This differential activity was further evident from selective influence on the expression of Bcl-2 (down regulation) and Bax (up regulated) proteins with GA treatment in cancer cells. There was no significant difference that was found on these proteins in L02 cells upon treatment. In SMMC-7721 xenograft tumor mice, GA (2, 4, 8 mg/kg) treatments resulted in significant growth inhibition (33.1%, 50.3% and 64.2%, respectively) compared with the vehicle group mice. In a subsequent study, the anti-cancer potential of GA on Hep3B (p53 deletion, IC50: 1.8 μM) and Huh7 (or p53 mutation, IC50: 2.2 μM) was acting through the mitochondrial pathway (activation of caspases-3/7, −8 and −9) [61]. This study suggests that GA acts as an independent of p53-associated pathway. Park *et al.,* [62] proved the anti-metastatic activity of GA in SK-HEO1 HCC cells. Thoroughly, this molecular study confirms its activity is regulated by the actin cytoskeleton and NF-κB-mediated MMP-2 and −9 expression.

A derivative of gambogic acid (GA, compound 3e) was showed an improved aqueous solubility and displayed potent inhibition of HCC cell proliferation [23]. In brief, this compound exhibited IC50 (μM) as 0.045, 0.73, 1.25, 0.12, and 0.067 in Bel-7402, SMMC-7721, Bel-7404, QGY-7701, and HepG2 cells, respectively. These values are relatively less compared to Taxol® (1.25, 0.88, 0.82, 0.04, and 0.05) and GA (0.59, 1.59, 1.99, 0.41, and 0.94) in those cell lines, respectively. Similarly, another GA-based cages ring system (compound 11a) showed comparable anti-cancer activities in BCG-823, SMMC-7721, and HepG2 cells and tumor xenograft model [63]. The IC50 of these compounds were noted as 3.59, 8.06, and 2.37 μM (GA compound 11a) and 2.35, 3.20, and 2.4 μM in these cell lines. This therapy outcome was even better in tumor inhibitory capacity. GA 11a compound showed dose dependent growth inhibition in HepG2 xenografts. At 25 mg/kg dose GA 11a showed weight of tumor ~1.36 g compared to GA 20 mg/kg dose, ~1.10 g. The advantage with these derivatives is easy administration *in vivo*. An oxidative analogue of GA was also found to have remarkable apoptosis capacity in HepG2 cells [64]. This was observed through multiple series of *in vitro* assays and the apoptosis induction is perhaps through the intrinsic mitochondrial pathway.

Like many other cancer cells, GA (1.5, 3, and 6 μM) also showed generation of ROS in SMMC-7721 cells that contributes to apoptosis [65]. Overall, this study demonstrated that the apoptosis process GA governed by the loss of MMP, decrease the levels of ATP/glutathione, and activate stress-responsive p38 and JNK. This data was verified with a positive control, rotenone. The activation of human telomerase (often regulated by the human telomerase reverse transcriptase, hTERT), is a vital stage of cellular immortalization and malignant conversion. Such conversion process can be reversed using GA treatment [66]. In another attempt, GA treatment shown a significantly reduced telomerase activity in SMMC-7721 cells which was confirmed by the suppressed expression of the hTERT gene.

In addition, GA treatments significantly reduced the expression of c-MYC in a time- and concentration-dependent manner.

Wu *et al.,* [67] identified an uncoordinated 119 or retinal protein 4 (UNC119) as a new target of GA which induce a sensitivity mechanism in HCC cells. UNC119 is reported to be an activator of SRC-type tyrosine kinase and its expression resulted on forbearance in apoptosis potential. Clinical evaluations suggest its positive correlation in HCC tissues represent poor prognosis. GA can tackle this protein by introducing cell cycle arrest *via* GSK3β/β-catenin signaling pathway (a known downstream of UNC119). A proteomic study proved that Stathmin 1 (STMN1) may be a major target for GA [68]. This investigation verified that the overexpression of STMN1 could reverse the inhibitory effect of GA in HCC cell growth. At the same time, siSTMN potentially enhances the sensitivity of cancer cells to GA treatment. This work has major implication in HCC because STMN1 found to be present in a large portion of HCC biopsies and has involved in the pathogenesis of disease [69–71]. Further, STMN has strong positive correlation with high α-fetoprotein, tumor size and stage, and degree of invasiveness/metastasis [72].

### 2.4. Colorectal cancer

Colorectal cancer is the second most common cause of cancer-related death in the US. Gambogic acid shown suppressive colon cancer cell activity in SW620 cells [73]. This *in vitro* study compared with 5-fluorouracil (5-FU) (10 μg/ml) treatments in proliferation, invasion, and apoptosis assays and found that the almost equivalent activity was observed for GA treatment (100 μg/ml) in cancer cells. GA also proven to bring down protein expression levels of PI3K, AKT, p-AKT, MMP-2 and −9 indicating it is a potent molecule affecting the PI3K/AKT pathway. GA+5-FU combination treatment was effective and significant in reducing tumor cells [74]. In a similar comparative study, GA showed dose-dependent apoptosis *via* mitochondrial pathway [75]. 1.25–5.00 μmol/L GA is sufficient to produce elevated levels of apoptosis-related factors Fas, FasL, FADD, cytochrome c, and Apaf-1. GA treatments (5, 10, and 20 mg/kg mice) resulted with 21, 15, 10.5 decreased relative tumor volumes (RTV) compared to control group. The GA treatment (20 mg/kg) was noticed to be close to docetaxel (10 mg/kg) treatment group tumor relative volume, 7 RTV.

Zhang *et al.,* [76] systematically evaluated that GA was responsible to induce ROS-mediated autophagy in HCT116 and SW620 colorectal cancer cells. In detail, this study disclosed that GA was able to produce a dysregulation of lipid metabolism and activate 5-lipoxygenase subsequently leading to induction of intracellular ROS. These events managed GA to gain an inhibitory potential of Akt-mTOR signaling. In a 12 day GA treatment (8 mg/kg), C26 tumor-bearing (100 $mm^3$) mice showed tumor volumes remained same whereas control group tumors grown to ~ 400 $mm^3$. Similarly, GA treatment also restricted tumor growth to its basal tumor volumes (~600 $mm^3$) but control groups reached to ~ 2300 $mm^3$.

The GA treatment could enhance the activation of phosphatase and tensin homolog (PTEN) besides suppressing PI3K/Akt in HT-29 cells [77] thus regulates the proliferation, migration, and invasion of cells. When miR-21 mimics supplementation along with GA reversed all the activities of GA. This confirmed miR-21 was the effector of GA and can block PI3K/Akt signaling pathway *via* enhancing PTEN activity. GA is able to tackle stem cell like cell of

colorectal cancer cells [78]. This was proven both in tumor spheroid assay and *in vivo* mouse bearing PNanog-GFP-T2A-Luc transgene (GFP+ cells, exhibited cancer stem cells characteristics). These results showed that GA can efficiently reduce the percent putative cancer stem cells (CD133+CD44+ cells), lowering stemness and EMT-associated markers. It was also evident that GA precisely impeded proliferation and stimulated apoptosis in both 5-FU sensitive (HCT-15P) and 5-FU resistant (HCT-15 R) colorectal cancer cells *via* activation of JNK signaling pathway [79].

### 2.5. Pancreatic cancer

Pancreatic cancer has the third highest mortality rate in US. This devastating disease pose five-year survival rate less than 7%. Thus, identification of a newer molecule(s) that can be used alone or in combination with gemcitabine or other therapeutic agents is highly warranted. The gambogic acid efficiently blocks the proliferation of pancreatic cancer cells in time- and dose-dependent manner [18]. In all tested pancreatic cancer cells (BxPC-3, MIA PaCa-2, PANC-1, and SW1990) GA showed IC50 values below 8.3, 3.8, and 1.7 μM for 12, 24, and 48 hrs treatment, respectively. GA induced apoptosis and affected the cell cycle distribution in the S-phase and was shown reducing the expression of Ribonucleotide Reductase Catalytic Subunit M1/M2 (RRM1/RRM2) while inhibiting the ERK-AKT signaling pathway at the same time. Youns *et al.*, [80] also seen similar inhibitory effects of GA on proliferation of pancreatic cancer cells. This study detailed on differential effect (IC50 μM) on normal (HPDE >15) and a panel of affected pancreatic cancer cells (Miapaca-2, ~1.71; Capan-1, ~3.98; Capan-2, ~4.56; Suit-007, ~3.50; BxPC-3, ~4.03; Colo-357, ~1.95; Panc-1, ~2.05; Suit-2, ~1.36). Its whole-genome transcription profiling analysis confirmed GA influenced for up-regulation of DDIT3, DUSP1, and DUSP5 and down-regulation of ALDOA, TOP2A, and ATG4B. Literature strongly advised that ribonucleotide reductase (RNR) is an enzyme that regulates the cell cycle and is composed of two subunits, the regulatory subunit RRM1 and catalytic subunit RRM2. The high levels of RRM2 in cancer cells not only indicate gemcitabine resistance but also associated with poor prognosis. A recent study confirmed that GA can limits the RRM2 expression in pancreatic cancer cells offering sensitization to gemcitabine therapy [18].

### 2.6. Prostate cancer

Literature indicated that GA reported as an apoptosis-inducer in many types of cancer cell lines by targeting transferrin receptor and modulating nuclear factor-kappa B signaling pathway. Angiogenesis is a crucial step in survival of cancer cells. Angiogenesis inhibitors are currently FDA-approved therapies for cancer treatment. Interestingly, GA was found to exhibit inhibitory actions against proliferation, migration, and invasion of human umbilical vascular endothelial cell (HUVEC) [81]. Particularly, GA is prone to inhibit the tube formation and micro vessel growth even at low nanomolar concentration. The possible molecular action was involved through the inhibition of the activation of vascular endothelial growth factor receptor 2 and its downstream protein kinases (c-Src, focal adhesion kinase, and AKT).

Like many other cancer cells, prostate cancer cells viability was also significantly affected by GA treatment [82]. GA treatment displayed dose-dependent (1–5 μM) cytotoxicity in

prostate cancer (PC3) cells. Additionally, it was able to inhibit the migration and invasion behavior of cancer cells that was induced by TNF-α. GA is a capable molecule able to block the TNF-αinduced activation of PI3K/Akt and NF-κB signaling pathways. The castration resistant prostate cancer (CRPC) cells are resistant to androgen (AR)-deprivation and conventional chemotherapies. These cells show homozygous alterations in phosphate and tension homology (PTEN) and TP53. GA showed potent sub-micromolar growth inhibitory activity on a panel of AR-independent Pten/Tp53 null prostate cancer-derived cell lines [83]. The GA treatment in CRPC patient derived xenograft organoid cultures was confirmed as an inhibitory molecular role in thioredoxin and as ROS-mediated apoptosis inducer. A following continuation study was focused how GA acts on CRPC (PTEN−/−/p53−/−PC and Los Angeles prostate cancer-4, LAPC-4) cells with deleted PTEN and p53 genes [84]. This *ex vivo* organoid study validated that GA causes time-dependent apoptosis, 11.78% and 29.94% in 6 hrs and 8 hrs, respectively. This is primarily due to mitochondrial caspase activation, light chain (LC)-3 conversion, decreased activity of MAPK pathway, and down-regulation of downstream c-fos. Together, this information suggests that GA inhibits growth and angiogenesis and thus may be a viable drug candidate in anti-cancer and antiangiogenesis therapies.

### 2.7. Melanoma

Malignant melanoma is an assertive type of cancer that can occur in skin, eyes, and central nervous system. The incidences of melanoma have been rising compared to other solid tumors. Metastatic melanoma can readily metastasize to other sites of the body and leads to multiple metastatic melanoma which has poor responsivity to existing chemotherapies. Thus, effective treatment for metastatic melanoma is highly rewarding in the medical setting. Gambogic acid treatments (5 and 10 μM) were able to inhibit proliferation of A375 malignant melanoma cells [85]. It was observed that GA exhibited a dose-dependent generation of intracellular ROS levels and lowered the oxygen consumption rate and the mitochondrial membrane potential. These events lead to GA-induced apoptosis. Through *in vitro* experiments it was proved that GA can efficiently induce apoptosis *via* mitochondrial p66shc/ROS-p53/Bax-mediated pathway. In addition, this study supports that GA (100 mg/Kg mice by intraperitoneal administration) could reduce tumor burden up to 40%. It is important to note that malignant melanoma is an aggressive form of cancer and resistant to chemotherapies, this is true in the case of gambogic acid. GA needed to treat these tumor cells requires a higher concentration than to treat other tumor cells. In another set of experiments, GA followed dose-dependent (1, 5, and 10 μM) influence on proliferation control and apoptosis activities [86]. This anti-tumor activity was observed *in vivo* in A375/CDDP xenograft mouse study *via* regulation through miR-199a-3p/ZEB1 signaling. Altogether, this investigation supports that GA treatment enables reduction in proliferative, migration, and invasion characteristics of cells while introducing apoptosis and chemo-sensitization features in melanoma cells. Besides these studies, GA happens to promote antimetastasis properties in melanoma cells by active inhibition of PI3K/Akt and ERK signaling pathways [87]. This study demonstrated GA’s role in the suppression behavior of cancer cells by inhibiting EMT and angiogenesis. Together, these outcomes laid the foundation for development of GA-based adjuvant therapies for melanoma.

### 2.8. Glioma

Glioblastoma is a common malignancy of the central nervous system in the body. Therapeutic strategies including combining of surgical resection with radiation and chemotherapies offers the mean survival time of patients is about 14 months. This type of tumors rapidly grows with a high proliferation rate and often infiltrate into adjacent brain tissue, causing invasive disease. Such disease condition disrupts brain tissue, its architecture thus impairs brain function. At this stage of the disease, the surgical removal of tumors is highly impractical, and combination of radiation or chemotherapy is applied. All FDA-approved chemotherapies such as cisplatin, carmustine, and temozolomide are the primary treatments but can't eliminate tumor cells completely. Therefore, recurrence is certain.

In 2008, Qiang *at al.*, [88] applied gambogic acid as a growth and angiogenesis inhibitor. This study demonstrated successful uptake of GA through blood-brain barrier (BBB) in primary cultured rBMEC cells. It was also shown GA can reduce cellular viability of rat C6 glioma cells and induce apoptosis (even at 1–2 μM). In *in vivo* anti-neoplastic effects of GA (1–2 μM)-treated cells generated <100 $mm^3$ tumors compared to control cells tumors (~400 $mm^3$). This study suggests that the GA treated cells proved a significant amount of apoptosis in tumor cells and a shrink angiogenesis from 40% to 20% level, while increased mice survival of 60–100% in a 14-day study. In another study, GA acts as an effective inducer of apoptotic cell death in T98G glioma cells even at sub-micromolar concentrations (200–400 nM) [89]. This behavior of GA was exerted by caspase-dependent apoptotic signaling effects, *via* activation of caspase-3, −8, and −9 and PARP cleavage. This cytotoxic effect was further supported by ROS generation. Additionally, GA not only efficiently cross the BBB but suppress the inflammatory signaling pathway(s) [90]. A subsequent study presented that GA promotes growth inhibition and apoptosis in U251 and U87MG glioblastoma cells *via* autophagy mechanism [91]. This was confirmed through increased LC3-II, Atg 5, and Beclin 1 presence in Western blot analysis. Confocal assay was a further supported autophagy property i.e., the punctate pattern of LC3 localization which was reduced in the presence of autophagy specific inhibitors (3-Methyladenine or bafilomycin).

