Pharmacogn. Res., 2024; 16(1):19-25.
https://www.phcogres.com

Review Article

Calotropin: Natural Phytomolecules for Cutting-edge Features

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ABSTRACT

Phytochemical is a collective term for plant chemicals with varied structure and function. The most common sources of phytochemicals are fruits, vegetables, whole grains, nuts and seeds, and other plant foods. Calotropin is a pharmacologically active compound isolated from milkweed plants like *Calotropis procera, Calotropis gigantea*, and *Asclepias curassavica* that belong to the Asclepiadaceae family which is used for medicinal purposes in many Asian countries. Calotropin is identified as a highly potent cardenolide that has a similar chemical structure to cardiac glycosides (such as digoxin and digitoxin). Among cardenolides, calotropin is identified as the most promising agent. Calotropin has cytotoxic and anti-tumor impacts, with cancers of the breast, colon, lung, and leukemia malignancies exhibiting the most significant effects. The effects of calotropin on cancer have been extensively studied in preclinical pharmacological studies *in vitro* using cancer cell lines and *in vivo* in experimental animal models that have targeted antitumor mechanisms and anticancer signaling pathways. During ancient times, calotropin was utilized in various techniques. A macerated bark extract is frequently utilized for de-hearing hides and tanning. Calotropin is a particularly effective abortifacient or interceptive agent in females. Cardenolide calotropin is poisonous. This critique focused on its chemistry and therapeutic activity in various cancer cells.

Keywords: *Calotropis gigantea*, *Asclepias curassavica*, *Calotropis procera*, Calotropin, Cardenolide.

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Received: 08-09-2023;
Revised: 18-10-2023;
Accepted: 09-11-2023.

INTRODUCTION

The term "phytoconstituent" is a general one that describes a large number of chemicals that occur naturally in plants.[1] Plant compounds with various structures and functions are collectively called phytochemicals. They may perform a variety of protective and reproductive roles in plants, such as those associated with color, scent, and insect attraction for pollination, phytoalexin defense against pathogens, hormonal functions for growth and signaling, antifeedants and toxins for insect defense, and allelochemicals for herbivore defense.[2,3]

We chose one of the most potent phytoconstituents, calotropin, found in the *Asclepiadaceae* family, from the many phytoconstituents exhibiting effective bioactivity. Calotropin is a pharmacologically active substance identified from milkweed plants of the Asclepiadaceae family, including *Calotropis procera, Calotropis gigantea*, and *Asclepias curassavica*.[4-8] All of these plants have been identified as being traditional Asian herbal medicines. Calotropin is a highly potent cardenolide with a chemical composition equivalent to cardiac glycosides (such as digoxin and digitoxin).[9-13]

Both species are widespread. However, *Calotropis procera* is more prevalent and has purple blooms, whereas *Calotropis gigantea* has whitish flowers.[14] The primary physical distinction between the two species, which allows for easy differentiating themselves, is the color of the buds or blooms on their flowers. So, if a plant doesn't have a blossom, it may be difficult to determine its species.[15,16] During ancient times, the calotropis was utilized in various techniques. A macerated bark extract is frequently utilized for de-hairing hides and tanning. Tannin is used as a dyeing agent. Arrow and spear poisons are commonly made from bark and latex.[17] Medicine: It has been discovered that compounds produced from the plant exhibit digitalis and emetic-cathartic effects. It is also used to cure lice, colic, whooping cough, diarrhea, headaches, painful lips and gums, toothaches, sterility, swellings, and ulcers.[18-20]

Calotropin appears to be a particularly effective abortifacient or interceptive agent in females. The term "interceptive" indicates a woman's pregnancy termination following implantation.[21] It may hamper the hormonal flow required to maintain the pregnancy's functioning. A study revealed that calotropin could be utilized as an effective abortifacient or interceptive drug for

DOI: 10.5530/pres.16.1.3

unexpected pregnancies and for the persistent suppression of testicular activity.[22,23]

APPROACH TO CALOTROPIN'S BIOSYNTHESIS

Calotropin is thought to be biosynthesized similarly to another cardenolide, digitoxin. Since digitoxin is a more well-known treatment for cardiac insufficiency, the production of the compound received more attention. Nevertheless, it is thought that many cardenolides are produced in plants through a similar process, although it is still being determined how. Precursors for steroidal alkaloids and sterols are comparable in this mechanism. The conversion of the sterol precursor to digitoxigenin, the precursor to digitoxin, is achievable by either two hypothesized means, the pregnane pathway or the norcholanic acid method. Progesterone 5β-reductases, specifically P5βR and P5βR2, are required for the norcholanic acid and pregnancy pathways. In the pregnane pathway, pregnenolone is assumed to be converted to progesterone by a plant analog of the mitochondrial cytochrome P450 (CYP11A in humans). P5βR converts progesterone further into digitoxigenin and 5β-Pregnane-3, 20-dione. On the norcholanic acid route, less is known. The analogies between digitoxin and calotropin stop here [Figure 1].[24,25]

