Research Article

Unravelling the Therapeutic Potential of Gambogic Acid: Deciphering Its Molecular Mechanism of Action and Emerging Role as an Anticancer Xanthone

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Abstract

Use of current anticancer therapies such as chemo-radiotherapy (CRT), biologics and immunotherapies are severely limited due to side-effects and systemic toxicity. Cancer metastasis, drug resistance, and recurrence of cancer are other important considerations to evolve targeted therapies. This highlights the urgent clinical need to explore natural compounds that are bioactive, biosafe and unquestionably efficient as immune-modulators for cancer prevention and anti-cancer therapy. Natural compounds are extremely successful in clinics as they have unique structures and complexity. Gambogic acid (GA), a plant derived caged-xanthone molecule extracted from *Garcinia hanburyi* tree as a dry resin that has emerged as a miracle molecule that exhibits multifarious biological activities against various cancers making it attractive for clinical applications. Since the molecular targets are still unclear, this review focuses on the therapeutic efficacy and its associated mechanistic interactions with its recognized targets involving anti-angiogenesis, anti-metastasis, synergistic effects and chemo-sensitization.

Introduction

Cancer still remains the deadliest form of disease, an estimated 10 million deaths that occurred due to cancer worldwide [1]. According to Globocan report, more than 2.26 million new cases were diagnosed with women breast cancer, lung cancer (2.21 million new cases, deaths 1.80 million), colon and rectum cancer (new cases 1.93 million, deaths 9.35 million) worldwide in 2020 [2]. The rising prevalence and incidence of cancer are associated with molecular alterations. Cancer encumbrance can be decreased considerably by detection at early-stage of cancer coupled with suitable regimens. Last five decades have witnessed an avalanche of literature that provides significant evidence proving phytochemicals as potent anti-cancer drugs. The phytochemicals can be derived from natural products that include leaves, bark, stem, roots, flowers of plants, micro-organisms as well as marine organisms. The phytochemicals may be organic

compounds like polyphenols, phytochemicals and xanthones that exhibit distinct anti-cancer properties [3]. Gambogic acid (GA) is the caged xanthone secreted as dry resin from the plants of genus Garcinia which is native to East Asia, India, America, Australia and southern Africa. Owing to abundance and ease of isolation *process of Garcinia hanburyi* in India and East Asia, it becomes an attractive molecule to exploit. The gamboge resin containing GA has been used as traditional folk medicine as phytomedicine for a variety of ailments in Ayurveda in countries like India and China over several decades [4].

GA has shown a wide variety of biological activities including anticancer, anti- cardiovascular disease (CVD) anti-inflammatory, anti-viral, anti-parasitic, anti-infectiousness, anti-oxidant, and a promising molecule to treat osteoarthritis [5]. The biocompatibility of GA was indicated by measuring heart rate, blood pressure and few parameters of the brain (CNS) indicating low toxicity on the cardiovascular and respiratory system [6].

GA is a solid, hydrophobic molecule that shows a maximum absorption wavelength of 365 nm as well as the unique xanthone skeleton. The activity relationship-based studies of GA indicate that the double bond of 9, 10 carbon of α, β-unsaturated ketone moiety plays a significant role in its biological activity. The ease of modification at 6-hydroxy and 3o-carboxy group enhances its efficacy. The electrophilic α, β-unsaturated carbonyl group in the bioactive compounds could specifically binds to the thiols. Structure and activity-based relationship of GA could provide a better understanding for developing GA as a potential candidate to exploit in clinics. Various structural alterations have been reported to increase bioavailability, enhance stability and improve the selective toxicity against targeted cancer cells [7].

Emerging studies reported that GA acts as potent anti-cancer agents against various form of cancer- breast, lungs, gastric, colon, head and neck cancer, brain tumor and prostate cancer [8]. Furthermore, China food and drug administration (CFDA) has approved GA for phase II clinical trials against lung cancer and other malignancies. Bone marrow suppression was not observed after the treatment with GA unlike other chemotherapeutic agents [9]. GA was reported to increase the intracellular concentration at lower doses of chemotherapeutic drug that helped develop it for combinatorial therapy to exploit its synergistic activity to selectively target cancer cells. Reports on the chemo-sensitization of GA indicated modulation of various pathways including PI3K/AKT, MAPK/ERK, and NF-kB [10]. Earlier reports suggest that GA exhibits its anti-inflammatory by suppressing NF-κB activity through modification of 179Cys of IKKβ moieties, resulting decreased expression of TNFα, COX-2, and iNOS. GA possibly exerts its inimitable anti-tumour activity via multiple cell death mechanisms such as apoptosis, autophagy and suppressing propagation and invasion of cancer cells. Despite its cited advantages, the clinical applications are severely limited due to the poor aqueous solubility (0.013mg/mL) requiring repeat injections. However, recent studies reported that the GA derivatives with higher aqueous solubility may show significant anti-tumor activity and could be crucial for clinical applications.

Herein, we summaries the recent advancement and mechanism of action of GA in facilitating the anti-cancer properties in various cancer cell lines types, animal models and human clinical trials through chemo sensitization and synergistic activity with other drugs to combat drug resistance in multiple cancers.

Herein, we review the challenges in combating cancer using innovative approaches based upon phytochemicals to permeate the hurdles associated with cancer incidence, metastasis and prognosis.