### 2.9. Other cancers

In addition to the above reports in various cancers, GA activity has been evaluated in other cancers, such as, cervical, renal, and ovarian cancers. For example, GA (3 μg/mL) proved to be a significant inhibitory activity in HeLa cervical cancer cells [92]. Such effects were observed due to ER stress induction in cells due to elevated levels of the transcription factors CHOP, GRP78, endoplasmic reticulum localized DnaJ homologues, growth arrest, and DNA damage-inducible protein. Furthermore, this study demonstrated that the apoptosis potential was induced by the ER response through up-regulation of JNK while down-regulation of ERK pathways. GA confirmed its apoptotic features in cervical cancer cells *via* vimentin cleavage (proteomics analysis) [93]. This was verified both *in vitro* (GA, 2 μM) and *in vivo* (GA, 2 mg/Kg mice) studies. The *in vivo* study shows 50% growth reduction over control groups. This biological growth inhibitory step occurs through an activation of p-38-MAPK → HSP27 → vimentin cleavage → cytoskeleton dysfunction → cell death. Similarly, GA (0–6 μM) exhibited a dose-dependent proliferation inhibition against three human oral squamous cell carcinoma cell lines (Tca8113, TSCC and NT) in cell viability assay [94].

The apoptotic behavior of these cells was confirmed due to the activation of NF-κB pathway.

Similarly, GA was shown to be prominent in inhibiting the growth of ovarian cancer cells *in vitro* and *in vivo* xenograft (SKOV-3) mouse model [95]. This activity was confirmed by regulation of p65, i.e, GA activated caspase 3 and 9 and lowers NF- κB p65 DNA binding. These effects were known for 0.8 to 3.2 μM of GA. In addition, GA happens to promote chemotherapy potential by introducing ROS-mediated apoptosis by JNK pathway in ovarian cancer cells [94].

Bromodomain- containing protein 4 (BRD4) exhibited oncogenic roles in various types of cancer including thyroid cancer. Higher BRD4 expression is linked to thyroid cancer tissues compared to its adjacent normal tissues. Interestingly, GA has been shown to decrease the expression of BRD4. The biological activity of GA acts against thyroid cancer cells *in vitro* and *in vivo* partly *via* downregulation of BRD4. These effects were also associated with pro-apoptotic activity [96]. All these findings imply that GA can be a prospective anti-cancer molecule for possible use as an alternative adjuvant therapeutic agent for cancer treatment.

## 3. Chemosensitization and synergistic actions of gambogic acid

Gambogic acid is known to have a chemo-sensitization effect on various cancer types by modulating different signaling pathway, such as, MAPK/ERK, PI3K/AKT and NF-κB. Therefore, GA has gained attention as an anti-cancer agent to be used for combination therapy. Table 3 document list of co-treatment of chemotherapeutic agent with GA resulting synergistic actions by suppressing multiple oncogenic pathways. Chemo-sensitization and synergistic actions of GA is considered to increase intracellular concentration of chemotherapy agent(s) in cancer cells as well as at the tumor site. It was noticed for doxorubicin, 5-FU, cisplatin, Imatinib, gefitinib, sunitinib, gemcitabine, irinotecan, paclitaxel, docetaxel, cabazitaxel, etc. For example, 1, 2, and 4 μM oxaliplatin treatment exhibited platinum levels of ~0.25, 0.45, 0.48 ng/$10^7$ cells, but in the presence of GA these levels increased to 0.45, 0.8, and 1.6 ng/$10^7$ cells [97]. It was about ~1.75 to 4-fold increase concentration of oxaliplatin. This effect was also dependent on time. With increase of time the ability to recruit more oxaliplatin in the presence of GA. This was verified by the increase of human copper transporter 1 whilst decrease in copper- transporting p- type adenosine triphosphatases 1 and copper- transporting p- type adenosine triphosphatases 2.

Gambogic acid with cisplatin combination treatment found deeper levels of the anti-cancer activity in cisplatin-resistant lung cancer (A549/DDP) cells [115]. This study was demonstrated that GA+cisplatin combination resulted in significant anti-growth activity by stimulating cell cycle arrest, apoptosis, and downregulation the LRP and MRP2 proteins in lung cancer cells. GA induces autophagy in A549 and NCI-H460 cells and ultimately causes cell death [58]. The induced autophagy suppresses Akt/mTOR signaling, thus enables a synergistic action with cisplatin and rapamycin. The significant effect of GA on TRAIL-induced apoptosis in HT-29 cancer cells by the generation of ROS and activation of caspases (caspase-3, −8, −9) due to expression of CHOP, DR4 and DR5 [116]. GA and chloroquine combination treatment also induced the expression of LC3-II and Beclin-1 proteins while it

also help to declined expression of p62 in pancreatic cancer cells [101]. Further, GA can efficiently reduce the mitochondrial membrane potential and aggressive production of ROS caused by activation of autophagy and apoptosis. GA and chloroquine synergistically reduced tumor growth. *In vivo* superior co-treatment efficacy of GA (1 mg/kg) and DOX (10 mg/kg) was noticed in a SKOV-3 xenograft mouse study [94]. This combination treatment (performed 15 mice per group) suppressed tumor growth to a markedly greater extent (~225 $mm^3$) than did treatment with either single agent, DOX (~265 $mm^3$) or GA (~350 $mm^3$) or control group mice (~360 $mm^3$). There was no apparent weight loss in mice during this co-treatment. This greater potential was achieved due to GA offered ROS-mediated apoptosis in cancer cells. GA demonstrated significant therapeutic benefit on paclitaxel (PTX)- resistant TNBC cells [117]. GA+PTX combination targets a sonic hedgehog signaling pathway. PTX-resistant cells show sensitivity to this combination treatment by the inhibition of expression of SHH, GLI1 and PTCH1compared to PTX.

A combination administration of gefitinib, an EGFR tyrosine kinase (EGFR-TKI) inhibitor, and GA in NCI- H1975 xenografts mice study demonstrated a marked inhibition of ~70% in tumor growth compared to their parent free drug molecules [109]. This is an important outcome for NSCLC because EGFR-TKI inhibitors have been known to be widely used for NSCLC, but its acquired resistance is observed in 50% patients. The combined treatment showed an influence on downstream molecules of the PI3K and ERK pathways. The gefitinib+GA combination inhibits p- AKT, p- MEK1/2, and p- ERK1/2 (but there is no change in their total protein levels). There were only limited effects on these proteins found with either gefitinib or GA. Similarly, gemcitabine is an approved chemotherapy for lung and pancreatic cancer. Studies confirmed that the expression of Ribonucleotide reductase (RNR, the regulatory subunit RRM1 and catalytic subunit RRM2) enables poor gemcitabine therapeutic outcome. In fact, higher levels of RRM1 or RRM2 confirmed that it is associated with a poor prognosis. A study reported that GA+GEM combination showed a tumor inhibition rate of 72.9% compared to 49.8%, and 30.2% in GA and GEM treatment, respectively [18]. GA could efficiently inhibit RRM2 by reducing the activation of the MAPK/ERK/E2F1 pathway in pancreatic cancer cells PANC-1 and BxPC-3. Our data is also supporting that GA can efficiently reduce RRM2 and enhance GEM-induced apoptosis potential in NSCLC [118]. This combination effect was noticed as synergistic which was determined by combination index using Chou and Talalay method for different concentration ratio of GEM and GA in A549 and H1299 NSCLC [118]. GA can also interact with dihydrofolate reductase (DHFR) and it leads to a synergistic anti-cancer effect with methotrexate [119]. DHFR assays revealed GA can slightly inhibit DHFR activity but the affinity of the enzyme for dihydrofolate was significantly altered.

Radionuclide therapy is the primary adjuvant treatment for many cancers, but, systemic side effects perimeter for its multiple dosages in clinical setting. Apart from chemo-sensitization potential, GA also involved in sensitization for radiation treatments [74]. A series of *in vitro* experiments was able to prove that GA effectively inhibit cisplatin-resistant non-small cell lung cancer cells (A549/DDP) when combined with $NaI^{131}$ radiosensitizer. GA can also significantly offer radio sensitization potential in cancer cells even under hypoxic environments [120]. GA and a radiation (X-ray radiation, 2–8 Gy) combination treatment in nasopharyngeal carcinoma cells promotes apoptosis due to G2/M-phase arrest and HIF-1α/

cyclin B1/cdc2 pathway. Another investigation delineates its superior radio-sensitivity in esophageal cancer cell line, TE13 [121] in which it involved both autophagy (ROS generation) and apoptosis (Akt/mTOR inhibition) mechanisms.

## 4. Nanotechnology based strategies for delivery of GA

Gambogic acid exhibited anti-cancer potential in multiple *in vitro* and *in vivo* evaluations. Additionally, its application has been translated into human cancer treatments in China. However, its poor aqueous solubility, poor biodistribution, and multi-targeting capacity can introduce unavoidable systemic toxicity issues. To minimize such un-invited side effects and enhance its clinical translation, nanotechnology approaches can be helpful. Nanotechnology based delivery systems with therapeutic agents considered as “Nanomedicine”, are an innovative approach to common medicinal results, which is commonly employed to overcome the disadvantages of conventional drug molecules [122, 123]. The value of nano drugs expected to be developed by 2025 has been estimated at $350.8 billion according to a report released by Grand View Research, Inc. To date, over 75 nanomedicine formulations have been approved by the FDA, and they are currently being utilized in clinical practice [124, 125]. There are various types of carrier designs, which can be utilized in GA delivery that are listed below in the schematic representation and a number of investigations that are currently available in the literature as well (Figure 3). In general, various preparative approaches can be followed to achieve uniform nanoparticle distribution. This information can be referred to our previous reports [126–129]. Additionally, Kumar *et al.,* [130] rationally presented number of advanced techniques, such as, solvent-antisolvent interaction, acoustic cavitation, mechanical milling, template-based, aerosol, electrospinning, supercritical fluid, and ice segregation induced assembly approaches to generate nanoparticles. The nanoparticle can broadly divide into two categories, namely, organic and inorganic nanoparticles. Forthcoming sections delineates how these nanoparticles helps to deliver gambogic acid specifically tumor cells.

Nanomedicine for cancer therapies have gained significant attention, as chemotherapeutic drugs loaded on nanocarriers have shown to 1) reduce the side effects of conventional chemotherapy *via* more efficient drug delivery to the tumor sites, 2) lower the dosage needed for administration, 3) reduce the possibility of acquiring drug resistance toward chemotherapeutic agents, 4) sustain release and ease of administration can minimize frequent clinical visits and the number of administrations in patients [123, 131, 132]. The rationale behind using NPs is based on their delivery approach, which increases accumulation at the tumor site *via* the enhanced permeation and retention (EPR) effect [133–135]. NPs prolong circulation time and improve tolerability concerns by decreasing toxicity issues. These advantages relate to their appropriately designed size (~100–400 nm), which allows them to easily bypass the reticuloendothelial system (RES) yet take advantage of the EPR effect due to leaky tumor vasculature. Consequently, the pharmacokinetic, pharmacodynamic and bioavailability profiles of therapeutic agents encapsulated in NPs is favorably enhanced by expanding their half-life and blood circulation time. NPs allow the drug enough time to be in close contact with the tumor microenvironment to efficiently penetrate and accumulate in tumor tissues and release the drug cargo at the tumor site. [136, 137]. Nanoparticles usually target cells through two main mechanisms: 1) Passive targeting;

whereby nanoparticles are able to take advantage of the EPR effect and passively diffuse to the tumor site and accumulate in the tumor tissue due to enhanced circulation time, and 2) active targeting; whereby attaching specific affinity ligands to the nanoparticle surface facilitates its recognition by receptors on the cancer cell surface and assists its uptake by tumor cells *via* a receptor-mediated endocytosis mechanism [123, 132, 138].

### 4.1. Organic nanoparticles

**4.1.1. Lipid and micelle-based nanocarriers—**In general, nanomedicine for cancer therapy consists of a carrier vehicle and a drug. Some NPs may be conjugated with a targeting moiety as well. Most of lipid or micelle-based nanoparticles are often composed from phospholipids or block copolymers. These nanoparticles can facilitate encapsulation of hydrophobic or hydrophilic drug or combination of both due to their lipid/hydrophobic polymer and hydrophilic/aqueous layers, respectively. Various parameters influence its structure and stability and loading efficiency of drugs in lipid structures [139–141]. Liposomal drug delivery systems are well established and FDA-approved nanoplatforms to deliver various drugs. A simple polyethylene glycol (PEG) conjugation on liposome system can significantly enhance the bio-compatibility, the EPR effect, and tumor accumulation. The PEGylation method also improve aqueous solubility of GA [142]. A positively charged PEGylated liposomal formulation of GA (GAL) can lead to spherical particle size of 107.3 ± 10.6 nm. This GAL formulation was able to inhibit growth of cancer cells (MDA-MB-231, Hep G2 and MIA PaCa-2 cells, IC50 values of ~3.2, ~4.06, and ~5.29 μM, respectively) compared to free GA. The improved potency was confirmed in TNBC xenograft tumors. GAL formulation showed >50% reduction in tumor volume and 1.7-fold lower in tumor weights. Further, this formulation proved its superior inhibition of cancer cell growth by the increased expression of p53 and Bax, and reduced expression of bcl-2, cyclin D1, surviving, and CD31 compared to free GA. This study also advised that GA nanoformulation could inhibit angiogenesis in TNBC. Similarly, GA grafted low molecular weight heparin (GA-LMWH) can form instantaneous self-assemblies which leads to the formation of stable micelle nanoparticles in aqueous solution that can efficiently increase GA solubility and activity at the tumor site [143]. This formulation was established to be liver targeting for HCC treatment. In the experimental procedure, a 27.5% substitution on heparin with the GA lead micelles with mean particle size of ~190 nm was observed. The area under the curve (AUC) and mean retention time of GA-LMWH treatment represented a significantly higher level in liver than in plasma, heart, spleen, lung, and kidney. Its liver targeting efficiency was ~51.57% compared to free GA ~24.30% indicating 2.1-times higher targeting. Liposomal drug delivery of GA and retinoic acid (RA) (weight ratio of GA to RA = 1:2, GRL) revealed synergistic combination index and enhanced *in vivo* anti-tumor efficacy in 4T1 cell line and xenograft models [105].

Liu group [144] developed a series of DSPE–PEG2000 lipid-based nanocarriers with GA and cell penetrating peptides (cRGD and RGERPPR). These formulations show unique small particle size range from 20 to 26 nm. GA's activity was more pronounced when two cell penetrating peptides combined on lipid nanoparticles in MDA-MB-231 tumors. The RGE peptide mediated delivery of GA showed superior inhibition of tumor growth 83.3% compared to cRGD (66.67%), RGE/cRGD (65.00%), and free GA (45%). This effect is

superior than cisplatin treatment (73.33%). More importantly, RGE peptide modified lipid-GA formulation did not show any signs of systemic toxicity in vital organs. This group also applied GA loaded lipids with monomeric and dimeric c(RGD) tumor targeting peptides (c(RGDfK) and E-[c(RGDfK)2]) for treating highly metastatic breast tumors [145]. A LyP-1 peptide-modified low-molecular-weight heparin-quercetin conjugate formulation was developed for co-delivery of GA to induce combinatorial chemo and angiostatic therapy to reverse drug resistance phenomenon in BC treatments [102]. This approach can provide significant outcome in metastatic BCs.

