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Review Article

Anti-Tumor Mechanisms of *Calotropis procera* in Cancer: A Comprehensive Review

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Abstract

Understanding processes that underlie cancer initiation, progression, and maintenance requires perceiving its distinct features. These characteristics serve as a roadmap for creating innovative treatment approaches that target the core characteristics of cancer, leading to more specialized treatments that may have longer-lasting effects but fewer side effects. Medicinal plants are becoming increasingly prevalent worldwide because of their widely acknowledged safety, affordability, and therapeutic potential. They include naturally occurring chemicals that are highly effective in herbal medicine for some cancer-related processes, such as prevention, mutagenesis suppression, antioxidant defense, and cell proliferation inhibition. The *Calotropis procera* is one recommended target for cancer treatment. Numerous cellular interactions in both healthy growth and illness were regulated by *C. procera*. In addition, recent research has elucidated the distinct anticancer activities of *C. procera* and their relationships with other cellular pathways. These benefits have increased expectations for the development of an effective treatment plan for all types of cancer, particularly the breast, prostate, colorectal, and glioblastoma multiforme cancers.

Keywords: *Calotropis procera*, Breast Cancer, Prostate Cancer, Colorectal Cancer, Glioblastoma

Introduction

Herbal medicine is a key element of globally traditional medical systems in which materials derived from plants are used to prevent and treat illnesses. Herbal treatments have long been the primary source of healthcare for many populations, from Ayurveda and Traditional Chinese Medicine to numerous tribal customs. The therapeutic potential of several phytochemicals has been brought to light by an increasing amount of scientific research in recent years, which has rekindled interest in incorporating herbal treatments into contemporary medical systems.[1,2] The increased prevalence of chronic illnesses, the drawbacks of synthetic medications, and the growing demand for natural and holistic remedies contribute to this comeback. The bioactive components of therapeutic plants are still being investigated in modern pharmacological research in order to clarify their modes of action, effectiveness, and safety profiles.[3] Despite encouraging results, issues including compositional heterogeneity, a lack of standardization, and possible herb-drug interactions call for strict regulatory monitoring and thorough clinical validation. Therefore, in addition to being a priceless cultural heritage, herbal therapy holds promise for future medication development and integrative healthcare approaches.

In arid and semiarid areas, *Calotropis procera*, also known as *Al-Ashkhar*, is a xerophytic perennial flowering shrub belonging to the Apocynaceae family. Numerous Ayurvedic systems of medicinal preparations use various portions of the leaves, roots, flowers, and latex of this plant.[4] Migraines can be effectively treated with *C. procera* leaves. In addition, *C. procera* leaves and stem bark preparations are frequently utilized to treat bronchial and dermatological illnesses in West African and Asian traditional medicine. Furthermore, the aqueous stem bark extract has the ability to suppress coughing in guinea

pigs that have bronchial irritation from the organic solvent ammonia.[5] Moreover, traditional folk medicine occasionally uses *C. procera* to treat colds, fevers, asthma, eczema, leprosy, dyspepsia, and dysentery.[6] According to reports, the extract of *C. procera* contains antibacterial, antifungal, anthelmintic, anticancer, anti-inflammatory, antidiabetic, gastroprotective, antioxidant, and analgesic propoerties.[7,8] Additionally, *C. procera*, in particular, has demonstrated promising results in testing its anti-cancer activities in glioblastoma multiforme, lung cancer, breast cancer (BC), prostate cancer (PCa), and colon cancer. In this regard, *C. procera* has been shown to significantly enhance apoptosis, reduce invasion and migration, and impede cell survival and proliferation in cancer cells. The anticancer effects of *C. procera* are due to the reduction of numerous signaling molecules, including nuclear factor kappa B (NF-κB), P27, cyclin-dependent kinase (CDK), reactive oxygen species (ROS), tumor necrosis factor-alpha (TNF-α), Wnt/β-catenin, protein kinase B (Akt), mechanistic target of the rapamycin (mTOR), and mitogen-activated kinase (MAPK). Research on *C. procera*'s anticancer effects in various tissues, both in vitro and in vivo, is covered in the current review [9,10] (Table 1, Figure 1).

Breast Cancer

BC ranks as the most prevalent cancer among women globally and is the second leading cause of cancer-related fatalities in this demographic.[22] Surgical intervention, chemotherapy, radiation therapy, and immunotherapy are some of the treatment interventions for primary BC; however, the presence of metastases in other organs significantly diminishes survival rates.[23,24] Despite ongoing research efforts over the years, the global incidence and mortality rates associated with BC remain alarmingly high.[25] Recently, monoclonal antibody therapies targeting specific oncoproteins overexpressed in triple-negative BC (TNBC) have demonstrated promising patient outcomes. Additionally, immunotherapy, immune-based combination treatments, and immune checkpoint inhibitors are becoming increasingly vital in the management of TNBC.[26-29] Nevertheless, these therapeutic agents may lead to adverse effects, including hearing impairments, fatigue, nausea, diarrhea, and liver damage.[30,31] Consequently, there is an urgent need for a deeper understanding of BC and the formulation of innovative strategies aimed at enhancing patient survival rates.