INSIGHT INTO CALOTROPIN'S FUNDAMENTAL CHEMICAL STRUCTURE

It is a cardenolide glycoside. chemical formula for calotropin is $C_{29}H_{40}O_9$ and melting point is 221°C and Molar mass-532.630 g·mol^{-1}.[23,26] IUPAC name-(2R,3R,5S,8R,9S,10R,13R,14S,17R)-2,3,14-trihydroxy-13-methyl-17-(5-oxo-2H-furan-3-yl)-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthrene-10-carbaldehyde. This structure contains a Hydrogen Bond Donor (HBD) 3 and a Hydrogen Bond Acceptor (HBC) 9 [Figure 2]. Calotropin, a cardiac glycoside of the cardenolide type, is hazardous. These substances contain a carbon backbone with steroids and are related to them.[27] Calotropagenin, the precursor of these compounds, is the basis for calotropin, calactin, calotoxin, and uscharin. In plants of the genus calotropis, they are frequently found together and exhibit comparable activities.[24,28]

When compared to gomphoside derivatives, calotropin (1) and related cardenolides (2-7) have more of an aldehyde functionality at C-19, which only typically occurs in some cardenolide classes, also including in k-strophanthin [Figure 3].[5,21,29] Most cardiac glycosides embrace one to four sugar residues connected to the genin, increasing their capability to bind to the heart muscle and make them water-soluble.[30,31] Numerous distinct substitution patterns were discovered at the sugar moiety in the calotropis family [Figure 3]. The hydroxyl group can be altered by being acetylated or epimerized at C-3′ and subjected to its oxidation.[4] As an additional prevalent modification of the sugar building block, an attachment of a thiazolidine or a dihydro thiazolidine moiety was identified at C-3′, illustrating the vast diversity of the presented cardenolide class.[32-34] Several distinct substitution patterns have been identified at the sugar moiety in the calotropin family. Apocannoside (II) and cymarin (III), which have recently been established to have cytotoxic activity, have significant structural similarity to calotropin, which was only recently given in the structure I [Figure 4].[35-38]

CALOTROPIN'S THERAPEUTIC ACTIVITY AND MECHANISMS IN GLAZED TYPES OF CANCER

From recent research, calotropin was discovered by fractionating two active extracts and isolating and characterizing a cytotoxic component.[39] The significant and initial mode of action of cardenolides, notably calotropin, was the inhibition of Na^+/K^+ P-type Adenosine Triphosphatase (ATPase), a sodium-potassium exchanger.[40] Calotropin affects the myocardium more than the skeletal muscles because myocytes have more active Na^+/K^+ adenosine triphosphatase [Figure 5].[10,24,34] Cardenolide calotropin is poisonous. Calotropin overdose can occasionally result in cardiac and respiratory collapse.[25,41,42]

Speculate about brain cancer

Calotropin confirmed a potential anticancer mechanism that includes G_2/M phase cell arrest by having the most significant cytotoxicity on the A172 and U251 glioma tumor cell lines.[43,44] Calotropin's ability to inhibit the enzyme Na^+/K^+-P-type Adenosine Triphosphatase (ATPase) provides additional proof that it may have cytotoxic effects on cancer cell lines. All mammalian cells contain Na^+/K^+ P-type Adenosine Triphosphatase (ATPase), a crucial transmembrane protein responsible for maintaining cell ion homeostasis.[45] In an investigation, commercial brain porcine Na^+/K^+ P-type Adenosine Triphosphatase (ATPase) was used to analyze the anticancer effects of calotropin.[46] Calotropin suppressed the activity of Na^+/K^+-P-type adenosine triphosphatase (ATPase) in a dose-dependent manner. Calotropin has the same IC_{50} value on porcine cerebral cortex Na^+/K^+-P-type Adenosine Triphosphatase (ATPase) activity, according to a prior study that determined the IC_{50} value. Calotropin's IC_{50} value is similar to other renowned cardenolides like ouabain and digoxin.[47,48]