Monomethyl poly(ethylene glycol)-block-poly(ε-caprolactone) (MPEG-*b*-PCL) copolymer was capable of encapsulate GA molecules with high encapsulation efficiency (92.1±0.3%) without compromising its nanostructure (29±2 nm) [146]. This polymer micelle system provides sustained long term drug release, superior dispersity, cellular uptake, enhanced apoptosis, and superior tumor targeting potential. GA-loaded mixed polymeric micelles [PEG-poly(L-histidine)-PLGA) and D-α-tocopheryl polyethylene glycol 1000 (TPGS)] was also shown to overcome drug resistance in cancer cells which promotes its value for effective clinical translation in cancer therapeutics [147].

**4.1.2. Polymer-based nanoparticles—**Polymeric NPs are often constructed from bio-degradable and bio-compatible polymers either from synthetic or natural origin. The most frequently used biodegradable synthetic polymers are poly(lactide), poly(caprolactam), poly(lactide-*co*-glycolide) copolymer, poly(acrylates). On the other hand, chitosan, alginate, albumin, and biomacromolecules are among other natural polymers. Majority of polymeric nanoparticles which falls in this category consists of polymersomes, polyplexes, polymer hybrid systems, and protein nanoparticles. Two main strategies, the “top-down” approach (the dispersion of preformed polymers) and the “bottom-up” approach (polymerization of monomers) are being used to produce and prepare polymeric NPs. Various methods, such as, solvent evaporation, micro-emulsion, mini-emulsion, surfactant-free emulsion, nanoprecipitation, salting-out, dialysis, fluid technology, and interfacial polymerization can produce desired nanoparticles.

Poly($_{D,L}$-lactide) (PDLLA) was able to produce uniform polymer NPs with different size range, ~70 nm, ~186 nm, ~358 nm, and 7.6 μm (GA-P1 to GA-P4) using an electrospray technique [148]. This approach offered high entrapment efficiencies of GA, 82–90%. These NPs can increase up to 1.78-times in the liver compared to GA solution indicating its use for HCC therapy. Gambogic acid was identified as a noncompetitive ligand specific to the transferrin receptor, a receptor that is present on various cancer conditions. PLGA NPs has been conjugated with GA to prove its non-competitive affinity to TfR in cell/cell-free systems [149]. *Ex vivo* and *in vivo* study exhibited superior transport of PLGA-GA through intestinal barrier. At the same time GA lead PLGA NPs to reach higher serum and brain levels while minimal levels in liver concentration. In a formulation of PLGA NPs, an optimal molar ratio of GA/docetaxel was encapsulated to achieve enhanced cellular apoptosis and the downregulation of P-gp expression [150]. The co-delivery using PLGA NPs are feasible and holds possible therapeutic implications to treat multidrug-resistant tumors.

A hyaluronic acid-*g*-glutamic acid based precision medicine has been created for successful targeted delivery of GA in HCC xenograft mouse model [151]. In this construction, hyaluronic acid was chosen with a disulfide bond linker (hyaluronic acid-all-trans retinoid acid, HA-SS-ATRA) which can be grafted at different rates (Fx, 60 or 100%). These nanostructures are considered as a novel reduction-activated charge-conversional core-shell NPs. The strongest reduction-responsive drug release with tumor penetrating formulation was optimized for therapeutic delivery. A drastic growth reduction was achieved with this formulation, i.e., 150 $mm^3$ compared to more than 3500 $mm^{3.}$ A layer-by-layer construction approach from protamine, hyaluronic acid, and lecithin resulted a self-assembly nanoparticle formulation which was used to load GA. A rapid HA detachment can occur *via* a "proton sponge" effect in the presence of hyaluronidase-rich tumor microenvironment [152]. This has been seen in tumor targeting in A549 xenograft tumors and exhibited superior anti-cancer potential.

A new nanoparticle concept was proposed to generate *N*-octyl-*N*-arginine-chitosan (OACS) for oral delivery of GA [153]. The selection of arginine was based on its complexation capacity with GA and its guanidyl groups which can promote the gastrointestinal uptake of drugs by transient opening of the tight junctions. Chitosan accounts for the increasing solubility of drugs and for increasing the transepithelial absorption. To achieve an optimized GA-OACS formulation with higher GA loading with superior absorption capacity, four different preparative methods (direct addition, emulsion solvent evaporation, dialysis, and thin-film rehydration) and orthogonal designs were utilized. [153] However, a superior construct was achieved by evaluating their absorption parameters in duodenum, jejunum, ileum, and colon for effective oral GA delivery for colorectal cancer. The extension of this work was also reported to examine pharmacokinetics and biodistribution in rats [154]. This data showed 1.5-fold and 2.0-fold increased for GA-OACS formulation in AUC and elimination half-life compared with GA–arginine, respectively. Moreover, GA-OACS formulation end up primarily in liver as seen reduced levels in kidney and heart.

#### 4.1.3. Inclusion complex- and pluronic polymer-based nanoparticles—

The inclusion complexes are often formed by a series of host-guest molecule(s) complexation and forms supramolecular nanostructures. Among many of these nanocarriers, cyclodextrins (CDs) are widely used to form inclusion complexes/self-assemblies with a number of hydrophilic/hydrophobic drug moieties. Ji *el al.,* [155] developed a well-defined biodegradable *β*-cyclodextrin grafted hyaluronic acid (HA) (HA-*g*-CD), phenylalanine-based poly(ester amide)s nanostructures with special arrangement of gambogic acid molecules. This unique nanostructure with HA enables for targeted delivery of GA to tumor cells by targeting CD44 receptors that are present on tumor cells present in patients with cancer. This formulation also exhibited enhanced cytotoxic and apoptosis potential in MDA-MB-435/MDR melanoma cells compared to free GA. Further, inclusion complexes of GA demonstrated repressed matrix metalloproteinase activities, implying potential tumor metastasis characteristics.

The pluronic F-68, poly(oxyethylene)-*b*-poly(oxypropylene) copolymer (MW 8350) was covalently linked with linoleic acid (LA) by esterification reaction to obtain F68-LA, which was efficient to form nano-spheres that can encapsulate GA by the film hydration method

[156]. This approach offers to the formulation: particle size (159.3 ± 2.2 nm), polydispersity index (0.076), negative zeta potential (- 23.2 mV), and GA loading (8.4% w/w) with loading efficiency (92%). F68-LA/GA nano-spheres exhibited similar inhibition of cell growth but outperformed inducing apoptosis in Annexin-V-PI and caspase 3/7 activity assays. A structurally fine-tuned 30 nm nanoarchitectures of vitamin E-based PEG-dendrimer self-assemblies were developed to obtain higher GA loading capacity (3:10 drug/nanoformulation, w/w). Although *in vitro* cytotoxicity test shows similar activity for both free GA and this novel GA-self-assemblies, but achieved better anti-tumor effects compared to GA in HT-29 tumor xenograft mouse model [157]. In general, poloxamers and *D-α*-Tocopheryl polyethylene glycol 1000 succinate (TPGS) has inherent P-gp inhibition characteristic. Moreover, their mixed micelles can increase therapeutic delivery of GA 2.9-times in NCI-ADR-RES cells which induce significant toxicity [158].

**4.1.4. Biomimetic nanoparticles—**Biomimetic nanoparticles often referred to as a combination of synthetic nanocarriers that are cloaked with cellular bio membranes. These nanoparticles in specific are targeted as a method of delivery for anti-cancerous agents. This method offers improved flexibility, functionality, and targetability. The major advance of these biomimetic NPs includes an efficient escape from immune infiltration/evasion, prolonged circulation time, and accumulation at the tumor sites. Among many cellular cloaked biomimetic NPs, red blood cell (RBC) membrane derived particles shown promising effects in *in vivo*. A strategically designed nanoparticle formulation that combined with PLGA parent core (preserve GA) with RBC-membranes. This formulation not only render GA's antitumor activity against colorectal tumor cells but exhibits enhanced biocompatibility and passive targeting while offering limited side effects [159]. Similar concept was also applied for an optimal targeting for colorectal cancer with high EGFR expression [160]. In this method, bispecific recombinant protein anti-EGFR-iRGD (contains tumor penetrating peptide, RGD peptide and EGFR single-domain antibody, sdAb) was decorated on GA containing PLGA-RBC-membrane biomimetic NPs which can introduce long-term tumor inhibition as well as safer antitumor efficacy than free GA. ABI 007, a human serum albumin-bound paclitaxel nanoparticle formulation called, Nab-paclitaxel technology (Abraxane®)) was approved by the US Food and Drug Administration (FDA) in 2005. A recent study followed Nab™ technology to produce human serum albumin (HSA)-GA NPs by simple HSA aqueous solution and GA organic solution mixing under sonication condition and solvent evaporation process [161]. All HSA-GA NPs generated in various organic solvent systems produced particle size <200 nm. The GA release profile is very slow under pH 7.4 in sodium dodecyl sulphate solution. It is important to note that tumor growth inhibition of HSA-GA NPs (2 mg/kg) in A549-bearing mice was 69.53 ± 5.23% which can be highly comparable with cabazitaxel (2 mg/kg), 65.86 ± 4.69%.

**4.1.5. Dendrimers—**Dendrimers are a class of nanocarriers with broad functional properties, ranging from drug delivery to nanodevice. For drug delivery applications, they can either entrap the drug *via* noncovalent interactions (formulation approach), or covalently couple with drugs (nano-construct approach). Dendrimers consist of multiple branches, providing enough surfaces for drug conjugations. They have been used frequently for platinum-drug delivery, and have been able to increase solubility and loading payload and

also decrease the toxicity and selective penetration of the drug in tumor tissue, leading to improvement in anti-cancer efficacy [162, 163]. A delivery through dendrimer is also a useful strategy [157]. This telodendrimer system composed of linear polyethylene glycol, dendritic oligomer of cholic acid, and vitamin E which self-assembled into ~ 30 nm stable cylindrical/spherical nanoparticles These dendrimers were preferentially accumulated into tumor tissue and deliver GA in HT-29 human colon cancer xenografts.

### 4.2. Inorganic nanoparticles

Another category of the nanocarriers, such as, metal-based NPs, silica, carbon nanotubes, and quantum dots, are designed for biomedical application focusing on diagnostic studies, for instance, discrimination of the histological aspects of cancer cells and healthy cells. Magnetic nanoparticles falls under this category and generally shown the encouraging properties for cancer therapy [129]. Magnetic nanoparticles, particularly, super paramagnetic nanoparticles ($Fe_3O_4$) exhibits excellent biocompatibility and cellular internalization, magnetic resonance, and magnetic hyperthermia characteristics enables several human medicine applications including cancer. $Fe_3O_4$ NPs combined with GA co-treatment facilitated GA-induced apoptosis in LOVO cells through PI3K/Akt/Bad pathway [164]. This combination study advices a higher anti-cancer potential may be expected when GA in encapsulated in $Fe_3O_4$ and in this regards further investigations are warranted. Through the magnetic $Fe_3O_4$ NPs it was possible to deliver GA more efficiently into Capan-1 pancreatic cancer cells and achieve synergistic anticancer activity by reducing protein expression of Bcl2 and enhancing the activation or levels of Bax, caspase 9, and caspase 3 [165]. Similarly, magnetic nanoparticle based-GA formulation can suppress Panc-1 pancreatic cancer cell proliferation and stops cell migration activity [166]. These effects were achieved due to decreased expression of ETS1 and its downstream target genes (cyclin D1, uPA, and VEGF). A hybrid magnetic NPs system composed of a reducible hexadecanol-modified chitosan oligosaccharide polymer micelle (CSO-SS-Hex) that is decorated with hyaluronic acid layers and grafted sheddable PEG-PLL (sPEG) copolymers in which iron oxide NPs and GA was encapsulated [167]. This nanoconstruct exhibited a superior targeting tumor by targeting CD44 receptors and prompt delivery of theranostics into tumors. Under external magnetic field, it produces enhanced cellular targeting and selective tumor cell killing. This formulation promotes a tumor-targeted delivery of therapeutics with redox/pH dual-responsivity which have superior implications in chemo and hyperthermia therapy for solid tumors.

Graphene and single-walled carbon nanotubes were utilized in number biomedical applications. An earlier attempt presented feasibility of GA loading and delivery to cancer cells [168]. GA-nanocomplex showed greater anti-proliferative apoptosis potential in breast and pancreatic cancer cells compared to free GA. These superior activity of GA-nanoassemblies was assessed through a). lactate dehydrogenase release, b). mitochondrial membrane depolarization, c). mitochondria dehydrogenase activity, d). intracellular lipid content, e). DNA fragmentation, and f). membrane permeability/caspase activities.

Quantum dots are larger representation for semiconductor nanomaterials. These nanomaterials widely being used for labeling of cells, imaging cells and organs, targeted

drug delivery purpose, and photodynamic therapies due to their unique optical properties. Cadmium tellurium (CdTe) are commonly used in biological cell labelling and drug delivery applications. Cysteamine (Cys), a positively-charged amine can be used to modify CdTe QDs that can efficiently be used for both labeling of cancer cells and drug delivery of GA[169]. These quantum dots exhibited a dose- and time-dependent anti-proliferation activity against HepG2 liver cancer cells. This nanosystem showed IC50 values of 1.35, 0.28, and 0.15 μM for 24, 48, and 78 hrs treatments, respectively. The GA in Cy-CdTe QDs could not affect the fluorescence potential for cell labeling and imaging. This system can also be extended for dual drug loading, GA and daunorubicin for efficient treatment of malignant lymphoma [170]. Such nanosystem not only provides a built in imaging and drug delivery characteristics but introduces a minimized drug resistance in cancer cells, which is a promising strategy in cancer treatment.

### 4.3. Multi-functional nanoparticles

Recent developments in nanomedicine and their unique applications are motivated in a big part for the development of multifunctional nanoparticles [171]. The major goal of multi-functional nanoparticles [172, 173] includes 1) early and accurate detection/diagnosis of disease, 2) enabling precise targeting of therapeutic agents, 3) Integrating multiple components or introducing physico-chemical responsive advantage in the treatment modality, and 4) no additional complications or side effects.