Cytotoxic Activity of Gambogic acid

In this study, bibliographic investigations were conducted on anti-cancer properties of GA in various malignancies from their discovery to till date by analysing journals and peer-reviewed papers indexed on PubMed link, Scopus and Google scholar. Only relevant studies are used to illustrate the remarkable role of GA in biomedical field in the form of Pie -chart in **figure 1.** However, the scientific studies on GA began in 1966 and the mechanistic studies are on-going. Therefore, it has been widely studied during recent years as there were only four scientific reports on GA from 1966 to 2004 but a sudden escalation was observed after 2004 and many papers exploring the potency of GA as an anticancer, anti-inflammatory agent have been reported. These studies mainly include breast, liver and lung cancer. (Figure 1). The standards used for the selection of data and information in this review deliberate *in vitro* and *in vivo* cytotoxicity of Xanthone derived Gambogic acid.
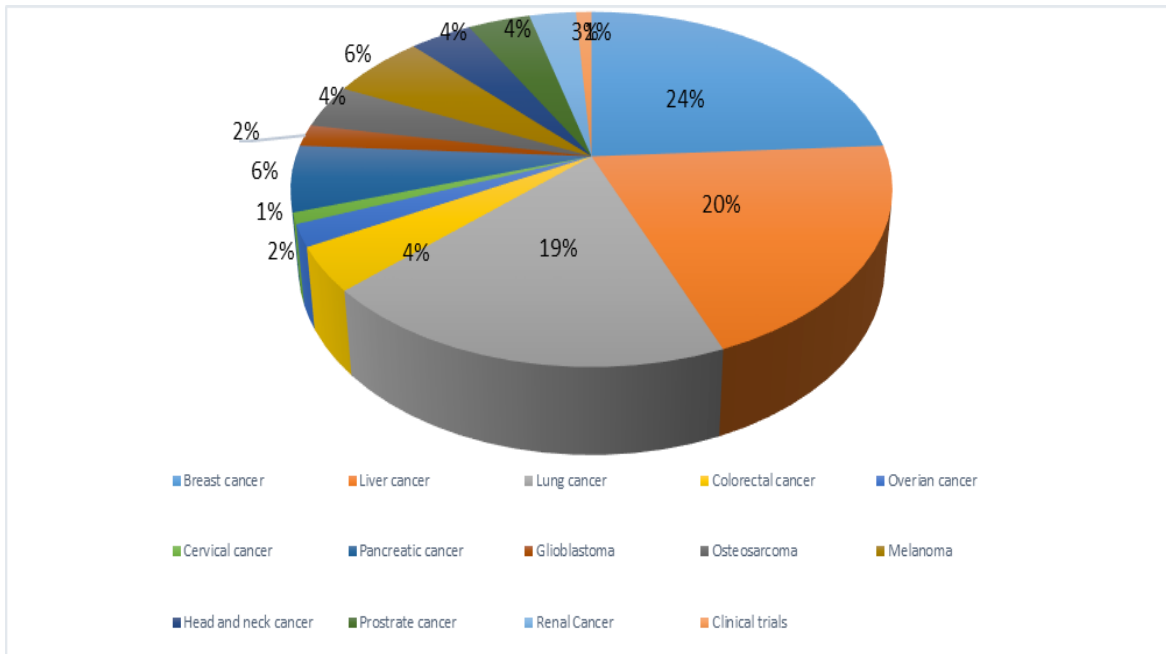
Figure 1: Schematic illustration of remarkable role of GA in medical research. Pie chart depicting the studies associated with GA in multiple cancer treatment (breast, liver, lung, colorectal, ovarian, cervical, pancreatic, glioblstoma, melanoma, head and neck, prostrate, renal and in clinical trials. Data generated inclusive of GA in studies on pubmed search link using key words: "Gambogic Acid and respective cancer" Only this criterion has been followed for this search.
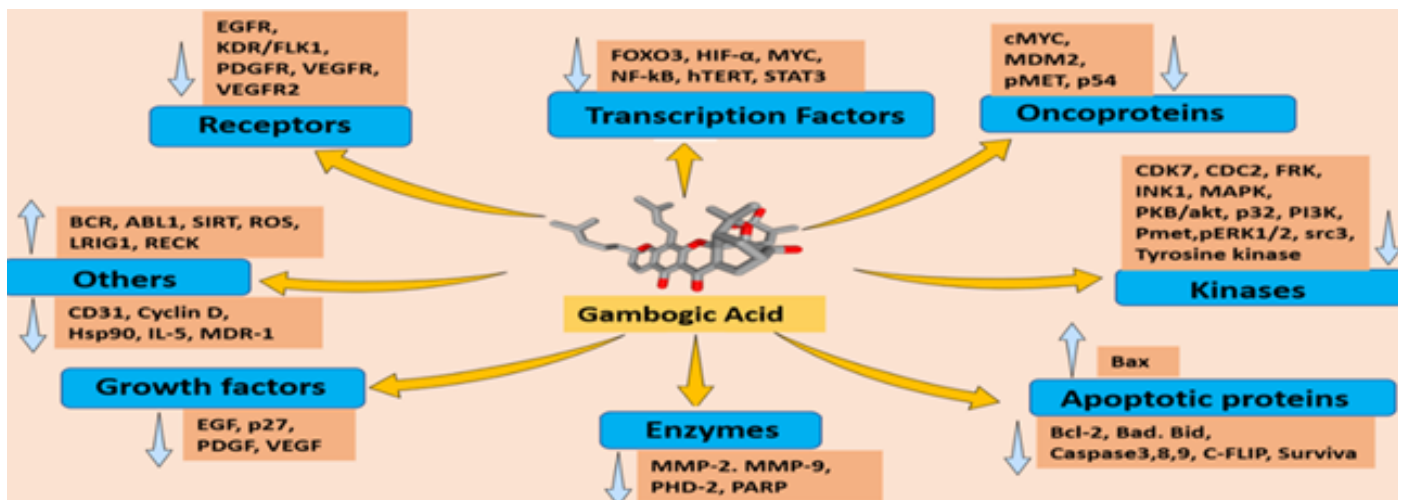

Figure 2: Overview depicting the modulation of signalling pathways by Gambogic Acid.