Calotropis procera Has Shown Inhibitory Effects on Cathepsin L

In this regard, cathepsins, which belong to the papain superfamily of lysosomal proteases, facilitate cell migration and invasion by breaking down the components of the extracellular matrix. CTSL, classified as a C1A-type protease, is found to be overexpressed in various types of cancers, including breast, glioblastoma, colon, and lung cancers. The elevated levels of CTSL are significantly associated with poor prognoses in affected patients.[32,33] Moreover, cancer cells exhibiting high levels of CTSL demonstrated greater proliferation, enhanced colony formation, and increased metastatic potential.[34,35] Consequently, targeting CTSL activity may be a viable approach to mitigating tumor aggressiveness. Endogenous protein inhibitors, such as cystatins (I25A), stefins (I25B), and kininogens (I25C), which contain an I25 domain, are known to reversibly inhibit CTSL activity and compete for its substrates.[36,37] Then, a disruption in the balance between CTSL and these endogenous inhibitors is a notable form of dysregulation observed in BC.[32] In addition, earlier investigations have identified the I29 domain of the papain-like cysteine protease, SnuCalCp15, derived from *C. procera*, referred to as SnuCalCpI15.[38,39] This protease has shown inhibitory effects on papain and CTSL, with its kinetic properties indicating a promising role as an anti-cancer agent. Nevertheless, the absence of data regarding the cytotoxic effects, appropriate dosages, and the role of SnuCalCpI15 in tumor suppression has limited investigations into its anti-cancer efficacy and therapeutic applications. Kwon et al assessed the inhibitory properties of SnuCalCpI15 against four C1A-type cysteine proteases and calculated its inhibitory constant for CTSL in order to evaluate its effectiveness as a selective inhibitor and potential anti-cancer compound. Consequently, they conducted molecular docking simulations, followed by cytotoxicity assays and tumor migration and invasion evaluations in MDA-MB-231 BC cells. Overall, their findings indicated that SnuCalCpI15 may serve as a novel agent in preventing BC metastasis [11] (Table 1).

Calotropis procera Exerts Anti-Angiogenic Effects

The cellular microenvironment, including the immune system, endothelial cells, and macrophages, synergistically interacts to promote cancer progression. Natural products have been identified as possessing a range of biological activities, notably their potential to combat cancer. Among various plant species, *C. procera* has demonstrated significant anticancer and anti-angiogenic effects, warranting further investigation to create new cancer therapies.[40] In addition, extracts from the roots of *C. procera* exhibited anti-angiogenic properties and inhibited angiogenesis induced by vascular endothelial growth factor (VEGF). According to some studies, these extracts may hinder the formation of new blood vessels by disrupting the proliferation of endothelial cells.[41,42] The cytotoxic effects of crude extracts from *C. procera* and various isolated phytoconstituents have been examined across multiple cancer cell lines.[43] Further, apoptosis, cell cycle arrest, and inhibition of angiogenesis have been linked to its anticancer properties [12,44,45] (Table 1). Due to its anti-angiogenic and anticancer properties, *C. procera* is a promising candidate for developing novel anticancer therapies. Moreover, research has demonstrated that *C. procera* exhibited anticancer properties against a variety

Table 1. *Calotropis procera* Affects Breast, Prostate, Glioblastoma, Colorectal, Lung, and Hepatocellular Carcinoma Cancers

Cancer Type	Cell Line	Mechanisms and Results	Reference
Breast	MCF-7 and MDA-MB-231	1) ↓ Proliferation 2) ↑ Apoptosis 3) ↓ NF-κB 4) ↑ Antioxidant activity 5) ↓ Inflammatory cells	10
Breast	MDA-MB-231	6) ↓ CTSL 7) ↓ Migration 8) ↓ Invasion 9) Targeting EMT	11
Glioblastoma	U87 MG and U251 MG	10) Epigenetic regulation 11) Angiogenesis modulation 12) ↑ Apoptosis and cell cycle arrest	12
Breast	MDA-MB-231 and MCF7	1) ↓ Angiogenesis, 2) Targeting HDAC 3) Modulating the activity of the p53-binding protein Mdm-2	13
Breast	4T1 and MCF-10A	1) ↑ Apoptosis, 2) ↓ Akt/mTOR 3) ↓ MAPK/ERK1/2 4) ↓ Angiogenesis 5) Modulation of tumor suppressor proteins (PTEN and TSC2)	14
Breast	MCF-7	1) ↑ Antioxidant activity 2) ↓ Cell proliferation	15
Breast	MCF-7	1) ↑ Apoptosis 2) ↑ Cell cycle arrest 3) Protein expression alterations 4) ↓ AKT/mTOR pathway	16
Prostate	PC-3, 22Rv1, and RWPE-1	1) ↓ Cell viability 2) ↓ ROS levels 3) Regulation of autophagy 4) Alteration of NF-κB and p27 levels	17
Lung cancer	H1299 lung cancer cells	1) Scavenge OH, H_2O_2, and NO radicals	18
Colorectal	Not mentioned	2) ↓ Carcinogenesis markers 3) ↑ Apoptosis 4) ↓ ACF	19
Colorectal	Not mentioned	1) ↓ Inflammation 2) ↑ Antioxidant activity 3) ↓ Carcinogenesis	20
Hepatocellular carcinoma	Hepatoma (Huh7) and non-hepatoma (COS-1) cell lines	1) ↑ Apoptosis	21