A new multi-component PLGA NPs system composed of chemo-/anti-angiogenesis and immunoadjuvant therapy [174] has promising advantage over other projects in the fight against cancer. For this, a complex NPs system was created using a PLGA-based core−shell nanoparticle loaded with GA, heparin (HP), and the immunoadjuvant cytosine-phosphate-guanine oligonucleotide (CpG ODN). Compared to CpG and GH (GA+HP) NPs treatment, GHC (GA+HP+CpG ODN) NPs treated group mice shown livers absence of tumor margins with no significant weight loss of mice. Additionally, the survival of mice was increased from 14 days to 30 days. This could be achieved due to the assistance of GA and HP, and CpG in GHC NPs which effectively upregulate cytotoxic T cell, promote helper T cell differentiation, and induce Th1 immune responses compared to single CpG ODN. Albumin-based tumor microenvironment-responsive “smarticles” were developed by self-assembly process. This system is composed of HSA, gambogic acid [as a heat shock protein 90 (HSP90)-inhibitor], and photothermal/cyanine dye (dc-IR825) as a way to achieve imaging, photo-thermal and anti-cancer therapy actions [175]. This system displays a pH-responsive tumor accumulation (at pH 6.8 tumor *vs* pH 7.4 normal physiological condition), which shows a release of theranostic molecules enabling synergistic actions of chemo/photo thermal therapy. Another construction was based on hyaluronic acid grafted with gambogic acid molecules (HA-*g*-GA) with reactive oxygen species sensitive attachment. This amphipathic prodrug was encapsulated with photosensitizer chlorin e6 (Ce6) to achieve complementary photodynamic therapy along with chemotherapy [176]. This formulation efficiently depleted intracellular glutathione and induced ROS and enhanced the photodynamic therapy potential in the cells. In addition, a multi-functional delivery system with LyP-1 peptide-modified low-molecular-weight heparin-quercetin conjugate (PLQ) is highly efficient to deliver a combination of chemotherapy and angiostatic therapy agents

[102]. LyP-1 peptide drive these nanoparticles specifically to breast cancer cells. This composite formulation was able to disrupt the lymphatic formation of tumor and inhibited the P-glycoprotein (P-gp) expression enabling reversing the drug resistance and preventing metastasis of cancer cells.

## 5. Future Perspective of GA delivery

The overall elements of this review suggest that GA-based nanomedicine is efficient in targeting tumors, capable to inhibit tumor growth, metastasis, angiogenesis, and reverse drug resistance (Figure 4).

It is important to consider the use of already FDA approved nanotechnology carriers for delivering GA which enables easy ways of conducting clinical trials and a step closure for translation in clinic usage. Most of the nanoparticle developed for GA delivery are in the form of suspensions that can be administered orally or parenterally. Often such type of nanoparticle formulations requires sterile preparations and highly stable formulations. Like Bayer® fast relief pain medication (Aspirin™, acetylsalicylic acid, microparticles that are on average 10 percent of the size of particles found in previous Aspirin™ tablets) development of GA nanoparticles in tablet form can provide easy access for administration and patient compliance. Such formulation may open favorable viewpoints for efficient delivery of GA as an adjuvant molecule in cancer treatments.

## 6. Conclusions

Over the past two decades implications of natural compounds has been multi-fold in cancer research. Gambogic acid is a promising natural molecule involved in multiple targets in cancer progression, invasion, metastasis, and angiogenesis. This review provides systematic elucidation of its ability of selective apoptosis induction in cancer cells. This molecule affects a great number of oncotargets and offer synergistic actions with chemotherapy and radiation therapies. GA has potential to reduce the numbers of cancer-associated deaths and introduce pain free medication for prolonged lives of patients. This review also recognized efficient ways to deliver GA using nanoparticles for enhanced solubility, bioavailability, adsorption and tumor imaging and targeting. Together, this work advices GA is a shining compound which can be translated for cancer therapy as a nanomedicine.

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## References

[1]. Siegel RL, Miller KD, Jemal A, Cancer statistics, 2020, CA Cancer J Clin 70(1) (2020) 7–30. [PubMed: 31912902]

[2]. Dias DA, Urban S, Roessner U, A historical overview of natural products in drug discovery, Metabolites 2(2) (2012) 303–36. [PubMed: 24957513]

[3]. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, Capaccioli S, Natural compounds for cancer treatment and prevention, Pharmacol Res 59(6) (2009) 365–78. [PubMed: 19429468]

[4]. Liesenklas W, Auterhoff H, The constitution of gambogic acid and its isomerization. 4. Chemistry of gum-resin, Arch Pharm Ber Dtsch Pharm Ges 299(9) (1966) 797–8. [PubMed: 5234498]

[5]. Han QB, Cheung S, Tai J, Qiao CF, Song JZ, Xu HX, Stability and cytotoxicity of gambogic acid and its derivative, gambogoic acid, Biol Pharm Bull 28(12) (2005) 2335–7. [PubMed: 16327177]

[6]. Lu GB, Yang XX, Huang QS, Isolation and structure of neo-gambogic acid from Gamboge (Garcinia hanburryi), Yao Xue Xue Bao 19(8) (1984) 636–9. [PubMed: 6549529]

[7]. Tisdale EJ, Slobodov I, Theodorakis EA, Unified synthesis of caged Garcinia natural products based on a site-selective Claisen/Diels-Alder/Claisen rearrangement, Proc Natl Acad Sci U S A 101(33) (2004) 12030–5. [PubMed: 15210986]

[8]. Wang X, Chen W, Gambogic acid is a novel anti-cancer agent that inhibits cell proliferation, angiogenesis and metastasis, Anticancer Agents Med Chem 12(8) (2012) 994–1000. [PubMed: 22339063]

[9]. Zhang HZ, Kasibhatla S, Wang Y, Herich J, Guastella J, Tseng B, Drewe J, Cai SX, Discovery, characterization and SAR of gambogic acid as a potent apoptosis inducer by a HTS assay, Bioorg Med Chem 12(2) (2004) 309–17. [PubMed: 14723951]

[10]. Kashyap D, Mondal R, Tuli HS, Kumar G, Sharma AK, Molecular targets of gambogic acid in cancer: recent trends and advancements, Tumour Biol 37(10) (2016) 12915–12925. [PubMed: 27448303]

[11]. Luo H, Vong CT, Chen H, Gao Y, Lyu P, Qiu L, Zhao M, Liu Q, Cheng Z, Zou J, Yao P, Gao C, Wei J, Ung COL, Wang S, Zhong Z, Wang Y, Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine, Chin Med 14 (2019) 48. [PubMed: 31719837]

[12]. Chi Y, Zhan XK, Yu H, Xie GR, Wang ZZ, Xiao W, Wang YG, Xiong FX, Hu JF, Yang L, Cui CX, Wang JW, An open-labeled, randomized, multicenter phase IIa study of gambogic acid injection for advanced malignant tumors, Chin Med J (Engl) 126(9) (2013) 1642–6. [PubMed: 23652044]

[13]. Zhao L, Zhen C, Wu Z, Hu R, Zhou C, Guo Q, General pharmacological properties, developmental toxicity, and analgesic activity of gambogic acid, a novel natural anticancer agent, Drug Chem Toxicol 33(1) (2010) 88–96. [PubMed: 20001662]

[14]. Zhou Z WJ, Phase I human tolerability trial of gambogic acid, Chin J New Drugs 16 (2007) 79–83.

[15]. Banik K, Harsha C, Bordoloi D, Lalduhsaki Sailo B, Sethi G, Leong HC, Arfuso F, Mishra S, Wang L, Kumar AP, Kunnumakkara AB, Therapeutic potential of gambogic acid, a caged xanthone, to target cancer, Cancer Lett 416 (2018) 75–86. [PubMed: 29246645]

[16]. Davenport J, Manjarrez JR, Peterson L, Krumm B, Blagg BS, Matts RL, Gambogic acid, a natural product inhibitor of Hsp90, J Nat Prod 74(5) (2011) 1085–92. [PubMed: 21486005]

[17]. Duan D, Zhang B, Yao J, Liu Y, Sun J, Ge C, Peng S, Fang J, Gambogic acid induces apoptosis in hepatocellular carcinoma SMMC-7721 cells by targeting cytosolic thioredoxin reductase, Free Radic Biol Med 69 (2014) 15–25. [PubMed: 24407164]

[18]. Xia G, Wang H, Song Z, Meng Q, Huang X, Huang X, Gambogic acid sensitizes gemcitabine efficacy in pancreatic cancer by reducing the expression of ribonucleotide reductase subunit-M2 (RRM2), J Exp Clin Cancer Res 36(1) (2017) 107. [PubMed: 28797284]

[19]. Wang S, Wang L, Chen M, Wang Y, Gambogic acid sensitizes resistant breast cancer cells to doxorubicin through inhibiting P-glycoprotein and suppressing survivin expression, Chem Biol Interact 235 (2015) 76–84. [PubMed: 25824409]

[20]. Salmon SE, Dalton WS, Grogan TM, Plezia P, Lehnert M, Roe DJ, Miller TP, Multidrug-resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer, Blood 78(1) (1991) 44–50. [PubMed: 1676918]

[21]. Chen J, Zhou M, Zhang Q, Xu J, Ouyang J, Anticancer Effect and Apoptosis Induction of Gambogic Acid in Human Leukemia Cell Line K562 In Vitro, Med Sci Monit 21 (2015) 1604–10. [PubMed: 26049398]

[22]. Hua X, Liang C, Dong L, Qu X, Zhao T, Simultaneous determination and pharmacokinetic study of gambogic acid and gambogenic acid in rat plasma after oral administration of Garcinia

hanburyi extracts by LC-MS/MS, Biomed Chromatogr 29(4) (2015) 545–51. [PubMed: 25159917]

[23]. He L, Ling Y, Fu L, Yin D, Wang X, Zhang Y, Synthesis and biological evaluation of novel derivatives of gambogic acid as anti-hepatocellular carcinoma agents, Bioorg Med Chem Lett 22(1) (2012) 289–92. [PubMed: 22153338]

[24]. Pandey MK, Karelia D, Amin SG, Gambogic Acid and Its Role in Chronic Diseases, Adv Exp Med Biol 928 (2016) 375–395. [PubMed: 27671824]

[25]. Yang LJ, Chen Y, New targets for the antitumor activity of gambogic acid in hematologic malignancies, Acta Pharmacol Sin 34(2) (2013) 191–8. [PubMed: 23274413]

[26]. Xu Y, Liu J, Wu Y, Guo Q, Sun H, Chen G, Natural products against hematological malignancies and identification of their targets, Sci China Life Sci 58(12) (2015) 1191–201. [PubMed: 26566803]

[27]. Wang YY, Zhang XJ, Yang YR, Sun HP, You QD, Progress in research of the structural optimization of natural product-like Garcinia caged xanthones, Yao Xue Xue Bao 49(3) (2014) 293–302. [PubMed: 24961098]

[28]. Wang S, Wu X, Tan M, Gong J, Tan W, Bian B, Chen M, Wang Y, Fighting fire with fire: poisonous Chinese herbal medicine for cancer therapy, J Ethnopharmacol 140(1) (2012) 33–45. [PubMed: 22265747]

[29]. Anantachoke N, Tuchinda P, Kuhakarn C, Pohmakotr M, Reutrakul V, Prenylated caged xanthones: chemistry and biology, Pharm Biol 50(1) (2012) 78–91. [PubMed: 22196584]

[30]. Klein-Junior LC, Campos A, Niero R, Correa R, Vander Heyden Y, Filho VC, Xanthones and Cancer: from Natural Sources to Mechanisms of Action, Chem Biodivers 17(2) (2020) e1900499.

[31]. Avila-Carrasco L, Majano P, Sanchez-Tomero JA, Selgas R, Lopez-Cabrera M, Aguilera A, Gonzalez Mateo G, Natural Plants Compounds as Modulators of Epithelial-to-Mesenchymal Transition, Front Pharmacol 10 (2019) 715. [PubMed: 31417401]

[32]. Mishra S, Verma SS, Rai V, Awasthee N, Chava S, Hui KM, Kumar AP, Challagundla KB, Sethi G, Gupta SC, Long non-coding RNAs are emerging targets of phytochemicals for cancer and other chronic diseases, Cell Mol Life Sci 76(10) (2019) 1947–1966. [PubMed: 30879091]

[33]. Aksorn N, Chanvorachote P, Integrin as a Molecular Target for Anti-cancer Approaches in Lung Cancer, Anticancer Res 39(2) (2019) 541–548. [PubMed: 30711928]

[34]. Sun R, Zhang HM, Chen BA, Anticancer activity and underlying mechanism of neogambogic acid, Chin J Nat Med 16(9) (2018) 641–643. [PubMed: 30269840]

[35]. Rajagopal C, Lankadasari MB, Aranjani JM, Harikumar KB, Targeting oncogenic transcription factors by polyphenols: A novel approach for cancer therapy, Pharmacol Res 130 (2018) 273–291. [PubMed: 29305909]

[36]. Shahabipour F, Caraglia M, Majeed M, Derosa G, Maffioli P, Sahebkar A, Naturally occurring anti-cancer agents targeting EZH2, Cancer Lett 400 (2017) 325–335. [PubMed: 28323035]

[37]. Hientz K, Mohr A, Bhakta-Guha D, Efferth T, The role of p53 in cancer drug resistance and targeted chemotherapy, Oncotarget 8(5) (2017) 8921–8946. [PubMed: 27888811]

[38]. Sung B, Prasad S, Yadav VR, Aggarwal BB, Cancer cell signaling pathways targeted by spice-derived nutraceuticals, Nutr Cancer 64(2) (2012) 173–97. [PubMed: 22149093]

[39]. Pandey MK, Gupta SC, Karelia D, Gilhooley PJ, Shakibaei M, Aggarwal BB, Dietary nutraceuticals as backbone for bone health, Biotechnol Adv 36(6) (2018) 1633–1648. [PubMed: 29597029]

[40]. Ruan J, Zheng C, Liu Y, Qu L, Yu H, Han L, Zhang Y, Wang T, Chemical and Biological Research on Herbal Medicines Rich in Xanthones, Molecules 22(10) (2017).

[41]. Han QB, Xu HX, Caged Garcinia xanthones: development since 1937, Curr Med Chem 16(28) (2009) 3775–96. [PubMed: 19747141]

[42]. DeSantis CE, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL, Breast cancer statistics, 2019, CA: a cancer journal for clinicians 69(6) (2019) 438–451. [PubMed: 31577379]

[43]. Gu H, Rao S, Zhao J, Wang J, Mu R, Rong J, Tao L, Qi Q, You Q, Guo Q, Gambogic acid reduced bcl-2 expression via p53 in human breast MCF-7 cancer cells, J Cancer Res Clin Oncol 135(12) (2009) 1777–82. [PubMed: 19582475]

[44]. Chen J, Gu HY, Lu N, Yang Y, Liu W, Qi Q, Rong JJ, Wang XT, You QD, Guo QL, Microtubule depolymerization and phosphorylation of c-Jun N-terminal kinase-1 and p38 were involved in gambogic acid induced cell cycle arrest and apoptosis in human breast carcinoma MCF-7 cells, Life Sci 83(3–4) (2008) 103–9. [PubMed: 18586278]

[45]. Zhen YZ, Lin YJ, Li KJ, Yang XS, Zhao YF, Wei J, Wei JB, Hu G, Gambogic Acid lysinate induces apoptosis in breast cancer mcf-7 cells by increasing reactive oxygen species, Evid Based Complement Alternat Med 2015 (2015) 842091.