Leveraging the potential of GA

In cell cycle regulation

The phases of cell cycle are the key regulators of all cellular processes and cell growth that are also considered as the major anticancer mechanisms. Literature survey suggested the influence of GA on the different cell cycle phases in various types of cancers. GA reduces the level of phosphor-cdc2 (Thr 161) and cdc25B to promote G2/M arrest in MG63 osteosarcoma cells and also reduces the phosphor-GSgsK3-β (ser9) and the expression of cyclin D1 in U2OS cells [11]. Depolymerization of microtubules induced by treatment of GA was observed in MCF-7 cell line [12]. Decreased CDK7 kinase activity leading to inactivation of cdc2/p34 kinase in BGC-823 human gastric carcinoma cells were observed post treatment with GA [13]. Further, downregulation of SRC-3 expression leading to inhibition of AKT signaling pathway was observed that resulted in G0/G1 arrest in K562 myelogenous leukemic cells [14].

In elucidating cell death mechanisms

Apoptosis

Apoptosis or programmed cell death is a cascade of events through a number of intrinsic or extrinsic apoptotic proteins. p53 known as the "guardian of the genome" plays a crucial role in tumor suppression, but when mutated it leads to apoptosis, redox resetting, cell cycle arrest and senescence. Various studies suggested that GA upregulates p53 during translation by downregulating expression of the mdm2 gene in (non-small cell lung cancer H1299 cells [15]. Earlier reports suggested that GA inhibits Bcl-2 antiapoptotic proteins in cervical cancer [16]. Furthermore, GA could also promote p21waf1/cip1 levels to induce apoptosis in (MCF-7 cells) by inhibiting MDM2 either through p53 pathway or p53-independent pathway suggesting that MDM2 activation is not solely dependent on the mutated p53 stability in cancer [17]. GA reportedly has the ability to degrade the mutant p53 in MDA-MB-435 cells by interacting with Hsp70 and Hsp90 via the ubiquitin/proteasomal system. Hsp90 and Hsp70 modulate the mutated p53 to CHIP (chaperone-associated ubiquitin ligase carboxy terminus of Hsp70-interacting protein) resulting in its proteasomal mode of degradation [18] GA facilitates mutant p53 to interact with hsp70 and restricts Hsp90/mutantp53 complex formation resulting in apoptotic cell death [19]. GA induces cytotoxicity in melanoma cells by directly inhibiting the 20S ubiquitin-proteasome system [20]. GA has the potential to activate mitochondrial-dependent (intrinsic pathway) as well as mitochondria independent (extrinsic apoptotic pathway). Moreover, GA enhances production of reactive oxygen species (ROS) by collapsing the mitochondrial transmembrane potential (MMP), increasing downregulation of SIRT1 in multiple myeloma, and enhancing phosphorylation of c-Jun-N-terminal protein kinase (JNK) and p38 in hepatoma SMMC-7721 cells. Interaction of GA with thioredoxin reductase 1(TrxR1) possibly produces ROS accumulation in hepatocellular carcinoma [21]. Interaction between the transferring receptor and GA induces a specific signal for prompt apoptosis in cancer [22]. Suppression of IkBα and p65 phosphorylation by GA could possibly inhibit the NF-kB pathway to revoke NF-kB-dependent reporter gene expression [23-25]. GA down regulates mitogen-activated protein kinases (MAPK) signaling pathway, and c-fos induces cell apoptosis by deletion of phosphate and tension homolog (PTEN) and p53 gene in prostate cancer [26]. GA promotes apoptosis by regulating AKT/FOXO/BIM signaling pathway by increasing the expression of miR-21 in multiple myelomas [27]. In lung cancer (NSCLC), inhibition of bcl-2 and PI3K *via* the notch signaling pathway was induced by GA [28] Similarly, GA was found to target various types of cancer, including SRC-3 (steroid receptor coactivator-3) and hERG in leukemia [29], DDIT3, GADD45B, DUSP5, TOP2B, TOP2A, DUSP1, ALDOA and TOP3A in pancreatic cancer [30] and BRD4 in anaplastic thyroid cancer [31]. GA inhibits AKT/mTOR complex 1 (mTORC1) by upregulating (AMP-activated protein kinase) AMPK and LRIG1 (leucine-rich repeats and immunoglobulin-like domains 1) via enhancing epidermal growth factor receptor (EGFR) in glioma cells [32]. Interestingly, GA also activates T lymphocytes in H22 transplanted mice to cell apoptosis [33].
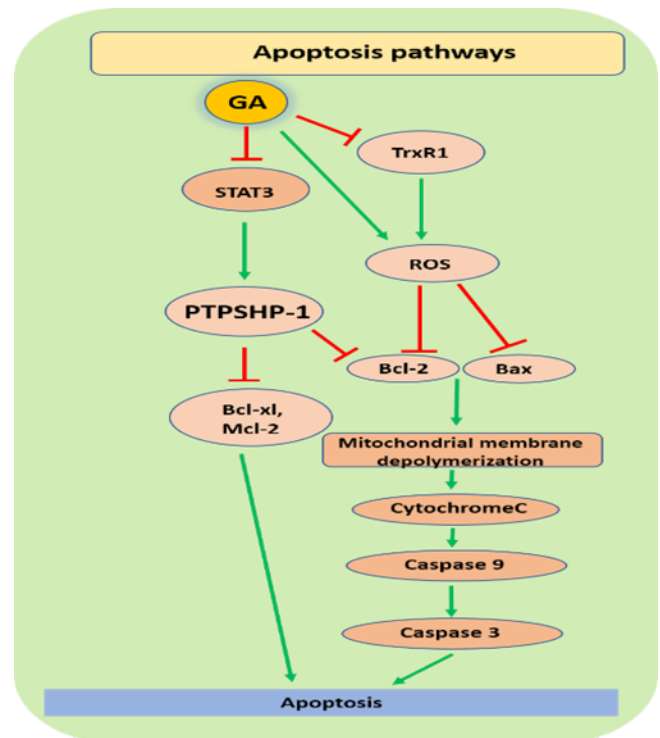

Figure3: Schematic representation of apoptosis signaling pathway induced by GA.