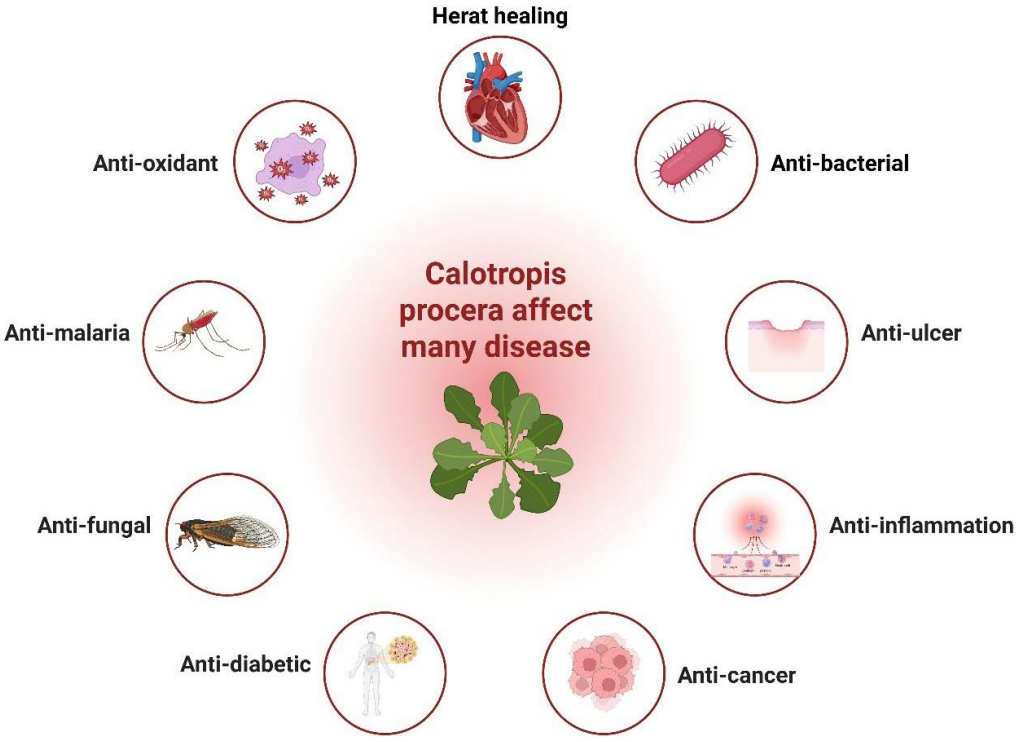

Figure 1. *Calotropis procera* Affects Many Diseases

of carcinomas, assessed in vitro and in vivo. The findings indicated that leaf extracts, fruit, stem, and seeds have shown activity against MDA-MB-231 (BC) cell lines. Additionally, *C. procera* extracts are effective and display low toxicity, positioning this plant as a potentially cost-effective option for cancer treatment[13] (Table 1).

Calotropis procera Regulates Phosphatidylinositol 3-Kinase/Protein Kinase B/Mechanistic Target of the Rapamycin Signaling Pathway

Apoptosis is recognized as a highly effective mechanism against cancer and is induced by numerous chemopreventive agents, including different plant extracts.[46] In this regard, caspase-3 is identified as the most significant component among the executioner caspases.[47] The application of phenolic extract from *C. procera* resulted in the upregulation of cleaved caspase-3 and a decrease in total PARP protein levels (Figure 2), suggesting that PARP cleavage by caspase-3 is occurring, thus promoting programmed cell death[14] (Table 1). In addition, the MAPK signaling pathway plays a crucial role in regulating protein functions through phosphorylation and is essential for the initiation, progression, and metastasis of a wide variety of tumors. Notably, phenolic extract from *C. procera* and quercetin influence ERK1/2 phosphorylation in opposing manners. Furthermore, this phenolic extract decreases phospho-ERK1/2 levels (Figure 2), a well-established mechanism that inhibits cell proliferation and metastasis by preventing interactions with the extracellular matrix and diminishing crosstalk with the PI3K/Akt pathway.[48,49] Overall, the results revealed that the phenolic extract of *C. procera* reduced the viability of 4T1 BC cells, partly by lowering oxidative stress (OS) and downregulating the Akt/mTOR (Figure 2) and MAPK/ERK1/2 signaling pathways associated with cell growth.[14]

Further, abnormalities in the PI3K/Akt/mTOR signaling pathway rank among the most prevalent genomic alterations observed in BC, significantly influencing tumor apoptosis, proliferation, and resistance to therapies.[50,51] The phosphorylation of Akt, which is essential for its complete activation, was inhibited by the phenolic extract of *C. procera*.[51] Additionally, this phenolic extract was found to suppress mTOR phosphorylation, a process that facilitates the growth and dissemination of TNBC cells.[52] Likewise, the insulin receptor (IR) is a cell membrane protein that, when activated by insulin, plays a role in the pro-oncogenic Akt/mTOR signaling pathway. The signaling through IR/IGF-IR is frequently disrupted in BC; thus, agents that improve insulin resistance linked to cancer therapies may offer promising avenues for cancer prevention and treatment.[53] Following phenolic extract from *C. procera* treatment, a decrease in IR levels was observed, which contributed to the downregulation of Akt. Furthermore, TSC genes are considered potential tumor suppressors; however, germline mutations in TSC genes have been found to be aberrantly expressed in human BC cell lines, and their expression in tumor tissues has been correlated with unfavorable clinical outcomes in BC patients.[54] In this respect, phenolic extract from *C. procera* treatment led to a reduction in total protein levels of TSC2. These findings highlight further investigation into the specific role of phenolic extract from *C. procera* in regulating TSC gene products and mechanisms in 4T1 BC cells. Additionally, scientists examined the anti-cancer effects and the underlying anti-metastatic mechanisms of the phenolic extract from *C. procera* in vitro, explicitly using the 4T1 BC cell line. Their results demonstrated that the phenolic extract of *C. procera* treatment decreased ROS levels (Figure 2), contributing to a decline in cell viability.[14] The growth of BC cells is maintained by low and consistent levels of ROS, particularly hydrogen peroxide; therefore, a decline in ROS levels results in a slower growth rate of these cells.[55]