[46]. Qi Q, Lu N, Wang XT, Gu HY, Yang Y, Liu W, Li C, You QD, Guo QL, Anti-invasive effect of gambogic acid in MDA-MB-231 human breast carcinoma cells, Biochem Cell Biol 86(5) (2008) 386–95. [PubMed: 18923540]

[47]. Kasibhatla S, Jessen KA, Maliartchouk S, Wang JY, English NM, Drewe J, Qiu L, Archer SP, Ponce AE, Sirisoma N, Jiang S, Zhang HZ, Gehlsen KR, Cai SX, Green DR, Tseng B, A role for transferrin receptor in triggering apoptosis when targeted with gambogic acid, Proc Natl Acad Sci U S A 102(34) (2005) 12095–100. [PubMed: 16103367]

[48]. Jian J, Yang Q, Huang X, Src regulates Tyr(20) phosphorylation of transferrin receptor-1 and potentiates breast cancer cell survival, J Biol Chem 286(41) (2011) 35708–15. [PubMed: 21859709]

[49]. Li D, Song XY, Yue QX, Cui YJ, Liu M, Feng LX, Wu WY, Jiang BH, Yang M, Qu XB, Liu X, Guo DA, Proteomic and bioinformatic analyses of possible target-related proteins of gambogic acid in human breast carcinoma MDA-MB-231 cells, Chin J Nat Med 13(1) (2015) 41–51. [PubMed: 25660287]

[50]. Theodoraki MA, Rezende CO Jr., Chantarasriwong O, Corben AD, Theodorakis EA, Alpaugh ML, Spontaneously-forming spheroids as an in vitro cancer cell model for anticancer drug screening, Oncotarget 6(25) (2015) 21255–67. [PubMed: 26101913]

[51]. Chantarasriwong O, Milcarek AT, Morales TH, Settle AL, Rezende CO Jr., Althufairi BD, Theodoraki MA, Alpaugh ML, Theodorakis EA, Synthesis, structure-activity relationship and in vitro pharmacodynamics of A-ring modified caged xanthones in a preclinical model of inflammatory breast cancer, Eur J Med Chem 168 (2019) 405–413. [PubMed: 30831408]

[52]. Chantarasriwong O, Dorwart TJ, Morales TH, Maggio SF, Settle AL, Milcarek AT, Alpaugh ML, Theodoraki MA, Theodorakis EA, Chiral resolution of a caged xanthone and evaluation across a broad spectrum of breast cancer subtypes, Bioorg Chem 93 (2019) 103303.

[53]. Pandey MK, Sung B, Ahn KS, Kunnumakkara AB, Chaturvedi MM, Aggarwal BB, Gambogic acid, a novel ligand for transferrin receptor, potentiates TNF-induced apoptosis through modulation of the nuclear factor-kappaB signaling pathway, Blood 110(10) (2007) 3517–25. [PubMed: 17673602]

[54]. Zhu X, Zhang H, Lin Y, Chen P, Min J, Wang Z, Xiao W, Chen B, Mechanisms of gambogic acid-induced apoptosis in non-small cell lung cancer cells in relation to transferrin receptors, J Chemother 21(6) (2009) 666–72. [PubMed: 20071291]

[55]. Qi Q, Lu N, Li C, Zhao J, Liu W, You Q, Guo Q, Involvement of RECK in gambogic acid induced anti-invasive effect in A549 human lung carcinoma cells, Mol Carcinog 54 Suppl 1 (2015) E13–25. [PubMed: 24532189]

[56]. Ye L, Zhou J, Zhao W, Jiao P, Ren G, Wang S, Gambogic acid-induced autophagy in nonsmall cell lung cancer NCI-H441 cells through a reactive oxygen species pathway, J Cancer Res Ther 14(Supplement) (2018) S942–S947. [PubMed: 30539827]

[57]. Zhu M, Jiang Y, Wu H, Shi W, Lu G, Cong D, Liu K, Song S, Ren J, Gambogic Acid Shows Anti-Proliferative Effects on Non-Small Cell Lung Cancer (NSCLC) Cells by Activating Reactive Oxygen Species (ROS)-Induced Endoplasmic Reticulum (ER) Stress-Mediated Apoptosis, Med Sci Monit 25 (2019) 3983–3988. [PubMed: 31138775]

[58]. Zhao T, Wang HJ, Zhao WW, Sun YL, Hu LK, Gambogic acid improves non-small cell lung cancer progression by inhibition of mTOR signaling pathway, Kaohsiung J Med Sci 33(11) (2017) 543–549. [PubMed: 29050671]

[59]. Li X, Tang X, Su J, Xu G, Zhao L, Qi Q, Involvement of E-cadherin/AMPK/mTOR axis in LKB1-induced sensitivity of non-small cell lung cancer to gambogic acid, Biochem Pharmacol 169 (2019) 113635.

[60]. Yang Y, Yang L, You QD, Nie FF, Gu HY, Zhao L, Wang XT, Guo QL, Differential apoptotic induction of gambogic acid, a novel anticancer natural product, on hepatoma cells and normal hepatocytes, Cancer Lett 256(2) (2007) 259–66. [PubMed: 17693016]

[61]. Lee PN, Ho WS, Antiproliferative activity of gambogic acid isolated from Garcinia hanburyi in Hep3B and Huh7 cancer cells, Oncol Rep 29(5) (2013) 1744–50. [PubMed: 23426995]

[62]. Park MS, Kim NH, Kang CW, Oh CW, Kim GD, Antimetastatic effects of gambogic acid are mediated via the actin cytoskeleton and NF-kappaB pathways in SK-HEP1 cells, Drug Dev Res 76(3) (2015) 132–42. [PubMed: 25959042]

[63]. Wang X, Lu N, Yang Q, Gong D, Lin C, Zhang S, Xi M, Gao Y, Wei L, Guo Q, You Q, Studies on chemical modification and biology of a natural product, gambogic acid (III): determination of the essential pharmacophore for biological activity, Eur J Med Chem 46(4) (2011) 1280–90. [PubMed: 21334116]

[64]. Mu R, Lu N, Wang J, Yin Y, Ding Y, Zhang X, Gui H, Sun Q, Duan H, Zhang L, Zhang Y, Ke X, Guo Q, An oxidative analogue of gambogic acid-induced apoptosis of human hepatocellular carcinoma cell line HepG2 is involved in its anticancer activity in vitro, Eur J Cancer Prev 19(1) (2010) 61–7. [PubMed: 19934761]

[65]. Nie F, Zhang X, Qi Q, Yang L, Yang Y, Liu W, Lu N, Wu Z, You Q, Guo Q, Reactive oxygen species accumulation contributes to gambogic acid-induced apoptosis in human hepatoma SMMC-7721 cells, Toxicology 260(1–3) (2009) 60–7. [PubMed: 19464570]

[66]. Guo QL, Lin SS, You QD, Gu HY, Yu J, Zhao L, Qi Q, Liang F, Tan Z, Wang X, Inhibition of human telomerase reverse transcriptase gene expression by gambogic acid in human hepatoma SMMC-7721 cells, Life Sci 78(11) (2006) 1238–45. [PubMed: 16257012]

[67]. Wu L, Guo H, Sun H, Zhang W, Sun C, Wang J, UNC119 mediates gambogic acid-induced cell-cycle dysregulation through the Gsk3beta/beta-catenin pathway in hepatocellular carcinoma cells, Anticancer Drugs 27(10) (2016) 988–1000. [PubMed: 27669172]

[68]. Wang X, Chen Y, Han QB, Chan CY, Wang H, Liu Z, Cheng CH, Yew DT, Lin MC, He ML, Xu HX, Sung JJ, Kung HF, Proteomic identification of molecular targets of gambogic acid: role of stathmin in hepatocellular carcinoma, Proteomics 9(2) (2009) 242–53. [PubMed: 19086098]

[69]. Hsieh SY, Huang SF, Yu MC, Yeh TS, Chen TC, Lin YJ, Chang CJ, Sung CM, Lee YL, Hsu CY, Stathmin1 overexpression associated with polyploidy, tumor-cell invasion, early recurrence, and poor prognosis in human hepatoma, Mol Carcinog 49(5) (2010) 476–87. [PubMed: 20232364]

[70]. Imura S, Yamada S, Saito YU, Iwahashi S, Arakawa Y, Ikemoto T, Morine Y, Utsunomiya T, Shimada M, miR-223 and Stathmin-1 Expression in Non-tumor Liver Tissue of Patients with Hepatocellular Carcinoma, Anticancer Res 37(10) (2017) 5877–5883. [PubMed: 28982915]

[71]. Li C, Tan YX, Zhou H, Ding SJ, Li SJ, Ma DJ, Man XB, Hong Y, Zhang L, Li L, Xia QC, Wu JR, Wang HY, Zeng R, Proteomic analysis of hepatitis B virus-associated hepatocellular carcinoma: Identification of potential tumor markers, Proteomics 5(4) (2005) 1125–39. [PubMed: 15759316]

[72]. Zhang R, Gao X, Zuo J, Hu B, Yang J, Zhao J, Chen J, STMN1 upregulation mediates hepatocellular carcinoma and hepatic stellate cell crosstalk to aggravate cancer by triggering the MET pathway, Cancer Sci 111(2) (2020) 406–417. [PubMed: 31785057]

[73]. Zhou Z, Ma J, Gambogic acid suppresses colon cancer cell activity in vitro, Exp Ther Med 18(4) (2019) 2917–2923. [PubMed: 31555380]

[74]. Wei J, Yang P, Li W, He F, Zeng S, Zhang T, Zhong J, Huang D, Chen Z, Wang C, Chen H, Hu H, Cao J, Gambogic acid potentiates the chemosensitivity of colorectal cancer cells to 5-fluorouracil by inhibiting proliferation and inducing apoptosis, Exp Ther Med 13(2) (2017) 662–668. [PubMed: 28352348]

[75]. Huang GM, Sun Y, Ge X, Wan X, Li CB, Gambogic acid induces apoptosis and inhibits colorectal tumor growth via mitochondrial pathways, World J Gastroenterol 21(20) (2015) 6194–205. [PubMed: 26034354]

[76]. Zhang H, Lei Y, Yuan P, Li L, Luo C, Gao R, Tian J, Feng Z, Nice EC, Sun J, ROS-mediated autophagy induced by dysregulation of lipid metabolism plays a protective role in colorectal cancer cells treated with gambogic acid, PLoS One 9(5) (2014) e96418.

[77]. Gao G, Bian Y, Qian H, Yang M, Hu J, Li L, Yu L, Liu B, Qian X, Gambogic acid regulates the migration and invasion of colorectal cancer via microRNA-21-mediated activation of phosphatase and tensin homolog, Exp Ther Med 16(3) (2018) 1758–1765. [PubMed: 30186399]

[78]. Wei F, Zhang T, Yang Z, Wei JC, Shen HF, Xiao D, Wang Q, Yang P, Chen HC, Hu H, Chen ZP, Huang Q, Li WL, Cao J, Gambogic Acid Efficiently Kills Stem-Like Colorectal Cancer Cells by Upregulating ZFP36 Expression, Cell Physiol Biochem 46(2) (2018) 829–846. [PubMed: 29627822]

[79]. Wen C, Huang L, Chen J, Lin M, Li W, Lu B, Rutnam ZJ, Iwamoto A, Wang Z, Yang X, Liu H, Gambogic acid inhibits growth, induces apoptosis, and overcomes drug resistance in human colorectal cancer cells, Int J Oncol 47(5) (2015) 1663–71. [PubMed: 26397804]

[80]. Youns M, ElKhoely A, Kamel R, The growth inhibitory effect of gambogic acid on pancreatic cancer cells, Naunyn Schmiedebergs Arch Pharmacol 391(5) (2018) 551–560. [PubMed: 29546614]

[81]. Yi T, Yi Z, Cho SG, Luo J, Pandey MK, Aggarwal BB, Liu M, Gambogic acid inhibits angiogenesis and prostate tumor growth by suppressing vascular endothelial growth factor receptor 2 signaling, Cancer Res 68(6) (2008) 1843–50. [PubMed: 18339865]

[82]. Lu L, Tang D, Wang L, Huang LQ, Jiang GS, Xiao XY, Zeng FQ, Gambogic acid inhibits TNF-alpha-induced invasion of human prostate cancer PC3 cells in vitro through PI3K/Akt and NF-kappaB signaling pathways, Acta Pharmacol Sin 33(4) (2012) 531–41. [PubMed: 22426696]

[83]. Pan H, Jansson KH, Beshiri ML, Yin J, Fang L, Agarwal S, Nguyen H, Corey E, Zhang Y, Liu J, Fan H, Lin H, Kelly K, Gambogic acid inhibits thioredoxin activity and induces ROS-mediated cell death in castration-resistant prostate cancer, Oncotarget 8(44) (2017) 77181–77194. [PubMed: 29100379]

[84]. Pan H, Lu LY, Wang XQ, Li BX, Kelly K, Lin HS, Gambogic Acid Induces Cell Apoptosis and Inhibits MAPK Pathway in PTEN(−/−)/p53(−/−) Prostate Cancer Cells In Vitro and Ex Vivo, Chin J Integr Med 24(2) (2018) 109–116. [PubMed: 28578487]

[85]. Liang L, Zhang Z, Gambogic Acid Inhibits Malignant Melanoma Cell Proliferation Through Mitochondrial p66shc/ROS-p53/Bax-Mediated Apoptosis, Cell Physiol Biochem 38(4) (2016) 1618–30. [PubMed: 27119348]

[86]. Liang L, Zhang Z, Qin X, Gao Y, Zhao P, Liu J, Zeng W, Gambogic Acid Inhibits Melanoma through Regulation of miR-199a-3p/ZEB1 Signalling, Basic Clin Pharmacol Toxicol 123(6) (2018) 692–703. [PubMed: 29959879]

[87]. Li CY, Wang Q, Wang XM, Li GX, Shen S, Wei XL, Gambogic acid exhibits anti-metastatic activity on malignant melanoma mainly through inhibition of PI3K/Akt and ERK signaling pathways, Eur J Pharmacol 864 (2019) 172719.