Table 1: Apoptotic cell death mechanisms.

Apoptosis			
Mitochondria Dependent (Intrinsic pathway)		**Mitochondria Independent (Extrinsic pathway)**	
• Reduces bcl-2 mediated release of cyt- c, directly targeting the mitochondria with rapid depolarization of mitochondrial membrane potential causing release of cyt- c and activation of caspase 3 and caspase 9, cleaved PARP, and increased ratio of Bax/Bcl-2.	[16]	• Enhances death receptor i.e Fas, FasL, FADD, and Apaf 1 facilitates DNA fragmentation, binds to transferrin receptors that might induce a special signal resulting in rapid Apoptosis.	[22] [34] [26]
• Induce ROS induced collapse of mitochondrial membrane potential which downregulated the SIRT1, enhanced the phosphorylation of c-Jun-n- terminal protein (JNK) and p38.	[21]	• Inhibits STAT3 phosphorylation through the activation of SHP-1. • Inhibit MAPK pathway PTEN -/-/ p53. • Enhance miR-21 through AKT/FOXO1/BIM. • Suppress notch-signaling induce Apoptosis. • Inhibit the growth of osteosarcoma.	[27] [28] [11]

Autophagy

Autophagy is a self-sustained mechanism of cells that is now being contemplated to treat diverse ailments like cancer, cardiovascular, and neurodegeneration. The majority of proteins including VPS34 (vesicular protein sorting 34), Beclin-1, LC3 (microtubule-associated protein chain 3) and Atg5 (autophagy-cognate genes), have been identified to regulate the autophagy process. Both, *in-vivo* as well as *in-vitro* studies apparently, suggested that the xanthone component of GA targets a variety of autophagy proteins by increasing the initiation factor for autophagosome formation (Beclin-1) to convert LC3 I to autophagosome marker (LC3 II) in non-small cell lung cancer NCI-441 cells [35]. GA also enhances autophagic vacuole formation and upregulation of Beclin 1, Atg5 and LC3-II in Glioblastoma (U251MG and U87MG) cells [36], GA was involved in the modulation of ROS induced autophagy and apoptotic protein expression including, Hsp90, Beclin-1, p62 and GRP-78 in bladder cancer cells (T24 and UMUC3) [37]. Similarly, GA could act as initiator of ROS dependent autophagy *via* resetting lipid metabolism and Akt-mTOR signaling in colorectal cells [38]. Foggetti et al. concluded that GA not only increases the expression levels of Beclin-1 and LC3 (autophagy-associated protein markers) but concomitantly promotes reduction of BCR-ABL, SQSTM1/ sequestosome-1 anti-apoptotic gene product [39]. GA was reported to increase the sensitivity of esophageal cancer cell lines towards radiotherapy to inhibit Akt/mTOR signal transduction by promoting increment in the expression of LC3 and caspase 3,8,9 resulting in autophagy induced apoptosis [40].
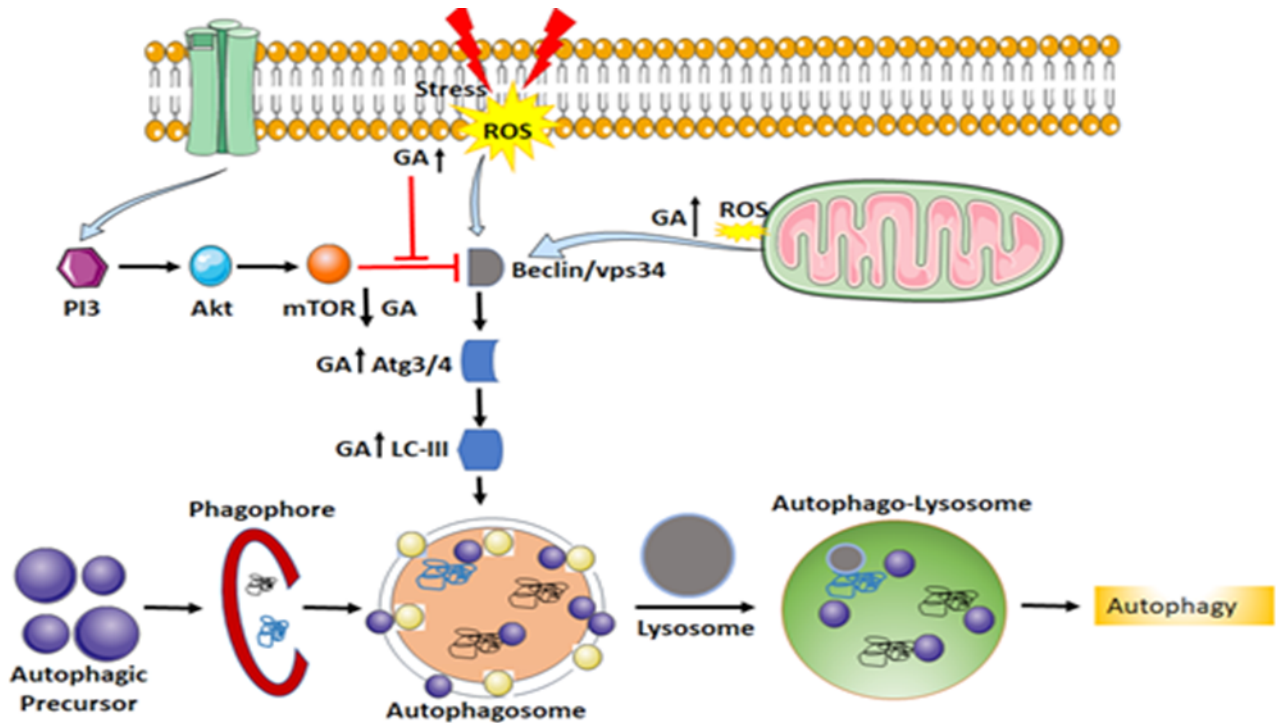
Figure 4: Schematic representation of Gambogic mediated autophagy.