Calotropis procera Modulates the Nuclear Factor Kappa B Activation Pathway

The *C. procera* protein (CP-P) has been shown to possess

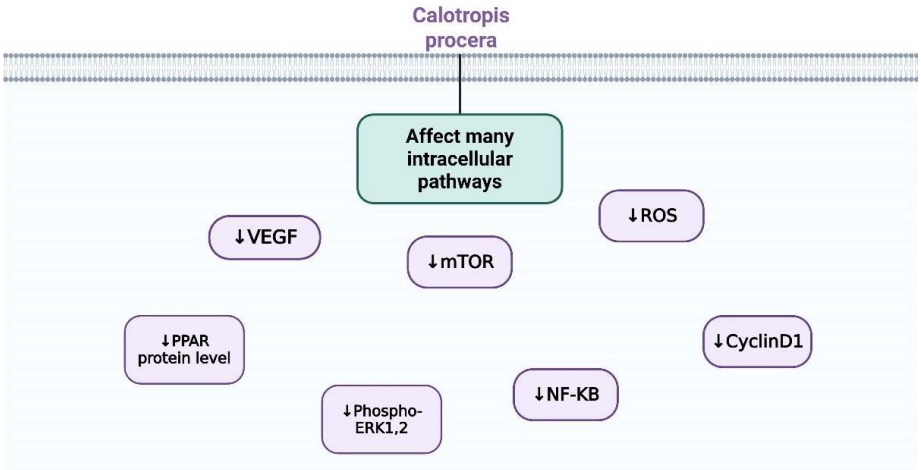

Figure 2. *Calotropis procera* Affects Various Intracellular Pathways
Note. ROS: Reactive oxygen species; VEGF: Vascular endothelial growth factor; NF-κB: Nuclear factor kappa B; mTOR: Mechanistic target of the rapamycin

antipyretic, antioxidant, and anti-inflammatory effects. According to reports, CP-P may mediate these effects by modulating the NF-κB activation pathway, which is significantly associated with inflammation, tumorigenesis, cell proliferation, invasion, angiogenesis, and metastasis. In this regard, the NF-κB pathway is activated by various inflammatory agents, carcinogens, tumor promoters, and growth factors. Samy et al sought to determine whether the pro-apoptotic and anti-proliferative effects of CP-P in vivo on BC induced by 7, 12-dimethylbenz (a)anthracene (DMBA) in rat models are facilitated through the suppression of NF-κB signaling.[10] Initially, they observed the notable in vitro cytotoxic effect of CP-P, both in the presence and absence of CYC, on BC cell lines MDA-MB-231 and MCF-7. Furthermore, CP-P was found to significantly induce apoptosis in MCF-7 cells, regardless of CYC's presence, which may contribute to its potential anti-cancer properties. Similarly, they noted that CP-P inhibited NF-κB activation (Figure 2) triggered by TNF-α, thereby reducing IkBa phosphorylation and degradation. This suppression of TNF-α-induced IKK activation also led to decreased IkBa phosphorylation and degradation in MCF-7 cells. Moreover, CP-P down-regulated the expression of several NF-κB-dependent genes associated with cell proliferation, such as cyclin D1 (Figure 2), and anti-apoptotic factors, including Bcl-2.[10] Numerous studies have indicated that NF-κB is crucial in the onset and advancement of BC. For instance, research has identified constitutively active NF-κB during the early stages of mammary cell neoplastic transformation.[56] Further, NF-κB activation has been confirmed to inhibit apoptosis in mouse mammary epithelial cells.[57] In addition, selective activation of NF-κB subunits has been documented in human BC cell lines and patient samples.[58] Furthermore, inhibiting NF-κB in BC cells can trigger spontaneous apoptosis.[59] Notably, BC tissues from women who do not respond to chemotherapy exhibited constitutively active NF-κB.[60] Therefore, CP-P's inhibition of NF-κB activation may explain its pro-apoptotic, anti-proliferative, and chemosensitizing effects. The possible inhibitory effects of CP-P on the progression of DMBA-induced BC in rats were noted as well. Animals treated with 0.2 mg/kg of CP-P alone displayed a tumor inhibition rate of only 60.5%. In contrast, rats receiving a 0.2 mg/kg dose of CYC alone demonstrated a tumor reduction of at least 70%. Remarkably, the combination treatment of CP-P resulted in a 74.5% decrease in tumor size, effectively inhibiting DMBA-induced tumors in the rat model.[10] Further, histopathological analysis revealed that the normal control rats exhibited typical neoplasm characteristics. At the same time, the CYC-treated group displayed low-grade differentiation, which is characterized by the presence of giant multinucleated cells, tissue infiltration, and necrotic effects attributed to CYC. Additionally, this group showed decreased cell density, increased tissue infiltration, elevated fibrosis levels, reduced inflammation, accelerated neovascularization, and preserved tissue architecture in CP-P-treated rats. Conversely, the group receiving both CP-P and CYC demonstrated glandular structures, indicating functional differentiation due to enhanced proliferative activity in the vasculature and epithelium because of the anti-tumor effects. These findings implied that the combination treatment significantly hinders tumor progression, thereby reducing the number and volume of tumors per rat in the treated groups. Briefly, the findings indicated that CP-P exhibited potential anticancer properties, primarily due to its influence on the proliferation, apoptosis, and redox balance in BC cells. The mechanism of its anticancer effect is linked to the suppression of the NF-κB activation pathway.[10]