[88]. Qiang L, Yang Y, You QD, Ma YJ, Yang L, Nie FF, Gu HY, Zhao L, Lu N, Qi Q, Liu W, Wang XT, Guo QL, Inhibition of glioblastoma growth and angiogenesis by gambogic acid: an in vitro and in vivo study, Biochem Pharmacol 75(5) (2008) 1083–92. [PubMed: 18070617]

[89]. Thida M, Kim DW, Tran TTT, Pham MQ, Lee H, Kim I, Lee JW, Gambogic acid induces apoptotic cell death in T98G glioma cells, Bioorg Med Chem Lett 26(3) (2016) 1097–1101. [PubMed: 26631318]

[90]. Fu Q, Li C, Yu L, Gambogic acid inhibits spinal cord injury and inflammation through suppressing the p38 and Akt signaling pathways, Mol Med Rep 17(1) (2018) 2026–2032. [PubMed: 29138827]

[91]. Luo GX, Cai J, Lin JZ, Luo WS, Luo HS, Jiang YY, Zhang Y, Autophagy inhibition promotes gambogic acid-induced suppression of growth and apoptosis in glioblastoma cells, Asian Pac J Cancer Prev 13(12) (2012) 6211–6. [PubMed: 23464433]

[92]. Krajarng A, Imoto M, Tashiro E, Fujimaki T, Shinjo S, Watanapokasin R, Apoptosis induction associated with the ER stress response through up-regulation of JNK in HeLa cells by gambogic acid, BMC Complement Altern Med 15 (2015) 26. [PubMed: 25887496]

[93]. Yue Q, Feng L, Cao B, Liu M, Zhang D, Wu W, Jiang B, Yang M, Liu X, Guo D, Proteomic Analysis Revealed the Important Role of Vimentin in Human Cervical Carcinoma HeLa Cells Treated With Gambogic Acid, Mol Cell Proteomics 15(1) (2016) 26–44. [PubMed: 26499837]

[94]. Wang J, Yuan Z, Gambogic acid sensitizes ovarian cancer cells to doxorubicin through ROS-mediated apoptosis, Cell Biochem Biophys 67(1) (2013) 199–206. [PubMed: 23436279]

[95]. Tang Q, Lu M, Zhou H, Chen D, Liu L, Gambogic acid inhibits the growth of ovarian cancer tumors by regulating p65 activity, Oncol Lett 13(1) (2017) 384–388. [PubMed: 28123571]

[96]. Wang Y, Wang W, Sun H, Bromodomaincontaining protein 4 is critical for the antiproliferative and proapoptotic effects of gambogic acid in anaplastic thyroid cancer, Int J Mol Med 42(1) (2018) 161–170. [PubMed: 29717765]

[97]. Wang Q, Wei J, Wang C, Zhang T, Huang D, Wei F, He F, Cai W, Yang P, Zeng S, Li W, Cao J, Gambogic acid reverses oxaliplatin resistance in colorectal cancer by increasing intracellular platinum levels, Oncol Lett 16(2) (2018) 2366–2372. [PubMed: 30008940]

[98]. Kang Y, Lu L, Lan J, Ding Y, Yang J, Zhang Y, Zhao Y, Zhang T, Ho RJY, Redox-responsive polymeric micelles formed by conjugating gambogic acid with bioreducible poly(amido amine)s for the co-delivery of docetaxel and MMP-9 shRNA, Acta Biomater 68 (2018) 137–153. [PubMed: 29288085]

[99]. Wang T, Wei J, Qian X, Ding Y, Yu L, Liu B, Gambogic acid, a potent inhibitor of survivin, reverses docetaxel resistance in gastric cancer cells, Cancer Lett 262(2) (2008) 214–22. [PubMed: 18248784]

[100]. Zou Z, Xie L, Wei J, Yu L, Qian X, Chen J, Wang T, Liu B, Synergistic anti-proliferative effects of gambogic acid with docetaxel in gastrointestinal cancer cell lines, BMC Complement Altern Med 12 (2012) 58. [PubMed: 22546220]

[101]. Wang H, Zhao Z, Lei S, Li S, Xiang Z, Wang X, Huang X, Xia G, Huang X, Gambogic acid induces autophagy and combines synergistically with chloroquine to suppress pancreatic cancer by increasing the accumulation of reactive oxygen species, Cancer Cell Int 19 (2019) 7. [PubMed: 30627053]

[102]. Tian F, Dahmani FZ, Qiao J, Ni J, Xiong H, Liu T, Zhou J, Yao J, A targeted nanoplatform co-delivering chemotherapeutic and antiangiogenic drugs as a tool to reverse multidrug resistance in breast cancer, Acta Biomater 75 (2018) 398–412. [PubMed: 29874597]

[103]. Wang LH, Li Y, Yang SN, Wang FY, Hou Y, Cui W, Chen K, Cao Q, Wang S, Zhang TY, Wang ZZ, Xiao W, Yang JY, Wu CF, Gambogic acid synergistically potentiates cisplatin-induced apoptosis in non-small-cell lung cancer through suppressing NF-kappaB and MAPK/HO-1 signalling, Br J Cancer 110(2) (2014) 341–52. [PubMed: 24300974]

[104]. Liu L, Qi XJ, Zhong ZK, Zhang EN, Nanomedicine-based combination of gambogic acid and retinoic acid chlorochalcone for enhanced anticancer efficacy in osteosarcoma, Biomed Pharmacother 83 (2016) 79–84. [PubMed: 27470553]

[105]. Na K, Liu K, Yu J, Wang X, Li M, Tian C, He H, He Y, Wang Y, A solvent-assisted active loading technology to prepare gambogic acid and all-trans retinoic acid co-encapsulated liposomes for synergistic anticancer therapy, Drug Deliv Transl Res 10(1) (2020) 146–158. [PubMed: 31529371]

[106]. Bishayee K, Habib K, Sadra A, Huh SO, Targeting the Difficult-to-Drug CD71 and MYCN with Gambogic Acid and Vorinostat in a Class of Neuroblastomas, Cell Physiol Biochem 53(1) (2019) 258–280. [PubMed: 31313541]

[107]. Shi X, Chen X, Li X, Lan X, Zhao C, Liu S, Huang H, Liu N, Liao S, Song W, Zhou P, Wang S, Xu L, Wang X, Dou QP, Liu J, Gambogic acid induces apoptosis in imatinib-resistant chronic myeloid leukemia cells via inducing proteasome inhibition and caspase-dependent Bcr-Abl downregulation, Clin Cancer Res 20(1) (2014) 151–63. [PubMed: 24334603]

[108]. Ning R, Wang XP, Zhan YR, Qi Q, Huang XF, Hu G, Guo QL, Liu W, Yang J, Gambogic acid potentiates clopidogrel-induced apoptosis and attenuates irinotecan-induced apoptosis through down-regulating human carboxylesterase 1 and −2, Xenobiotica 46(9) (2016) 816–24. [PubMed: 26750665]

[109]. Wang C, Wang W, Wang C, Tang Y, Tian H, Combined therapy with EGFR TKI and gambogic acid for overcoming resistance in EGFR-T790M mutant lung cancer, Oncol Lett 10(4) (2015) 2063–2066. [PubMed: 26622796]

[110]. Pfister C, Pfrommer H, Tatagiba MS, Roser F, Vascular endothelial growth factor signals through platelet-derived growth factor receptor beta in meningiomas in vitro, Br J Cancer 107(10) (2012) 1702–13. [PubMed: 23047550]

[111]. Wang Y, Xiang W, Wang M, Huang T, Xiao X, Wang L, Tao D, Dong L, Zeng F, Jiang G, Methyl jasmonate sensitizes human bladder cancer cells to gambogic acid-induced apoptosis through down-regulation of EZH2 expression by miR-101, Br J Pharmacol 171(3) (2014) 618–35. [PubMed: 24490857]

[112]. Liu N, Huang H, Liu S, Li X, Yang C, Dou QP, Liu J, Calcium channel blocker verapamil accelerates gambogic acid-induced cytotoxicity via enhancing proteasome inhibition and ROS generation, Toxicol In Vitro 28(3) (2014) 419–25. [PubMed: 24373880]

[113]. He D, Xu Q, Yan M, Zhang P, Zhou X, Zhang Z, Duan W, Zhong L, Ye D, Chen W, The NF-kappa B inhibitor, celastrol, could enhance the anti-cancer effect of gambogic acid on oral squamous cell carcinoma, BMC Cancer 9 (2009) 343. [PubMed: 19778460]

[114]. Jiang XL, Zhang Y, Luo CL, Wu XH, Targeting renal cell carcinoma with gambogic acid in combination with sunitinib in vitro and in vivo, Asian Pac J Cancer Prev 13(12) (2012) 6463–8. [PubMed: 23464475]

[115]. Zhang W, Zhou H, Yu Y, Li J, Li H, Jiang D, Chen Z, Yang D, Xu Z, Yu Z, Combination of gambogic acid with cisplatin enhances the antitumor effects on cisplatin-resistant lung cancer cells by downregulating MRP2 and LRP expression, Onco Targets Ther 9 (2016) 3359–68. [PubMed: 27330316]

[116]. Ye JL, Yu YJ, Wu AL, Wang DY, Liu YC, Liu YQ, [Sensitization of human colon cancer HT-29 cells to TRAIL-induced apoptosis by gambognic acid], Yao Xue Xue Bao 50(10) (2015) 1252–7. [PubMed: 26837170]

[117]. Wang Y, Sui Y, Tao Y, Gambogic acid increases the sensitivity to paclitaxel in drugresistant triplenegative breast cancer via the SHH signaling pathway, Mol Med Rep 20(5) (2019) 4515–4522. [PubMed: 31545492]

[118]. Hatami E, Design, Development and Evaluation of Dual Drug Nanomedicine for Non-Small Cell Lung Cancer, Pharmaceutic Sciences, The University of Tennessee Health Science Center, 2020, pp. 50–73.

[119]. Deschatrette J, Ng-Bonaventure K, Philippe L, Wolfrom C, Interaction between Gambogic acid and dihydrofolate reductase and synergistic lethal effects with methotrexate on hepatoma cells, Anticancer Res 33(1) (2013) 133–42. [PubMed: 23267138]

[120]. Yang M, Yang Y, Cui H, Guan Z, Yang Y, Zhang H, Chen X, Zhu H, Yang X, Cai J, Cheng H, Sun X, The natural compound gambogic acid radiosensitizes nasopharyngeal carcinoma cells under hypoxic conditions, Tumori 102(2) (2016) 135–43. [PubMed: 26357974]

[121]. Yang Y, Sun X, Yang Y, Yang X, Zhu H, Dai S, Chen X, Zhang H, Guo Q, Song Y, Wang F, Cheng H, Sun X, Gambogic acid enhances the radiosensitivity of human esophageal cancer cells by inducing reactive oxygen species via targeting Akt/mTOR pathway, Tumour Biol 37(2) (2016) 1853–62. [PubMed: 26318432]

[122]. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, AcostaTorres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin HS, Nano based drug delivery systems: recent developments and future prospects, J Nanobiotechnology 16(1) (2018) 71. [PubMed: 30231877]

[123]. Tran S, DeGiovanni PJ, Piel B, Rai P, Cancer nanomedicine: a review of recent success in drug delivery, Clin Transl Med 6(1) (2017) 44. [PubMed: 29230567]

[124]. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR, Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date, Pharm Res 33(10) (2016) 2373–87. [PubMed: 27299311]

[125]. Ventola CL, Progress in Nanomedicine: Approved and Investigational Nanodrugs, P T 42(12) (2017) 742–755. [PubMed: 29234213]

[126]. Yallapu MM, Jaggi M, Chauhan SC, Design and engineering of nanogels for cancer treatment, Drug Discov Today 16(9–10) (2011) 457–63. [PubMed: 21414419]

[127]. Yallapu MM, Jaggi M, Chauhan SC, Curcumin nanoformulations: a future nanomedicine for cancer, Drug Discov Today 17(1–2) (2012) 71–80. [PubMed: 21959306]

[128]. Yallapu MM, Nagesh PK, Jaggi M, Chauhan SC, Therapeutic Applications of Curcumin Nanoformulations, AAPS J 17(6) (2015) 1341–56. [PubMed: 26335307]

[129]. Chowdhury P, Roberts AM, Khan S, Hafeez BB, Chauhan SC, Jaggi M, Yallapu MM, Magnetic nanoformulations for prostate cancer, Drug Discov Today 22(8) (2017) 1233–1241. [PubMed: 28526660]

[130]. Kumar R, Gupta A, Chawla M, Aadil AR, Dutt S, Kumar VB, Chaudhary A, Advances in Nanotechnology based Strategies for Synthesis of Nanoparticles of Lignin, in: Sharma A, Kumar A (Eds.), Lignin Biosynthesis and Transformation for Industrial Applications, Springer, Cham, Springer Nature Switzerland AG 2020, 2020, pp. 203–22.

[131]. Bor G, Mat Azmi ID, Yaghmur A, Nanomedicines for cancer therapy: current status, challenges and future prospects, Ther Deliv 10(2) (2019) 113–132. [PubMed: 30678550]

[132]. Jin Q, Deng Y, Chen X, Ji J, Rational Design of Cancer Nanomedicine for Simultaneous Stealth Surface and Enhanced Cellular Uptake, ACS Nano 13(2) (2019) 954–977. [PubMed: 30681834]

[133]. Dai Q, Bertleff-Zieschang N, Braunger JA, Bjornmalm M, Cortez-Jugo C, Caruso F, Particle Targeting in Complex Biological Media, Adv Healthc Mater 7(1) (2018).

[134]. Haley B, Frenkel E, Nanoparticles for drug delivery in cancer treatment, Urol Oncol 26(1) (2008) 57–64. [PubMed: 18190833]

[135]. Yamashita F, Hashida M, Pharmacokinetic considerations for targeted drug delivery, Adv Drug Deliv Rev 65(1) (2013) 139–47. [PubMed: 23280371]

[136]. Jain RK, Stylianopoulos T, Delivering nanomedicine to solid tumors, Nat Rev Clin Oncol 7(11) (2010) 653–64. [PubMed: 20838415]

[137]. Wang J, Sui M, Fan W, Nanoparticles for tumor targeted therapies and their pharmacokinetics, Curr Drug Metab 11(2) (2010) 129–41. [PubMed: 20359289]

[138]. Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST, Challenges and strategies in anti-cancer nanomedicine development: An industry perspective, Adv Drug Deliv Rev 108 (2017) 25–38. [PubMed: 27137110]

[139]. Kumar R, Singh A, Sharma K, Dhasmana D, Garg N, Siril PF, Preparation, characterization and in vitro cytotoxicity of Fenofibrate and Nabumetone loaded solid lipid nanoparticles, Mater Sci Eng C Mater Biol Appl 106 (2020) 110184.

[140]. Kumar R, Singh A, Garg A, Acoustic Cavitation-Assisted Formulation of Solid Lipid Nanoparticles using Different Stabilizers, ACS Omega 4,(8) (2019) 13360–13370. [PubMed: 31460464]

[141]. Kumar R, Singh A, Garg N, Acoustic cavitation assisted hot melt mixing technique for solid lipid nanoparticles formulation, characterization, and controlled delivery of poorly water soluble drugs, J Drug Deliver Sci Technol 54 (2019,) 101277.