Paraptosis

Paraptosis is the mechanism of vacuolization-associated cell death. GA induces paraptosis in cancer cells by forming mega-mitochondria, which is characterized by the fusion of swollen mitochondria with endoplasmic reticulum derived vacuoles. The inhibition of proteasomal system by GA contributes to the dilation of ER and induces ER stress and mitochondrial membrane depolarization leading to the formation of mega-mitochondria in treated cancer cells. It was found that thiol-containing antioxidants blocked paraptosis, independent of ROS generation. Michael adducts may be formed by the reaction of GA with cysteinyl thiols exposing the potential of GA to covalently modify proteins causing protein misfolding and deposition of misfolded proteins within ER and mitochondria [41].

Ferroptosis

Ferroptosis is a regulated form of necrosis dependent upon iron. An aberrant elevation of Fe-dependent lipid peroxidation and oxidation of PUFA (polyunsaturated fatty acid), release of free radicals cause disturbance in homeostasis pushes the cells towards ferroptosis [42]. Accumulation of ROS in the cell leads to apoptotic cell death mechanism but the uncertain underlying mechanisms alter the tumor microenvironment ultimately enhancing the sensitivity of cells towards ferroptosis [43]. GA induced ferroptosis in HCT116 colon cancer cells was observed [44]. GA directly inhibits the expression of HSP90, a pleiotropic regulator of multiple signalling pathways, and increases the GSH depletion leading to an increased level of LPOs and ultimately the cell undergoes ferroptosis.
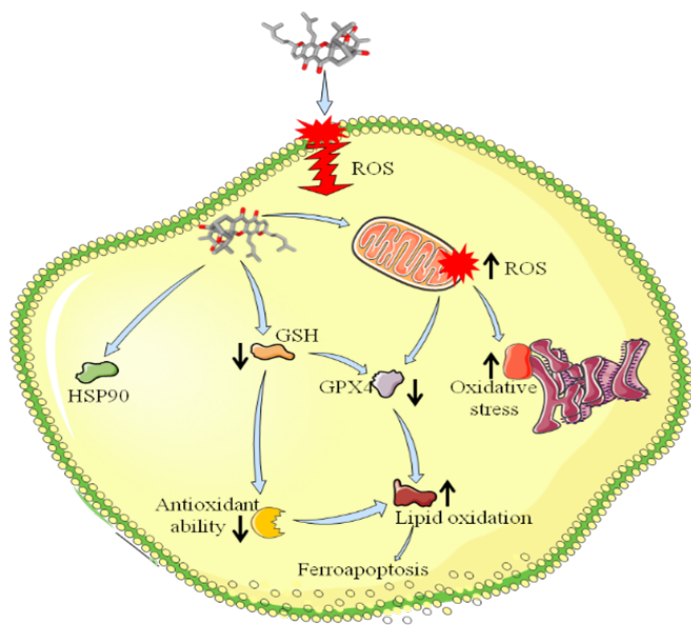
Figure 4: Schematic representation of Ferroptosis induced by the GA resulting in elevation of ROS generation and increased lipid peroxidation.

In regulation of Cell invasion and metastasis

Unregulated cell proliferation, tumour cell invasion and metastasis are the result of elemental aberration. In human prostate cancer cells (PC3 cells), GA suppressed cell proliferation, invasion and metastasis by regulating the action of TNF-λ by inactivating the PI3K/Akt and NF-kB signaling pathway. This resulted in the reduced expression of MMP-2 and MMP-9 [45]. Moreover, Zhou demonstrated that GA inhibited the proliferation, dispersion, invasion and migration of human colon cancer cells (SW620) in a dose dependent manner, through the PI3K/AKT/P21/MMP-2/9-dependent pathway as confirmed by the altered expression of levels of PI3K, AKT, phosphorylated-AKT, p21 and MMP-2 and MMP -9 [46]. Similarly, GA repressed the invasion and migration of transforming growth factor β1 (TG0F β1) induced EMT (epithelial -to mesenchymal transitions) in A549 cells (lung cancer cells) by impeding the NF-kB pathway. In addition, this study concluded that GA further inhibited the primary lesions and subsequent lung metastasis in *in vivo* orthotopic mice model [47]. Literature survey suggests the involvement of GA in regulating multiple signalling pathways that include PI3K/Akt, caspase-3 apoptosis and TNF-α/NF-κB to inhibit proliferation and migration in HT-29 cancer cells. Collectively, GA regimens also decreased the miR-21 expression and blocked PI3K/Akt signaling pathway by enhancing PTEN activity [48]. Simultaneously, GA inhibited the PTEN-PI3K-AKT-mTOR pathway in oesophageal squamous cell carcinoma [49]. In gastric cancer cells (AGS and HGC-27 cells), GA inhibited the proliferation, migration and invasion by downregulating the expression of ASAP2 and CDK7 [50].