Calotropis procera Modulates Cell Proliferation

In another study by Rasool et al, the cytotoxic effects of *C. procera* plant extract were examined, highlighting its correlation with various natural active compounds, including flavonoids, steroids, tannins, saponins, and alkaloids. The methanolic extract of *C. procera* is rich in flavonoids, tannins, saponins, and alkaloids, which exhibit antioxidant, antimicrobial, and anti-BC properties. Moreover, numerous researchers have reported that different types of flavonoids possess a range of biological activities, including anti-inflammatory, anti-allergic, antioxidant, and anti-cancer effects. Saponins are known for their antimicrobial properties, while tannins are recognized for their anti-cancer effects. Their findings revealed that the methanolic extract of *C. procera* was considerably effective against specific fungal strains and human BC MCF-7 cells [15] (Table 1). Likewise, Bou Malhab et al investigated the potential cytotoxic effects and underlying molecular mechanisms of *C. procera* on BC cell lines, specifically MCF-7. An ethanolic extract of the plant was employed for this purpose [16] (Table 1). Regulating the progression of the cell cycle in cancer cells is a recognized strategy for controlling tumor growth.[61] In addition, the findings confirmed that treatment with *C. procera* led to a notable arrest in the sub-G1 phase of MCF-7 cells. The ethanolic extract of *C. procera* also induced cell death in BC cells through various pathways.[62] Further, an analysis of the cell cycle and the molecular expression of various cyclins and their corresponding CDKs was conducted to clarify the cellular mechanisms behind the observed cytotoxicity and pro-apoptotic effects. The progression of the cell cycle is regulated by specific protein phosphorylation, with CDKs activated through their association with cyclins, which are expressed cyclically.[63] Cyclin D/CDK4 and CDK6 are linked to the transition from the G1 to the S phase, while cyclin B/CDK1 is associated with the M phase.[64] Furthermore, a significant reduction in cyclin D, CDK4, and CDK6 levels was observed in MCF-7 cells, which may account for the cell cycle arrest noted in the sub-G1 phase. Additionally, MCF-7 cells exhibited a response characterized by an arrest in the sub-G1 phase of the cell cycle, leading to cell death via necrosis.[65] In addition, survivin, a protein

composed of 142 amino acids, belongs to the family of apoptosis inhibitors and is essential for regulating cell proliferation. While survivin is typically not expressed in differentiated tissues, its levels are elevated in the majority of cancer cells, thereby diminishing cell death while promoting the uncontrolled growth of various tumors. Consequently, a reduction in survivin expression following treatment serves as a reliable marker for cell death.[66-69] Moreover, in the investigation of Bou Malhab et al, survivin expression was eliminated in MCF-7 cells when treated with *C. procera*. After a 24-hour treatment with *C. procera* ethanolic extract, they observed a decline in the phosphorylation of mTOR and AKT in both cell lines, indicating the inhibition of the AKT/mTOR pathway. Collectively, their findings suggested that *C. procera* extract may be a valuable tool in cancer treatment.[16]

Prostate Cancer

PCa ranks as the fourth most prevalent cancer in terms of new cases and eighth in cancer-related mortality across both genders, with a global death rate of 3.8%. Specifically, it is the second most frequently diagnosed cancer in men, exhibiting a death rate of 6.8% as of 2020.[70] Radiation therapy, androgen deprivation therapy, and surgical intervention are the primary treatment options for PCa patients.[71] However, these conventional treatments are associated with various adverse effects, such as neurotoxicity, cardiotoxicity, and musculoskeletal changes.[72,73] The bioactive compounds present in certain plants have demonstrated the ability to combat cancer cells by targeting a range of pro-oncogenic and anti-oncogenic molecules and signaling pathways.[74] Consequently, the therapeutic potential of plant derivatives and their bioactive constituents is currently under active investigation.[75,76]

Calotropis procera Modulates Cell Proliferation

In their study, Singh et al examined the impact of leaf extract from the herb *C. procera* on PCa cell lines in vitro and reported that the leaf extract significantly reduced cell viability and cells' ability to divide and migrate in androgen-independent and androgen-sensitive PCa cell lines. Notably, the androgen-sensitive 22Rv1 cell line exhibited greater sensitivity to *C. procera* extract treatment compared to the androgen-independent PC-3 cell line, as evidenced by the half-maximal inhibitory concentration values [17] (Table 1). In addition, the p27kip1 protein plays a crucial role in modulating inflammation by inhibiting NF-κB activation, thereby linking the cell cycle to inflammatory processes.[77] Furthermore, the absence of p27 has been associated with increased aggressiveness in PCa.[78] The results indicated that p27 levels increased in *C. procera* extract (CPE)-treated 22Rv1 cells, which may account for the observed decrease in cell division and migration capabilities following CPE treatment.[17] Additionally, the downregulation of NF-κB expression in both PCa cell lines post-CPE treatment aligns with the findings of previous research, demonstrating that *C. procera* can inhibit BC proliferation through NF-κB suppression.[10] Moreover, elevated levels of ROS are known to sustain tumorigenicity and contribute to genomic instability in cancer cells. Interestingly, the findings of Singh et al concluded that CPE treatment significantly lowers ROS levels, which may contribute to the observed reductions in cell viability and migration in PCa cells.[17] Furthermore, a decrease in ROS levels is typically linked to increased antioxidant levels.[79] Notably, recent findings revealed that the levels of antioxidant markers, such as catalase, superoxide dismutase 1 (SOD1), and thioredoxin, diminished following CPE treatment [18] (Table 1). Given that CPE is recognized for its substantial radical scavenging properties, it is noteworthy that SOD1 has been identified as a potential target for anticancer therapies, as it is often overexpressed in various cancers alongside elevated ROS levels, which helps mitigate damage from excessive ROS and supports tumorigenesis.[80] Similarly, thioredoxin, another antioxidant protein, has shown increased expression in androgen-independent PCa, and its inhibition has been associated with reduced cancer growth.[81] Therefore, the reduced levels of SOD1 and thioredoxin may play a role in the decreased proliferation of PCa cells.