[142]. Doddapaneni R, Patel K, Owaid IH, Singh M, Tumor neovasculature-targeted cationic PEGylated liposomes of gambogic acid for the treatment of triple-negative breast cancer, Drug Deliv 23(4) (2016) 1232–41. [PubMed: 26701717]

[143]. Yan X, Yang Y, He L, Peng D, Yin D, Gambogic acid grafted low molecular weight heparin micelles for targeted treatment in a hepatocellular carcinoma model with an enhanced anti-angiogenesis effect, Int J Pharm 522(1–2) (2017) 110–118. [PubMed: 28242375]

[144]. Huang R, Li J, Kebebe D, Wu Y, Zhang B, Liu Z, Cell penetrating peptides functionalized gambogic acid-nanostructured lipid carrier for cancer treatment, Drug Deliv 25(1) (2018) 757–765. [PubMed: 29528244]

[145]. Kebebe D, Wu Y, Zhang B, Yang J, Liu Y, Li X, Ma Z, Lu P, Liu Z, Li J, Dimeric c(RGD) peptide conjugated nanostructured lipid carriers for efficient delivery of Gambogic acid to breast cancer, Int J Nanomedicine 14 (2019) 6179–6195. [PubMed: 31447559]

[146]. Wang Y, Liang X, Tong R, Yang J, Yang Y, Zhong J, Wu Q, Gong C, Yu J, Cai L, Gambogic Acid-Loaded Polymeric Micelles for Improved Therapeutic Effect in Breast Cancer, J Biomed Nanotechnol 14(10) (2018) 1695–1704. [PubMed: 30041717]

[147]. Wang S, Yang Y, Wang Y, Chen M, Gambogic acid-loaded pH-sensitive mixed micelles for overcoming breast cancer resistance, Int J Pharm 495(2) (2015) 840–8. [PubMed: 26407923]

[148]. Yin D, Yang Y, Cai H, Wang F, Peng D, He L, Gambogic acid-loaded electrosprayed particles for site-specific treatment of hepatocellular carcinoma, Mol Pharm 11(11) (2014) 4107–17. [PubMed: 25290462]

[149]. Saini P, Ganugula R, Arora M, Kumar MN, The Next Generation Non-competitive Active Polyester Nanosystems for Transferrin Receptor-mediated Peroral Transport Utilizing Gambogic Acid as a Ligand, Sci Rep 6 (2016) 29501. [PubMed: 27388994]

[150]. Xu Y, Wang C, Ding Y, Wang Y, Liu K, Tian Y, Gao M, Li Z, Zhang J, Li L, Nanoparticles with Optimal Ratiometric Co-Delivery of Docetaxel with Gambogic Acid for Treatment of Multidrug-Resistant Breast Cancer, J Biomed Nanotechnol 12(9) (2016) 1774–81. [PubMed: 29345888]

[151]. Han L, Wang Y, Huang X, Liu B, Hu L, Ma C, Liu J, Xue J, Qu W, Liu F, Feng F, Liu W, A stage-specific cancer chemotherapy strategy through flexible combination of reduction-activated charge-conversional core-shell nanoparticles, Theranostics 9(22) (2019) 6532–6549. [PubMed: 31588234]

[152]. Ke Z, Yang L, Wu H, Li Z, Jia X, Zhang Z, Evaluation of in vitro and in vivo antitumor effects of gambogic acid-loaded layer-by-layer self-assembled micelles, Int J Pharm 545(1–2) (2018) 306–317. [PubMed: 29654896]

[153]. Yu F, He C, Waddad AY, Munyendo WL, Lv H, Zhou J, Zhang Q, N-octyl-N-arginine-chitosan (OACS) micelles for gambogic acid oral delivery: preparation, characterization and its study on in situ intestinal perfusion, Drug Dev Ind Pharm 40(6) (2014) 774–82. [PubMed: 23679668]

[154]. Yu F, Jiang F, Tang X, Wang B, N-octyl-N-arginine-chitosan micelles for gambogic acid intravenous delivery: characterization, cell uptake, pharmacokinetics, and biodistribution, Drug Dev Ind Pharm 44(4) (2018) 615–623. [PubMed: 29188736]

[155]. Ji Y, Shan S, He M, Chu CC, Inclusion complex from cyclodextrin-grafted hyaluronic acid and pseudo protein as biodegradable nano-delivery vehicle for gambogic acid, Acta Biomater 62 (2017) 234–245. [PubMed: 28859900]

[156]. Fang X, Xu Y, Wang S, Wan J, He C, Chen M, Pluronic F68-Linoleic Acid Nanospheres Mediated Delivery of Gambogic Acid for Cancer Therapy, AAPS PharmSciTech 18(1) (2017) 147–155. [PubMed: 26912357]

[157]. Huang W, Wang X, Shi C, Guo D, Xu G, Wang L, Bodman A, Luo J, Fine-tuning vitamin E-containing telodendrimers for efficient delivery of gambogic acid in colon cancer treatment, Mol Pharm 12(4) (2015) 1216–29. [PubMed: 25692376]

[158]. Saxena V, Hussain MD, Poloxamer 407/TPGS mixed micelles for delivery of gambogic acid to breast and multidrug-resistant cancer, Int J Nanomedicine 7 (2012) 713–21. [PubMed: 22359450]

[159]. Zhang Z, Qian H, Yang M, Li R, Hu J, Li L, Yu L, Liu B, Qian X, Gambogic acid-loaded biomimetic nanoparticles in colorectal cancer treatment, Int J Nanomedicine 12 (2017) 1593–1605. [PubMed: 28280328]

[160]. Zhang Z, Qian H, Huang J, Sha H, Zhang H, Yu L, Liu B, Hua D, Qian X, Anti-EGFR-iRGD recombinant protein modified biomimetic nanoparticles loaded with gambogic acid to enhance targeting and antitumor ability in colorectal cancer treatment, Int J Nanomedicine 13 (2018) 4961–4975. [PubMed: 30214200]

[161]. Zhang Y, Yang Z, Tan X, Tang X, Yang Z, Development of a More Efficient Albumin-Based Delivery System for Gambogic Acid with Low Toxicity for Lung Cancer Therapy, AAPS PharmSciTech 18(6) (2017) 1987–1997. [PubMed: 27933587]

[162]. Chauhan AS, Dendrimers for Drug Delivery, Molecules 23(4) (2018).

[163]. Madaan K, Kumar S, Poonia N, Lather V, Pandita D, Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues, J Pharm Bioallied Sci 6(3) (2014) 139–50. [PubMed: 25035633]

[164]. Fang L, Chen B, Liu S, Wang R, Hu S, Xia G, Tian Y, Cai X, Synergistic effect of a combination of nanoparticulate Fe3O4 and gambogic acid on phosphatidylinositol 3-kinase/Akt/Bad pathway of LOVO cells, Int J Nanomedicine 7 (2012) 4109–18. [PubMed: 22888247]

[165]. Wang C, Zhang H, Chen B, Yin H, Wang W, Study of the enhanced anticancer efficacy of gambogic acid on Capan-1 pancreatic cancer cells when mediated via magnetic Fe3O4 nanoparticles, Int J Nanomedicine 6 (2011) 1929–35. [PubMed: 21931488]

[166]. Wang C, Zhang H, Chen Y, Shi F, Chen B, Gambogic acid-loaded magnetic Fe(3)O(4) nanoparticles inhibit Panc-1 pancreatic cancer cell proliferation and migration by inactivating transcription factor ETS1, Int J Nanomedicine 7 (2012) 781–7. [PubMed: 22393285]

[167]. Sang MM, Liu FL, Wang Y, Luo RJ, Huan XX, Han LF, Zhang ZT, Feng F, Qu W, Liu W, Zheng F, A novel redox/pH dual-responsive and hyaluronic acid-decorated multifunctional magnetic complex micelle for targeted gambogic acid delivery for the treatment of triple negative breast cancer, Drug Deliv 25(1) (2018) 1846–1857. [PubMed: 30334478]

[168]. Saeed LM, Mahmood M, Pyrek SJ, Fahmi T, Xu Y, Mustafa T, Nima ZA, Bratton SM, Casciano D, Dervishi E, Radominska-Pandya A, Biris AS, Single-walled carbon nanotube and graphene nanodelivery of gambogic acid increases its cytotoxicity in breast and pancreatic cancer cells, J Appl Toxicol 34(11) (2014) 1188–99. [PubMed: 25220893]

[169]. Xu P, Li J, Shi L, Selke M, Chen B, Wang X, Synergetic effect of functional cadmium-tellurium quantum dots conjugated with gambogic acid for HepG2 cell-labeling and proliferation inhibition, Int J Nanomedicine 8 (2013) 3729–36. [PubMed: 24109183]

[170]. Zhou Y, Wang R, Chen B, Sun D, Hu Y, Xu P, Daunorubicin and gambogic acid coloaded cysteamine-CdTe quantum dots minimizing the multidrug resistance of lymphoma in vitro and in vivo, Int J Nanomedicine 11 (2016) 5429–5442. [PubMed: 27799767]

[171]. Habibi N, Quevedo DF, Gregory JV, Lahann J, Emerging methods in therapeutics using multifunctional nanoparticles, Wiley Interdiscip Rev Nanomed Nanobiotechnol (2020) e1625.

[172]. Babu A, Templeton AK, Munshi A, Ramesh R, Nanodrug delivery systems: a promising technology for detection, diagnosis, and treatment of cancer, AAPS PharmSciTech 15(3) (2014) 709–21. [PubMed: 24550101]

[173]. Srinivasan M, Rajabi M, Mousa SA, Multifunctional Nanomaterials and Their Applications in Drug Delivery and Cancer Therapy, Nanomaterials (Basel) 5(4) (2015) 1690–1703. [PubMed: 28347089]

[174]. Shao F, Zhang M, Xu L, Yin D, Li M, Jiang Q, Zhang Q, Yang Y, Multiboosting of Cancer Immunotherapy by a Core-Shell Delivery System, Mol Pharm 17(1) (2020) 338–348. [PubMed: 31793786]

[175]. Gao G, Jiang YW, Sun W, Guo Y, Jia HR, Yu XW, Pan GY, FG W, Molecular Targeting-Mediated Mild-Temperature Photothermal Therapy with a Smart Albumin-Based Nano Drug, Small 15 (2019) 1900501.

[176]. Liang H, Zhou Z, Luo R, Sang M, Liu B, Sun M, Qu W, Feng F, Liu W, Tumor-specific activated photodynamic therapy with an oxidation-regulated strategy for enhancing anti-tumor efficacy, Theranostics 8(18) (2018) 5059–5071. [PubMed: 30429886]

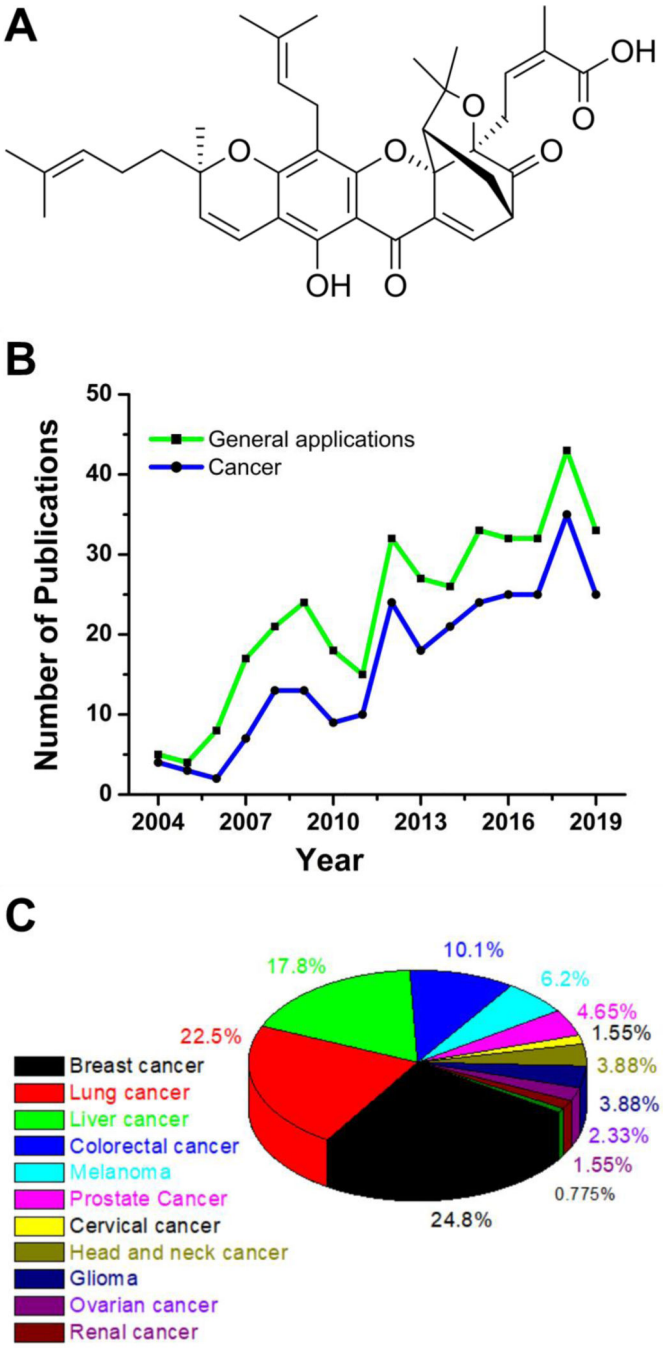


**Figure 1.**

Graphical representation of significant implications of “gambogic acid” in medicinal field. **A.** Chemical structure of gambogic acid. **B.** Area plot graphical illustration of scientific literature relevant to gambogic acid for general and cancer specific studies that are obtained using PubMed of National Center for Biotechnology Information, U.S. National Library of Medicine (http://www.ncbi.nlm.nih.gov/pubmed/) over a period of 2004–2019 (search was conducted April 2020). **C.** Pei chart patrician of studies related to gambogic acid for the treatment of various types of cancers (breast, lung, liver, melanoma, prostate, cervical, head

and neck, glioma, ovarian, renal, and oral cancers). Data was collected based on gambogic acid involved in studies directly or indirectly using PubMed link employing key words: “gambogic acid” for general application, “gambogic acid and cancer” for cancer application in Figure B, and “gambogic acid and respective cancer” for Figure C. There were no exclusion criteria applied for this search.