Calotropin is employed solely to combat breast cancer

Triple-Negative Breast Cancers (TNBC), responsible for 10%-20% of all breast cancers, develop when normal cells in the breast mutate and grow out of control. Breast cancer occurs with a frequency of 13% in the global population.[49] Several *Bacillus thuringiensis*-549(BT549) cells were selected for calotropin.[50] Calotropin's effects on human Triple-Negative Breast Cancer (TNBC) cell lines were demonstrated, coupled with its selectivity for multiple TNBC cell lines and varying IC_{50} values. Compared to MDA-MB-231, calotropin was more selective for *Bacillus thuringiensis*-459(BT459) and Hs578T cells. Due to the outcomes,

calotropin causes cell death in *Bacillus thuringiensis*-459(BT459) cells at lower concentrations than in other human Triple-Negative Breast Cancer (TNBC) cell lines due to increased intracellular Ca^{2+} levels. Calotropin's capacity to block the enzyme Na^{+}/K^{+}-P-type Adenosine Triphosphatase (ATPase), which affects crucial transmembrane cellular protein homeostasis, is another indication that it may have destructive effects on cancer cell lines [Figure 6].[51,52]

Touted as a therapy for lung cancer

Non-Small Cell Lung Cancer (NSCLC), one of the most prevalent types of cancer in humans, can be recognized by invasion, migration, rapid expansion, and recurrence.[53] Calotropin rehabilitation enhanced the expression of the pro-apoptosis genes Caspase-*3* and Caspase-*8* in Non-Small Cell Lung Cancer (NSCLC) *in vitro* experiments.[54] By diminishing the expression of Transforming Growth Factor-β (TGF-β)/Extracellular signal-regulated protein kinases 1/2 ($ERK_{1/2}$) and downregulating phosphorylation of Extracellular signal-regulated protein kinases 1/2 ($ERK_{1/2}$), calotropin treatment of cancer cells activates the transforming growth factor-β (TGF- β)/Extracellular signal-Regulated protein Kinases (ERK) signaling pathway. Calotropin administration dramatically raised the number of apoptotic cells, substantially elevated apoptosis markers, and significantly decreased tumor growth in Non-Small Cell Lung Cancer (NSCLC)-bearing mice, according to an *in vivo* assay: the Caspases 3 and 8.[55] Follow-up research has demonstrated that this treatment considerably increased the mice's survival duration. Transforming Growth Factor-β (TGF-β), a crucial element in the development of cancer was blocked by calotropin's effects on protein expression levels [Figure 7]. In non-small cell lung cancer cells, calotropin treatment controlled apoptosis, decreased tumor growth, and raised survival via the Transforming Growth Factor-β (TGF-β)/Extracellular signal-Regulated protein Kinases (ERK) signaling pathway.[56,57]

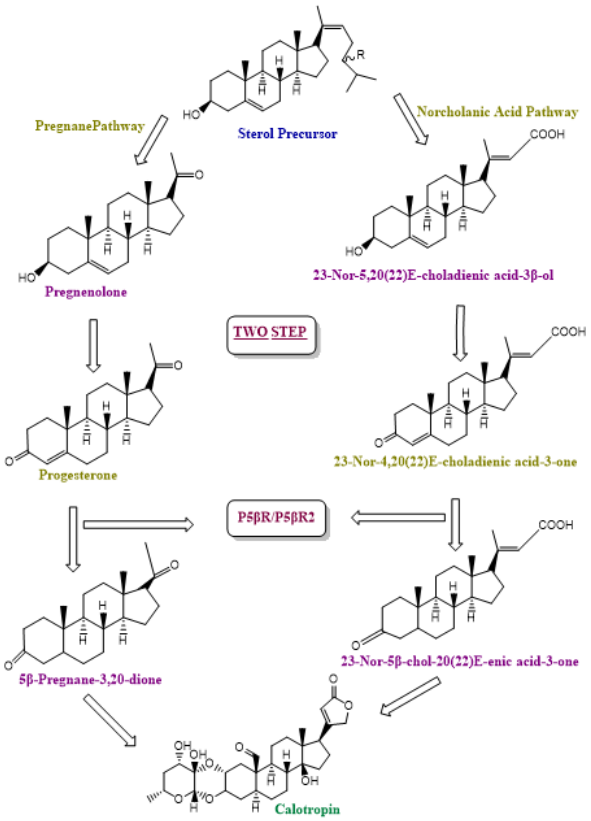

Figure 1: Biosynthesis Pathway of Calotropin.