Calotropis procera Modulated Autophagy

Cancer cells frequently employ autophagy to reallocate resources to enhance their survival.[82,83] According to research in PC-3 cells, the activation of autophagy correlates with an increased expression of Beclin-1, LC3B, and p62 at both the transcript and protein levels.[84-86] These proteins are essential for various critical phases of the autophagy process. Moreover, in the investigation by Singh et al, a significant upregulation of p62 and LC3-II expression was observed, indicating the induction of autophagy in PC-3 cells treated with CPE.[17] Conversely, CPE treatment led to a notable decrease in the expression of Beclin-1 and LC3-II in 22Rv1 cells, suggesting the suppression of autophagy. In conclusion, current findings demonstrated that CPE hampers the proliferation and migration of PCa cells by modulating autophagy and decreasing intracellular ROS levels. Additionally, androgen-sensitive and androgen-independent PCa cells exhibited distinct responses to herbal formulations, highlighting their unique characteristics that could be leveraged for targeted PCa therapies. This issue underscores the importance of evaluating herbal extracts across different types of PCa cells.[17]

Glioblastoma Multiforme

Glioblastoma multiforme (GBM) represents a significant portion of central nervous system tumors, constituting over 70% of all cancers related to the brain and nervous system.[87] Despite considerable funding for discovering anti-GBM drugs in the past decade, advancements have mainly remained within preclinical phases, with clinical

trials repeatedly facing numerous challenges.[88] Anti-angiogenesis therapies that target VEGF were initially considered the most promising treatment for GBM, given the highly vascularized nature of tumors.[89] To date, bevacizumab, an antibody that inhibits VEGF-A, has demonstrated notable efficacy in GBM through controlled clinical trials.[90] Nevertheless, various bevacizumab-related toxicities have been observed in patients with GBM.[91] These adverse effects are primarily due to the blockade of VEGF, which is crucial for several essential functions, including maintaining blood vessel integrity, ensuring vascular homeostasis, facilitating clotting, promoting wound healing, and supporting kidney filtration.[92] Concurrently, histone deacetylase (HDAC) inhibitors are being explored for their potential in cancer treatment. Several HDAC inhibitors have exhibited remarkable anti-glioblastoma effects by targeting multiple anticancer pathways, and many are currently undergoing clinical trials as either monotherapies or in combination with other treatments against GBM. However, developing most of these inhibitors into viable therapeutics has been hindered by undesirable side effects and toxicities.[93,94]

Calotropis procera Modulated Tumor Protein p53

The methanol extract derived from the leaves of *C. procera* had notable anticancer properties, exhibiting low half-maximal inhibitory concentration values against three distinct GBM cell lines in the current investigation.[12] Recent studies have highlighted the cytotoxic effects of calotropin on A172 and U251 GBM cells.[95] In addition, the crude extract comprises various bioactive compounds that collectively exert a synergistic effect by targeting multiple anticancer pathways. GBM harboring mutations in the TP53 are associated with a poor prognosis and exhibit resistance to conventional chemoradiotherapy. Therefore, targeting TP53 emerges as a promising approach to improve therapeutic outcomes in GBM. The selective cytotoxicity of the crude extract from *C. procera* toward U251 cells, along with findings related to calotropin, underscores its potential therapeutic benefits for GBM with TP53 mutations.[12,95]

Calotropis procera Inhibits Angiogenesis

Semaxanib has shown effectiveness in clinical trials for the treatment of acute colorectal cancer (CRC) and myeloid leukemia.[96] Trichostatin A, a recognized HDAC inhibitor, has been reported to inhibit angiogenesis in various cancer progression models, including GBM.[97] In this regard, both Semaxanib and Trichostatin A were found to obstruct the formation of angiogenic blood vessels in developing zebrafish embryos more effectively than *C. procera* leaf extract; however, they also caused significant toxicity and high mortality rates. Furthermore, in U251 cells, several angiogenesis-related proteins were explicitly regulated by the *C. procera* extract, leading to the downregulation of VEGF (Figure 2), activin A, coagulation factor III, serpin E1, basic fibroblast growth factors, endothelin, and insulin-like growth factor binding protein. Zhang et al revealed that activin A levels were significantly higher in GBM compared to normal brain tissue, correlating with an increased proliferative index of U87 cells relative to the control group [98], representing the potential role of the imbalance between activin A and follistatin in GBM pathogenesis. Moreover, HDAC inhibitors have yielded promising results in treating GBM.[99] In the mentioned study, the findings of real-time polymerase chain reaction indicated that *C. procera* extract led to the upregulation of class II and IV HDACs (specifically HDAC 5, 7, and 10) and the downregulation of HDAC4, HDAC6, and HDAC9 mRNA in U251GM cells. It was confirmed that *C. procera* extract functions as an HDAC inhibitor and a regulator of HDAC expression, downregulating isoforms that are elevated in GBM compared to normal brain tissue while enhancing the expression of isoforms that are reduced in GBM.[12]