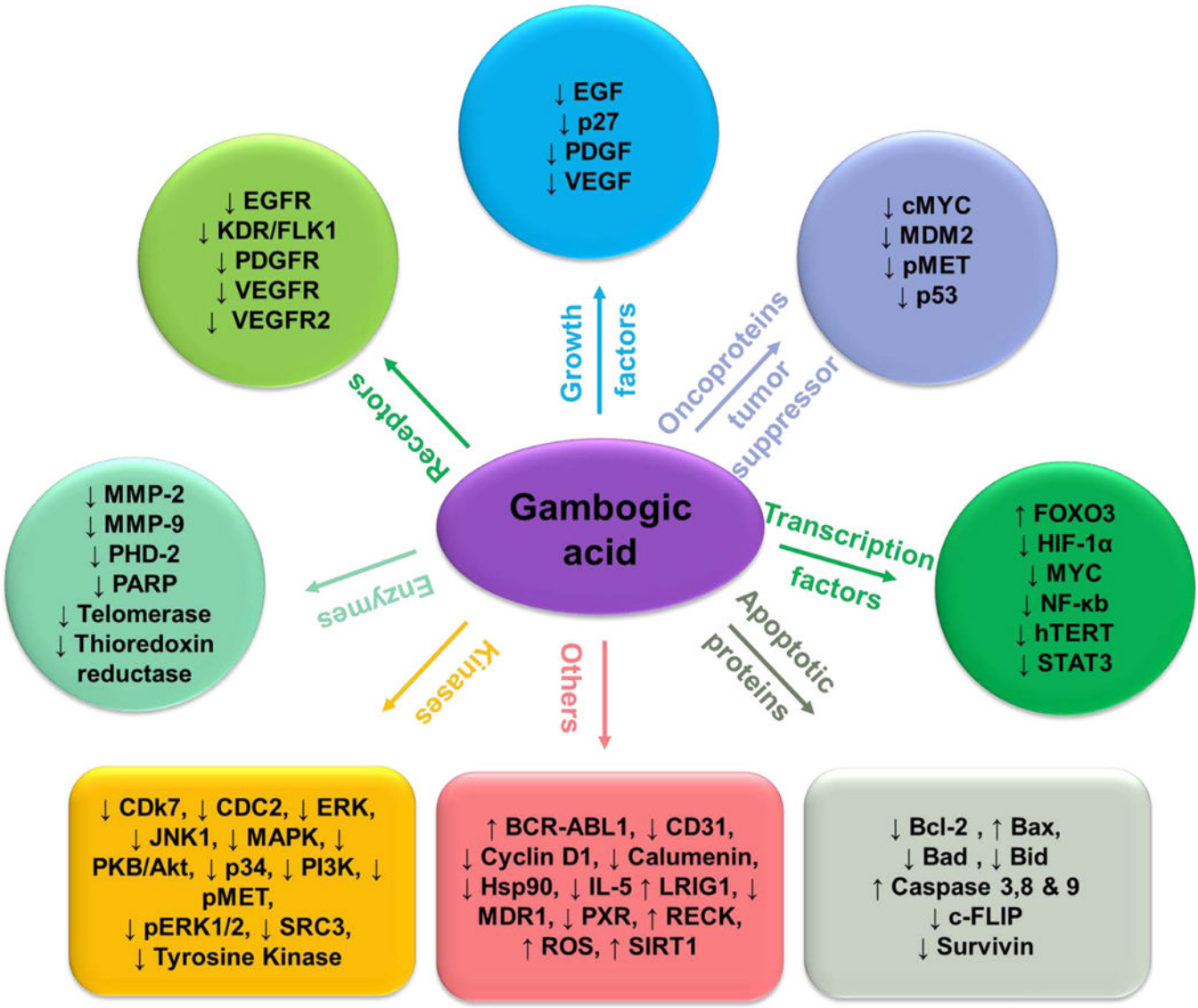


**Figure 2.**
Various molecular targets of gambogic acid in cancer cells [15].

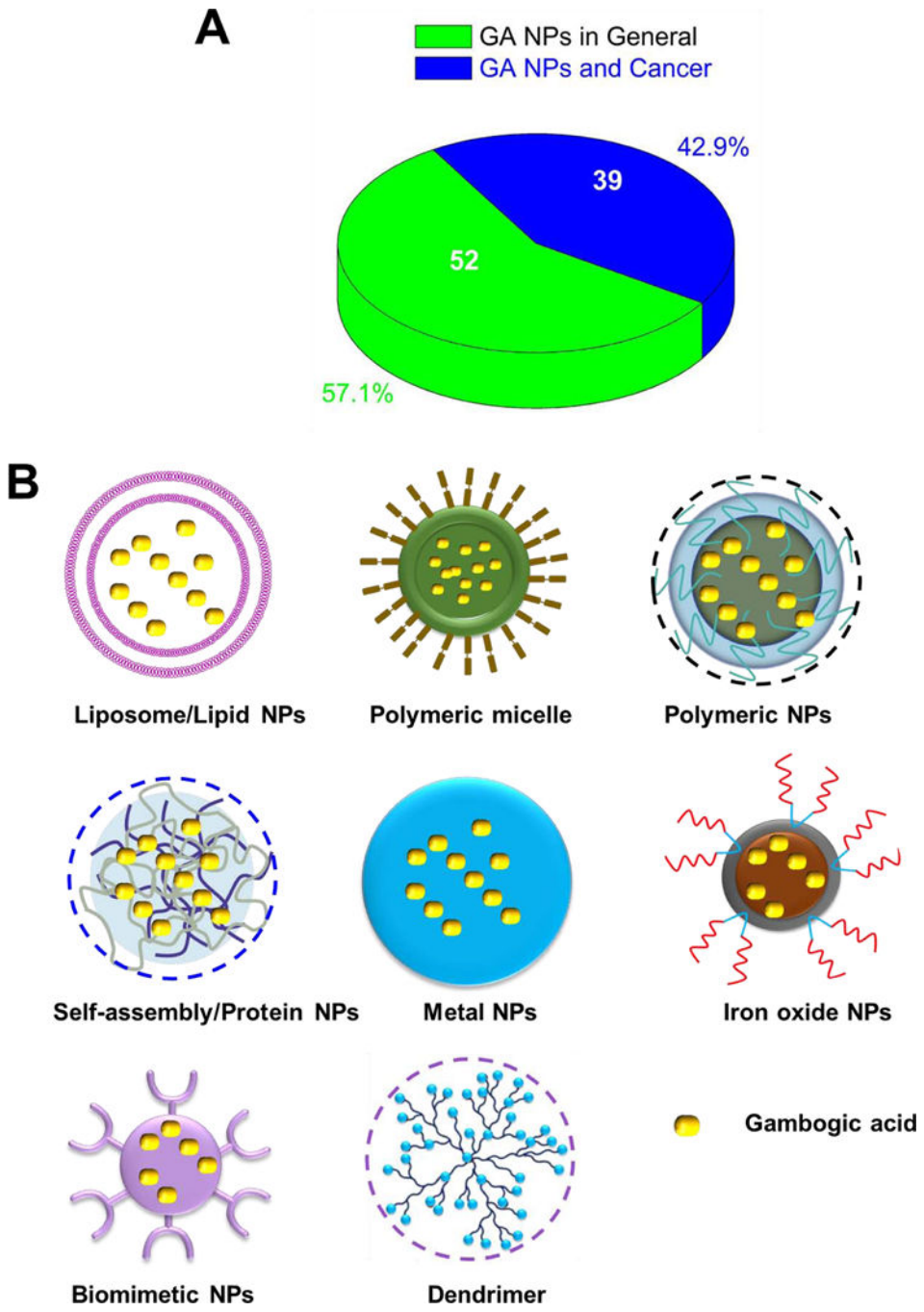


**Figure 3.**

GA-based nanomedicine in the literature. **A)** Literature support GA-based nanoparticle formulation for cancer and all general medical applications. PubMed data was retrieved in April 2020. Data was collected based on gambogic acid involved in studies directly or indirectly using PubMed link with key words: “gambogic acid and nanoparticles” for general application and “gambogic acid and nanoparticles and cancer” for cancer application in Figure A. There were no exclusion criteria applied for this search. **B)** Schematic representation of different types of nanoparticles that been used to generate gambogic acid-

based nanoparticles. All structures are hypothetical, color, scale, and morphologies are not to measure. These structures represent to liposome, lipid, polymeric micelle, polymeric, self-assembly, protein, metal, iron oxide, biomimetic, and dendrimer-based nanoparticles.

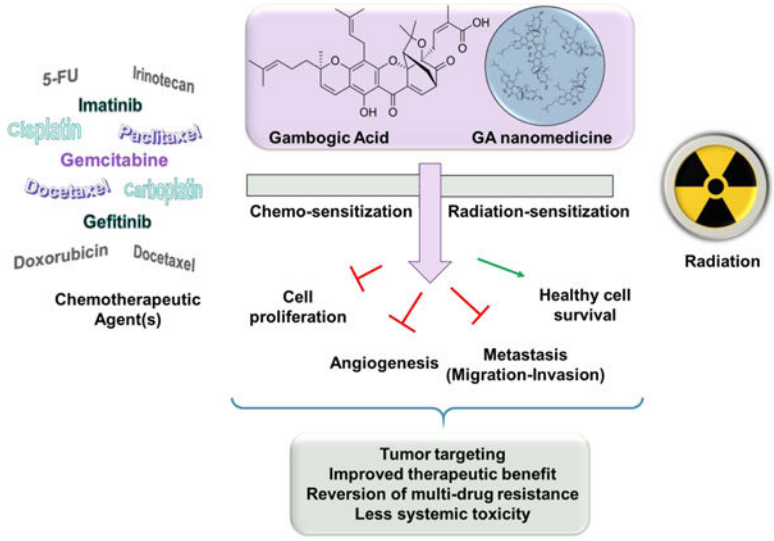


**Figure 4.**

Schematic representation of review concept of GA nanomedicine for improved therapeutic benefit in cancer treatment. This describes possible implications of GA/GA-nanoparticles to inhibit cell proliferation, angiogenesis, metastasis, chemosensitization, and radiation sensitization in cancer cells while restoring healthy cell function.

**Table 1.**

Published review article details of gambogic acid in cancer and its inhibitory roles in cancer associated pathways

| Title and author | Review article details pertained |
|---|---|
| Therapeutic potential of gambogic acid, a caged xanthone, to target cancer. Banik K *et al.*, 2018 [15] | This review provides information related to the molecular mechanisms underlying the targets of GA and as a pertinent candidate for cancer drug. |
| Gambogic Acid and Its Role in Chronic Diseases. Pandey MK *et al.*, 2016 [24] | Kokum (one of the major active components is gambogic acid) a spice derived from the fruit of the Garcinia hanburyi tree. This chapter discusses the sources, chemical components, mechanism of action, and disease targets of the kokum spice. |
| Molecular targets of gambogic acid in cancer: recent trends and advancements. Kashyap D *et al.*, 2016 [10] | This work uncovers the bio-therapeutic potential of GA along with the possible mechanistic interactions with cellular targets. |
| New targets for the antitumor activity of gambogic acid in hematologic malignancies. Yang LJ, Chen Y. 2013 [25] | This review focuses on the new mechanisms of GA inhibits proliferation and induces apoptosis in malignant hematological cells. |
| Gambogic acid is a novel anti-cancer agent that inhibits cell proliferation, angiogenesis and metastasis. Wang X, Chen W. 2012 [8] | In this review recapitulates the functional effects of GA on apoptosis and the prevention of angiogenesis and metastasis. |
| Natural products against hematological malignancies and identification of their targets. Xu Y, *et al.*, 2015 [26] | This article outlines various discoveries of natural compounds (adenanthin, oridonin, gambogic acid and wogonoside) and their targets with activity in hematological malignancies. |
| Progress in Research of the Structural Optimization of Natural Product-Like Garcinia Caged Xanthones. Wang Y-Y, *et al.*, 2014 [27] | This review provides GA-based pharmacophore for efficient design, structural optimization, and SAR for achieving potent anticancer activity. |
| Fighting Fire With Fire: Poisonous Chinese Herbal Medicine for Cancer Therapy. Wang S, *et al.*, 2012 [28] | This review aimed at documenting poisonous Chinese herbal medicine to exploit as potential anticancer molecules appropriate safety and molecular mechanism exploration. |
| Prenylated Caged Xanthones: Chemistry and Biology. Anantachoke N, *et al.*, 2012 [29] | Over 120 caged xanthones of (Garcinia, Cratoxylum, and Dascymaschalon) have been documented for their biological activities against cancer, HIV-1, bacterial, inflammatory, and neutropenia. |

**Table 2.**

Published review article details of gambogic acid and its associated molecules in cancer and other therapeutics.

| Title and author | Review article details pertained |
|---|---|
| Xanthones and Cancer: from Natural Sources to Mechanisms of Action. Klein- Júnior LC *et al.,* 2020 [30] | This review documents both natural and synthetic sources of xanthones (including GA) and reporting their mechanisms of action for the anti-cancer effects. |
| Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine. Luo H *et al.,* 2019 [11] | This provides a detailed perspective on the mechanism of action for therapy and immunomodulation. |
| Natural Plants Compounds as Modulators of Epithelial-to-Mesenchymal Transition. Avila-Carrasco L *et al.,* 2019 [31] | This article presents set of natural plant compounds and their influence on epithelial-to-mesenchymal transition related pathways. |
| Long non-coding RNAs are emerging targets of phytochemicals for cancer and other chronic diseases. Mishra S *et al.,* 2019 [32] | This work introduces the modulation of lncRNAs by various phytochemicals in cancer and other chronic diseases. |
| Integrin as a Molecular Target for Anticancer Approaches in Lung Cancer. Aksorn N & Chanvorachote P. 2019 [33] | This work was aimed to help in the understanding of key natural molecules for developing anti-cancer drugs. |
| Anticancer activity and underlying mechanism of neogambogic acid. Sun R *et al.,* 2018 [34] | This work underlines the anti-tumor potential of neogambogic acid (similar chemical structure as GA). |
| Targeting oncogenic transcription factors by polyphenols: A novel approach for cancer therapy. Rajagopal C *et al.,* 2018 [35] | This work summarizes multi-targeting capacity of natural compounds and their feasible application in cancer therapy. |
| Naturally occurring anti-cancer agents targeting EZH2. Shahabipour F *et al.,* 2017 [36] | This review documents some important pathways of naturally occurring anti-cancer agents. |
| The role of p53 in cancer drug resistance and targeted chemotherapy. Hientz K *et al.,* 2017 [37] | This work presents the role of p53-MDM2 signaling axis and how to tackle cancerous indexes with natural agents. |
| Cancer cell signaling pathways targeted by spice-derived nutraceuticals. Sung B *et al.,* [38] | This article revealed various transcription and growth factors, and inflammatory mechanism modulation by these spice-derived nutraceutical agents. |
| Dietary Nutraceuticals as Backbone for Bone Health. Pandey MK, *et al.,* 2018 [39] | This work discussed various inexpensive nutraceuticals (including GA) how they can modulate cell signaling pathways and reverse or slow down osteoporosis. |
| Chemical and Biological Research on Herbal Medicines Rich in Xanthones. Ruan J, *et al.,* 2017 [40] | This research presents various xanthones (including Garcinia) based plant resources for achieving useful bioactivity and the structure-activity relationships in the drug research and development. |
| Caged Garcinia Xanthones: Development Since 1937. Han Q-B, *et al.,* 2009 [41] | Caged xanthones of bioactive components derived from the garcinia family (~ 100 compounds) were characterized, tested for biological, mechanism of action, and anticancer drug development. |

**Table 3.**

Sensitization and synergistic actions of gambogic acid with various anti-cancer agents in various cancer types.

| Chemotherapy agent | Cancer type | Combination type | Mechanism of action | Reference |
|---|---|---|---|---|
| Docetaxel | Breast Cancer | Sensitization | Inhibiting P-gp, post translational proteasome | [98] |
| Docetaxel | Gastric cancer | - | Survivin inhibition | [99] |
| Docetaxel | Gastric cancer | Synergistic | β-tubulin III, tau and Survivin | [100] |
| Chloroquine | Pancreatic cancer | Synergistic | Accumulation of reactive oxygen species | [101] |
| Heparin- quercetin | Breast cancer | Synergistic | Inhibiting P-gp | [102] |
| Cisplatin | NSCLC | Synergistic | Downregulating MRP2 and LRP expression | [103] |
| 5-Fluorouracil | Colorectal cancer | Synergistic | P53, Survivin and thymidylate synthase (TS) | [74] |
| Retinoic acid chlorochalcone | Osteosarcoma | Synergistic | Increased apoptosis | [104] |
| Retinoic acid | Breast cancer | Synergistic | Apoptosis | [105] |
| Vorinostat | Neuroblastoma | Synergistic | JNK-IRE1-mTORC1 | [106] |
| Imatinib | Myeloid leukemia | Synergistic | Proteasome Inhibition | [107] |
| Irinotecan | Liver cancer | Synergistic | ERK/p38 MAPK activation | [108] |
| Gefitinib | NSCLC | Synergistic | ERK and p38 MAPK activation | [109] |
| Tandutinib | Meningiomas | - | Suppressed the PDGFRβ tyrosine phosphorylation | [110] |
| Doxorubicin | Breast cancer | Sensitization | Inhibiting P-gp & Survivin | [19] |
| Doxorubicin | Ovarian cancer | Sensitization | Accumulation of ROS | [94] |
| Gemcitabine | Pancreatic cancer | Sensitization | Downregulation RRM2 | [18] |
| Methyl jasmonate | Bladder cancer | Sensitization | Downregulation of EZH2 | [111] |
| Verapamil | Epithelial cancer | Synergistic | Proteasome inhibition | [112] |
| Celastrol | Oral carcinoma | Synergistic | NF-κB inhibition | [113] |
| Sunitinib | Renal cancer | Synergistic | NF-κB inhibition | [114] |