For exploiting the Tumor microenvironment

Altered tumour microenvironment leads to increased blood circulation within the tumour undergoing intense proliferation. Angiogenesis is a critical characteristic of cancer cells that plays a vital role in proliferation and tumorigenesis. Tumor progression is majorly regulated by Hypoxia-inducible factor-1α (HIF-1α). *in-vivo* studies have suggested that GA inhibits HIF-1α/VEGF to inhibit angiogenesis and reduced the progression of multiple myeloma [51]. GA also acts as an inhibitor of (VHL) propyl hydroxylase-2 (PHD2)- *von* Hippel-Lindau gene by suppressing angiogenesis[52]. GA was reported to shrink the blood vessels and reduced the density of vessels in rat microvascular endothelial cells (rBMEC) as evident by CD31-associated immuno-histological studies[53]. GA further suppresses vascular endothelial growth factor (VEGF) and inhibits the formation of tubes in (HUVECs) and aortic ring generation in the rat[54].

Combating drug resistance

Drug resistance is an established phenomena occuring in a variety of diseases including cancer. Several cancers are initially susceptible to chemotherapy but over time resistance develops through a multitude of mechanisms including DNA mutation, metabolic alterations, redox-resetting in the tumour microenvironment that can lead to drug inactivation, drug target alteration, drug efflux, DNA damage repair, cell death inhibition, or impact the epithelial-mesenchymal transition (EMT). GA has been suggested to reverse resistance to oxaliplatin in LoVo colorectal cancer cells by accumulating intracellular platinum levels in hCTR1 and reduce the expression of ATP7A and ATP7B receptors that are responsible for efflux of cisplatin from the cells[55]. Moreover, GA inhibits (ERK)/E2F pathway accompanied to reduce mRNA expression and the (RRM2) ribonucleotide reductase subunit- M2 protein and to diminish the resistance to gemcitabine [56]. Hypoxia-inducible factor (HIF)-1 is crucial in promoting resistance to anticancer therapy, istead GA has been reported to reverse hypoxia-induced resistance to cisplatin-mediated apoptosis independent of HIF-1α in osteosarcoma cells [57]. In a recent study, GA remarkably facilitates the activation of p38 MAPK pathway and ROS-mediated sensitization of cells in doxorubicin (DOX)-resistant breast cancer cells by inhibiting the P-glycoprotein pathway *via* suppression of survivin expression [58]. In gastric cancer cell lines (BGC-823/Doc), GA was found to downregulate the expression of survivin resulting in the reversal of docetaxel resistance [59]. Combinatorial treatment of gefitinib and GA inhibited gefitinib resistance due to EGFR T790M mutant lung cancer [52].

Repurposing and optimizing GA as combinatorial therapy

GA has been exploited for combinatorial therapy as it exhibits chemo-sensitization in multiple cancers by modulating a variety of biochemical signaling pathways such as, PI3K/AKT, MAPK/ERK and NF-kB. Co-treatment of GA aids in reducing cytotoxicity as well as enhanced intracellular concentration of chemotherapeutic drugs. Synergistic effects of GA were observed with many chemotherapeutic drugs including cisplatin, gemcitabine, docetaxel doxorubicin, irinotecan, paclitaxel, and cabazitaxel and with biologics mainly sunitinib, gefitinib, and imatinib. As reported, treatment with oxaliplatin at 1, 2, and 4μM concentration exhibited platinum levels of ~0.25, 0.45, and 0.48 ng/10^7 cells, while co-treatment with GA reduced the oxaliplatin concentration almost 4-folds and the platinum levels increased to 0.45, 0.8, and 1.6ng/10^7 cells in a time-dependent manner [60-66]. When GA was used in combination with cisplatin, it showed higher anticancer activity by downregulation of LRP and MRP2 proteins, thereby promoting cell cycle arrest and increased apoptosis in cisplatin-resistant (A549/DDP) lung cancer cells [67]. GA when combined with cisplatin and rapamycin induced autophagy which ultimately suppressed the Akt/mTOR signaling probably by synergistic effect. Further, chloroquine co-administration leads to decreased expression of p62 in colon cancer cells and pancreatic cancer cells, but upregulate the expression of Beclin-1 and LC3-II proteins [61]. Interestingly, chloroquine plus GA synergistically reduce the tumor burden. Moreover, *in-vivo* studies indicated delivery of GA at a dose of 1mg/kg plus Doxorubicin (DOX) (10 mg/kg) significantly reduced the tumor progression significantly (~225 mm^3) when compared to DOX or GA alone in a SKOV-3 xenograft mice model. Surprisingly, there were negligible adverse effects observed in tumor bearuing mice [68]. GA along with PTX exhibited upregulation of the sonic hedgehog signaling pathway by inhibiting the expression of SHH, GLI1, and PTCH1 than PTX alone [66]. Synergistic effect of GA and Geftinib in HCI-H1975 NSCLC xenograft mice model revealed a remarkable inhibition of ~70% tumor growth by suppressing p-MEK1, p-ERK1/2, and p-Akt /2 than treatment of GA and Gefitinib alone [52]. Similarly, GA with Gemicitabine (GEM) showed reduction in tumor burden by 72.9% to 49.8% and 30.2% upon treatment with GEM and GA *per se*. This study concluded that GA inhibited RRM2 by suppressing the E2F1/ERK/MAPK signaling in cancer cells of pancreas BxPC-3 and PANC-1 [56]. GA further triggers RRM2 to upregulate GEM-induced apoptosis in lung cancer [10]. Apart from chemo-sensitization, GA was found to be effective in radio-sensitization treatments as well. GA efficiently inhibits (A549/DDP) cell proliferation in cisplatin-resistant non-small cell lung cancer cells in combination with ^{131}NaI radio-sensitizer [67]. GA along with X-ray irradiation (2-8 Gy) promotes apoptosis in nasopharyngeal cancer cells by G2/M-phase arrest and cyclinB1/ HIF-1α /cdc2 pathway [69]. Further, the esophageal cancer cell line- TE13 was sensitized by radiowaves and GA it indicated ROS mediated autophagy and programmed cell death by apoptosis *via* inhibition of Akt/mTOR pathways [40].
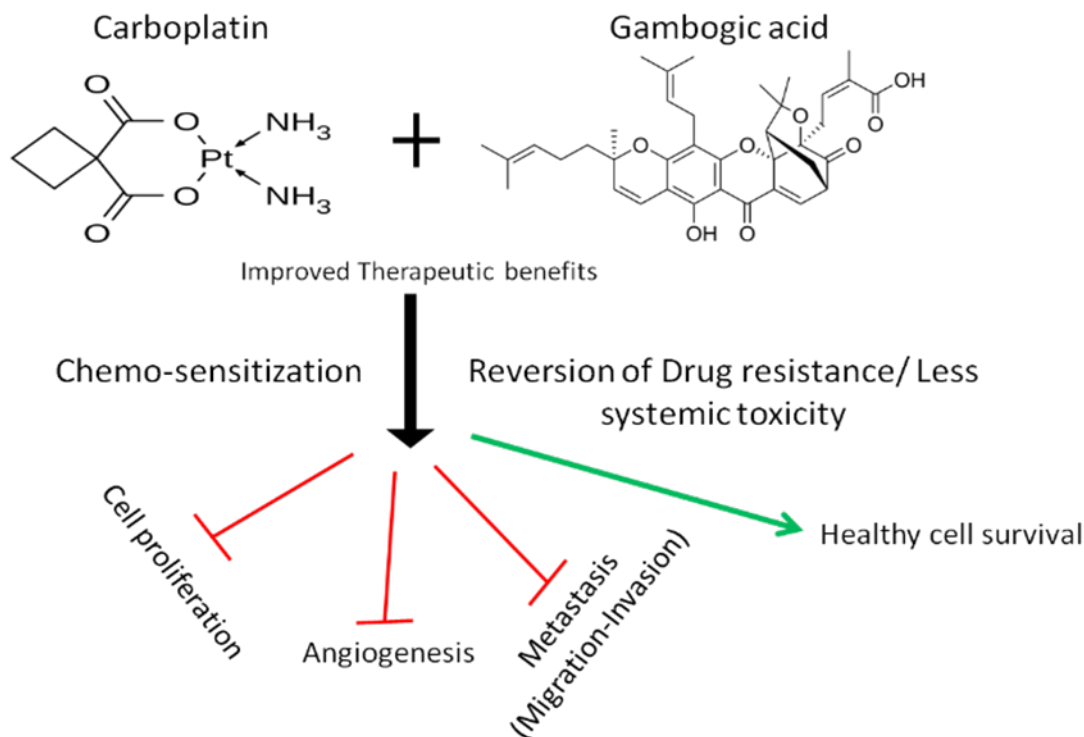
Figure 5: Diagrammatical illustration of review concept showing GA in combination with chemotherapeutic drugs (carboplatin and other) resulting in improved therapeutic benefits in cancer treatment.