Colorectal Cancer

CRC is acknowledged as a leading cause of cancer-related mortality worldwide. Numerous studies have indicated that long-term aspirin use significantly lowers the risk of developing CRC. Additionally, other anti-inflammatory medications have demonstrated positive effects on this disease.[100,101] Both traditional applications and various in vivo research findings highlight the anticancer and strong anti-inflammatory characteristics of the latex derived from *C. procera*.[79,102,103] In this regard, Kumar et al assessed the protective effects of the methanol extract of dried latex from this plant against the initial stages of colon carcinogenesis in a rat model. When administered orally daily, the methanol extract led to a dose-dependent decrease in the number of aberrant crypt foci, crypt multiplicity, and histological scores, similar to the effects observed with aspirin [19] (Table 1). Other components of the *C. procera* plant were also found to exhibit cytotoxic and anticancer effects.[104]

Calotropis procera Modulates Many Signaling Pathways

The process of colon carcinogenesis encompasses initiation and progression stages, both of which involve modifications in various signaling pathways, with the Wnt/β-catenin pathway being particularly significant. The overproduction of β-catenin, its accumulation in the cytoplasm, and subsequent movement into the nucleus lead to the activation of genes that regulate cell survival, proliferation, and differentiation, ultimately resulting in dysplastic changes.[105] Furthermore, hyperactive Wnt/β-catenin signaling is crucial for CRC adhesion, invasion, and metastasis.[106] Additionally, proliferating cell nuclear antigen serves as a marker for cellular proliferation linked to neoplastic progression and dysplasia in the human colon.[107-109] In the experimental group, there was an observed overexpression of β-catenin and proliferating cell nuclear antigen, along with increased levels of VEGF and MMP-9. The elevated expression of these markers

correlates with a poor prognosis in CRC, indicating the carcinogenic impact of 1,2-dimethylhydrazine (DMH) on the colon.[105,110,111] When orally administered to DMH-treated rats, the extract of air-dried latex of *C. procera* (MeDL) exhibited an anti-proliferative effect, as evidenced by the reduced expression of these markers. It was also revealed that both MeDL and aspirin significantly enhanced apoptotic DNA fragmentation, which was associated with increased DNA laddering and the upregulation of Bax, a pro-apoptotic marker, while downregulating Bcl2, an anti-apoptotic marker, leading to a higher count of apoptotic cells. Accordingly, MeDL may serve as a potential chemopreventive agent for CRC. Overall, this research demonstrated that the methanol extract of *C. procera* latex contains components that confer beneficial effects in the context of CRC.[19]

Moreover, the association between low-dose aspirin and other nonsteroidal anti-inflammatory drugs and a significant reduction in CRC risk is well established.[112] The latex from the plant *C. procera* displayed nonsteroidal anti-inflammatory drug-like properties, effectively alleviating inflammation, pain, and fever. Similarly, Kumar et al assessed the impact of MeDL on OS and inflammation markers in a rat model of CRC [20] (Table 1). The chemical DMH induced OS by producing ROS, leading to lipid peroxidation, which causes macromolecular damage and facilitates carcinogenesis.[113] This was evidenced by a notable decrease in antioxidant levels (e.g., glutathione and SOD), but an increase in thiobarbituric acid-reactive substances, a lipid peroxidation marker, in DMH-treated rats.[114] Furthermore, treatment with MeDL resulted in a dose-dependent normalization of OS markers, restoring homeostasis, similar to the effects observed with aspirin.[115] In addition, research indicated that both MeDL and aspirin effectively suppress OS and inflammation.[79,115] Furthermore, a high density of inflammatory cells has been documented in the colon tissue of rats treated with DMH.[113]

Calotropis procera Modulated Immunity

Mediators such as TNF-α and prostaglandin E2 (PGE2) are recognized for their roles in tumorigenesis, primarily due to their pro-inflammatory properties and participation in the Wnt/β-catenin signaling pathway.[116-119] Additionally, interleukin (IL-1β) (Figure 3), a cytokine produced in response to various pro-inflammatory signals, is well-established as a promoter of tumorigenesis. These inflammatory mediators interact with other factors that promote tumor development, thereby facilitating the initiation and continuation of carcinogenesis.[120] Elevated levels of these markers have been observed in patients with CRC, and their effects are known to be mediated through both cyclooxygenase-dependent and cyclooxygenase-independent pathways (Figure 3), as well as through the modulation of the host immune response.[121] Some research confirmed that daily administration of MeDL to rats treated with DMH inhibited carcinogenesis.[20] Moreover, another study demonstrated that MeDL reduced OS and inflammation in a rat model of CRC, resulting in a significant decrease in lipid peroxidation but an increase in glutathione and SOD levels, in addition to a notable reduction in nitrite, myeloperoxidase, TNF-α, PGE2, and IL-1β immunoreactivity.[20]

Conclusion

Medicinal plants are becoming increasingly prevalent globally due to their affordability, safety, and therapeutic