Probable neo-targets

Effects of GA on microRNA

MicroRNA is a single stranded non-coding RNA molecules participates in post-transcriptional regulation of gene expression. To date, over 2500 miRNAs have been recognized as cancer biomarkers specifically related to onset and progression of tumors. Reports suggested that in cervical cancer, GA suppressed epithelial-mesenchymal transition, migration and drug resistance by communicating with miRNA. The Neo-GA contributed in inhibiting the expression of miRNA 106b and miRNA93 in Hela cells, enhanced the sensitivity against cisplatin and downregulated the expression of N-cadherin, Snail and Vimentin [70]. Li et al reported that GA treatment enhanced the expression of BCRC4 and miR-101, that leads to the suppresssion of EZH2 expression level in T24T and UMUC3 cells (Bladder cancer) in a dose dependent manner [71]. Similarly, repressive effect of GA on gastric cancer was faciliated by the elevation of miR-26a-5p and downregulation of Wnt5a [72]. Collectively, GA inhibits cancer metastasis and invasion by corrdinating and regulating the expression of miRNAs. Hence, emerging evidences support that GA has the potential to suppress cell proliferation and progression by attaching to the miRNA sites. However, the molecular mechanisms showing their inhibitory effects still remain unexplored.

Discussions

Cancer still remains the most lethal form of disease that persistently affects the health of humans. This puts a tremendous urgency to develop therapies for cancer prevention and high through-put preparedness for advanced management. The paradox of conventional therapy that includes surgery, chemotherapy and radiotherapy are well known. Their success have been severely hampered owing to adverse side effects, drug resistance and subsequent recurrence leads to treatment failure. Drug resistance is primarily influenced by alteration in drug efflux transporters, impaired metabolism, inactivation and modification in targets and sequestering of drug *via* different channels. To overcome these limitations, several clinical investigation and laboratory research have been directed to scrutinize the efficacy of natural phytochemicals in cancer management. The broad-spectrum chemotherapeutic efficacy is the reason for the wide range of mechanisms of action by GA that is indicative of its remarkable potency. The activity- structure relationships unravelled the ease of modification of GA molecule without compromising its