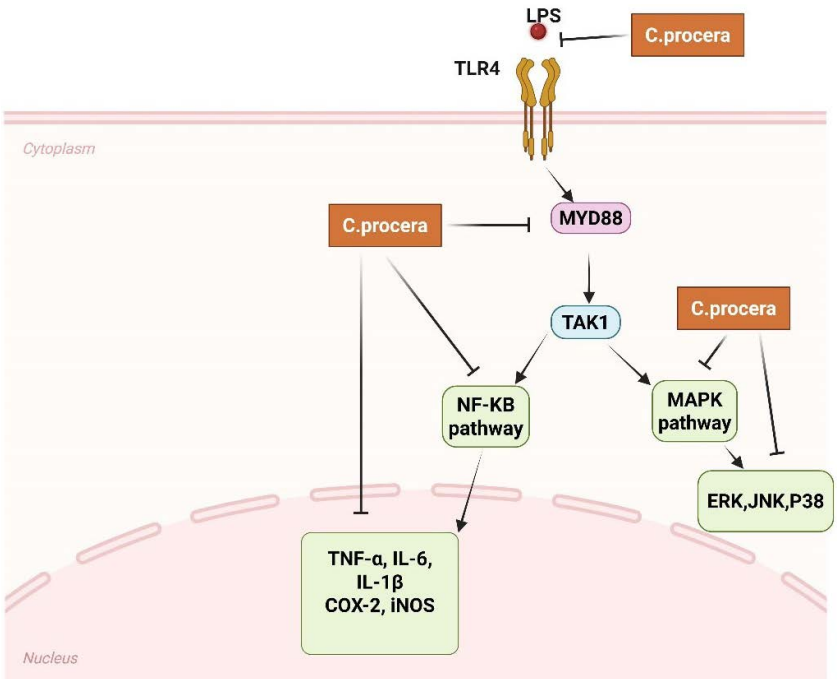

Figure 3. *Calotropis procera* Affects Inflammatory Pathways
Note. NF-κB: Nuclear factor kappa B; TLR: Toll-like receptor; LPS: Lipopolysaccharide; TNF-α: Tumor necrosis factor alpha; IL: Interleukin; INOS: Inducible nitric oxide synthase.

Table 2. *Calotropis procera* Exerts Anticancer Effects via Many Mechanisms

Anti-cancer mechanisms of *Calotropis procera*	↓ CTSL ↓ VEGF ↑ Cleaved caspase-3 ↓ Total PARP protein level ↓ Phospho-ERK1/2 level ↓ Cancer cell viability ↓ Oxidative stress ↓ AKT/MTOR ↓ MAPK/ERK1/2 ↓ Insulin receptor ↓ Total protein levels of TSC2 ↓ ROS ↑ Apoptosis ↓ NF-κB ↓ TNF-α ↓ IkBa phosphorylation ↑ IKK activation ↓ Cyclin D1 ↓ Bcl-2 ↑ Arrest in the sub-G1 phase ↑ Cell death ↓ Survivin ↓ Beclin-1 ↓ LC3-II ↓ Autophagy Alter TP53 ↓ Activin A ↓ Coagulation factor III ↓ Serpin E1 ↓ Basis FGF ↓ Endothelin ↓ IGFBP ↑ Bax ↑ Apoptotic DNA fragmentation ↓ Lipid peroxidation ↓ Nitrite ↓ Myeloperoxidase ↓ PGE2 ↓ IL-1β

Table 3. Abbreviations

Abbreviation	Definition
ROS	Reactive Oxygen Species
NF-κB	Nuclear Factor Kappa B
MAPK	Mitogen-Activated Kinase
CDK	Cyclin-Dependent Kinase
AKT	Protein Kinase B
mTOR	Mechanistic Target of the Rapamycin
TNF-α	Tumor Necrosis Factor-Alpha
ECM	Extracellular Matrix
VEGF	Vascular Endothelial Growth Factor
TNBC	Triple-Negative Breast Cancer
PCa	Prostate Cancer
TSA	Trichostatin A
CP-P	*Calotropis procera* Protein
GBM	Glioblastoma Multiforme
CRC	Colorectal Cancer
CPE	*Calotropis procera* Extract

potential. These plants contain naturally occurring chemicals that have been shown to be extremely effective in herbal medicine for several cancer-associated processes (e.g., prevention, antioxidant defense, mutagenesis suppression, and cell proliferation inhibition). According to the findings of this review, *C. procera* is a natural substance that can influence various mechanisms to hinder the proliferation and migration of cancer cells. This study demonstrated that *C. procera* affects numerous signaling pathways. (1) *C. procera*'s primary effect involves anti-inflammation mechanisms. This natural herb can change different ILs (e.g., IL-1β) and modulate additional immune pathways (e.g., TNF-α, NF-κB, and MAPK/ERK1,2). (2) Further, *C. procera* has anti-angiogenesis properties. Further studies have shown that this product inhibited VEGF, activin A, coagulation factor III, serpin E1, basic fibroblast growth factors, endothelin, and insulin-like growth factor binding protein. (3) In addition, *C. procera* induced apoptosis and led to the upregulation of cleaved caspase-3 and Bax while downregulating Bcl-2. (4) Moreover, it can inhibit cancer cells, such as down-regulating lipid peroxidation, nitrite, survivin, cyclin D1, the AKT/MTOR pathway, and the IR, while up-regulating cancer cell death (Table 2). This study offered several perspectives to improve our comprehension of the potential and constraints of the *C. procera* in cancer, particularly BC, PCa, CRC, and glioblastoma, based on the topics covered in earlier sections (Figure 3, Table 3).

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

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