pharmacological and biological activities. The potent anti-cancer property has been substantiated by the multiple death mechanisms induced by GA such as apoptosis, ferroptosis, and autophagy. This potency may further be due to enhanced reactive oxygen species (ROS), excess anti-proliferation, down regulation of key enzymes (e.g., telomerase), reduced secretion of growth factors such as vascular endothelial growth factor (VEGF) and intervention of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathways. GA was identified after a high-throughput screening of natural molecules/compound libraries having a potential for inhibition of heat shock protein 90 (Hsp90) [73]. Hsp90 is reported to have a binding site for GA that is a different site than Adenosine triphosphate (ATP) binding pocket. Additionally, Yang lj et al. have suggested that normal cells exhibit low GA sensitivity when compared to cancer cells, presumably due to change in the redox equilibrium [74]. Moreover, literature survey suggests that GA has been used for sensitization prior to chemotherapy/radiation in different types of cancers [56,58,66,68] to improve the therapeutic index. GA may be exploited as modulators of drug resistance, as they can downregulate or inhibit drug efflux in cancer cells through the P-glycoprotein (P-gp) pump. Inhibition of Pgp pump possibly enhances the concentrations of drugs inside the cells [75]. Wang et al. reported a phase I clinical trial on humans maximal tolerated dose (MTD) of a single injection was 55mg/m^2 and dose limiting toxicities (DLTs) were mainly concerned on liver dysfunction and pain [74]. Recently, GA has been approved in phase II trial in human metastatic breast cancer up to the promising dose of 45mg/m^2 [9].
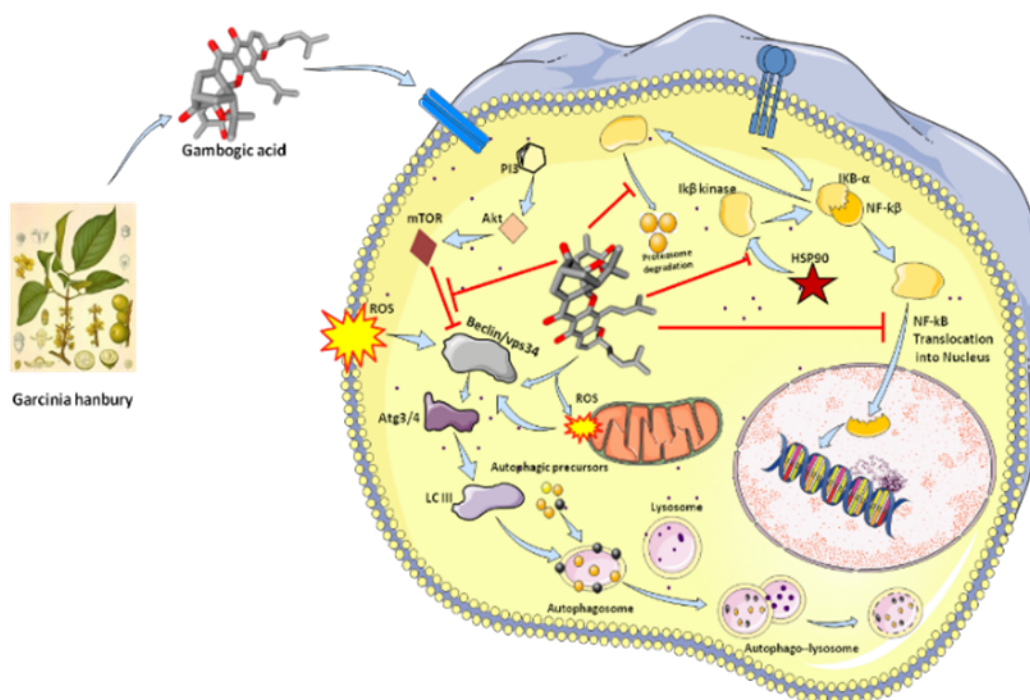

Figure 6: Pictorial representation of various signaling pathways including Autophagy and Apoptosis associated with GA.

Conclusion

Past few decades have witnessed a resurgence in use of phytochemicals to combat various cancers. GA is a promising novel anti-cancer phytochemical with innumerable targets to treat several cancers efficiently. The biological activity of GA includes its anti-inflammatory, anti-oxidant and anti-microbial efficacy. The complex structure of GA may restrict its absorption in the intestinal tract to enter the blood circulation. Therefore, prodrug strategies and special drug delivery systems may provide unique methods for the clinical application of GA and its derivatives. In addition, the modulation of oncotargets by GA to attain synergistic effects along with chemotherapeutic agents and radiation therapies would be an attractive strategy.

GA contributes in inhibition of cancer cell proliferation, invasion, metastasis, angiogenesis and chemoresistance as well as induction of apoptosis, autophagy, ferroptosis, paraptosis, cell cycle arrest has been demonstrated in *in vitro* and *in vivo* study. This review provides a systematic elucidation of the current understanding of GA in terms of its chemical and biological activities that include anti-cancer activities, underlying molecular mechanisms and impact of GA in overcoming drug resistance. Therefore, GA has the ability to decrease the number of cancer-associated mortality and increased patient compliance with prolonged life span. Undoubtedly, GA is considered to be a miracle compound owing to its unique pharmacological activities.

Future Perspectives

The futuristic studies should be focused on comprehensive view on mechanistic approach into numerous unexplored bioactivities, its entire pharmacokinetic and pharmacodynamics assessment to determine ADME patterns. The deep knowledge should be considered on biochemical targets and action of mechanisms of GA using QSAR techniques. It is critical to evaluate the biochemical and molecular targets to exploit the potential of GA. Detailed investigations are absolutely necessary to understand the concerns related to the isolation, biosynthesis of GA and other simplified xanthone moieties from plant cells, illustrating their physiological and endogenic role in plants and endogenous role in plants and the variation in content with environmental factors. A comprehensive analysis of GA structure and its simplified xanthone moieties requires aggressive research to elucidate the novel therapeutic derivatives to achieve therapeutic efficacy. The 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. Unravelling the potential therapeutic efficacy of GA necessitates in-depth pharmaceutical research to garner deep knowledge of its biodistribution and toxicological effects for both chemo-sensitization and synergistic actions. Experimental data as well as prognostic computational studies, done so far augment an opportunity that GA could be employed into a multi-functional drug.

Acknowledgement

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