Calotropin is implemented to treat hepatic carcinoma in accordance

Hepatocellular Carcinoma (HCC), the most prevalent kind of liver cancer, is one of the most aggressive malignancies with a terrible prognosis. According to a distinct liver carcinogenesis model, calotropin's anticancer action in Hepatocellular Carcinoma (HCC) was associated with regulating fatty acid levels via a decline in inflammatory cytokines and adipocytes shrinkage.[58] Calotropin's IC_{50} value on the human hepatocarcinoma cell line ($HepG_2$) and Raji cells (human B lymphoblastoid cell line) was revealed in a study, and values were reported as 0.04 μM and 0.02 μM, respectively; this validated the drug's cytotoxic action on tumor cell lines.[11,59-61]

Potentially having an impact on colorectal cancer

A malignant tumor that develops in the colon and rectum, Colorectal Cancer (CRC), can have a wide range of symptoms. In its early stages, Colorectal Cancer (CRC) is asymptomatic and is found through screening exams. By activating the Yes-Associated Protein (YAP), calotropin significantly reduces the growth of Colorectal Cancer (CRC) cells via the Hippo pathway.[62] Dysregulation of the Hippo pathway components frequently results in abnormal cell proliferation and tumor formation since the Hippo pathway is a highly complicated signaling network with over 30 branches. Colorectal cancer cells that have received calotropin treatment become dephosphorylated of the Yes-Associated Protein (YAP). Following calotropin treatment, Yes-Associated Protein (YAP) dephosphorylation led to its translocation into the nucleus of Colorectal Cancer (CRC) cells [Figure 8]. [11,63]

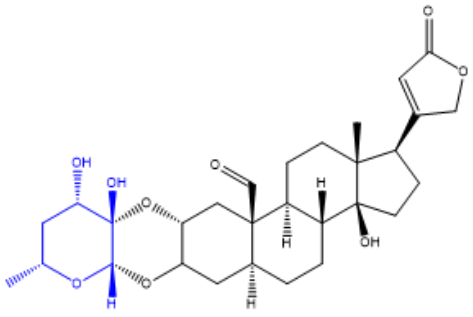

Figure 2: Structure of Calotropin.

Calotropin in light of the treatment for leukemia

The anti-cancer effects of calotropin were initially demonstrated in human chronic myeloid leukemia K562 cells and are most likely mediated in a caspase-dependent way. Because of the observed dose-dependent increases in the activity of cysteine-aspartic acid protease-3, cysteine-aspartic acid protease-8, and cysteine-aspartic acid protease-9 after calotropin treatment, it was hypothesized that calotropin may induce apoptosis through the caspase-dependent mechanism. Additionally, calotropin administration inhibits the production of anti-apoptotic and apoptotic protein inhibitors such as nuclear factor kappa B, p^{50}, protein kinase B, survivin, and X-linked Inhibitor of Apoptosis Protein (XIAP) in a dose-dependent manner [Figure 9]. Calotropin is being studied as a potential therapy for Adult T-cell Leukemia (ATL) and lymphoma.[7,11,64,65]

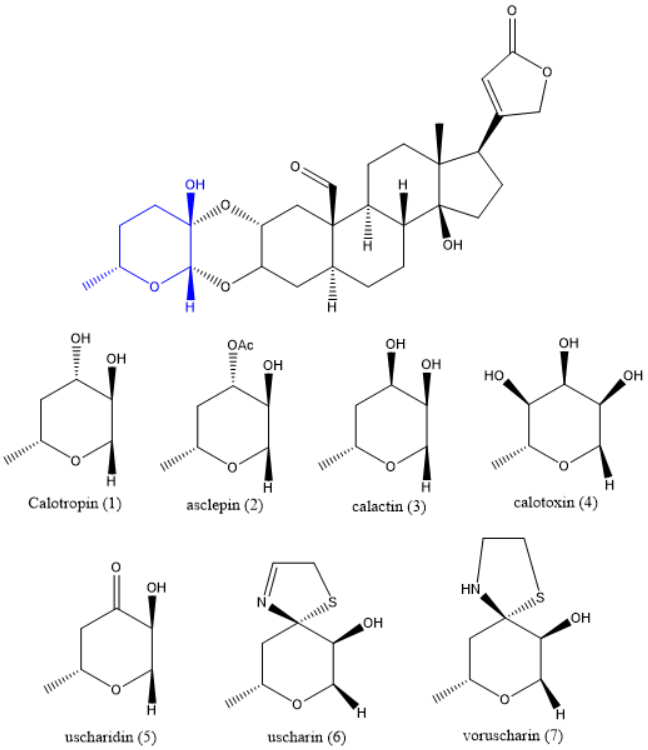

Figure 3: Calotropin (1) and selected cardenolide glycosides (2-7) isolated from the milkweed family *Asclepiadaceae*.

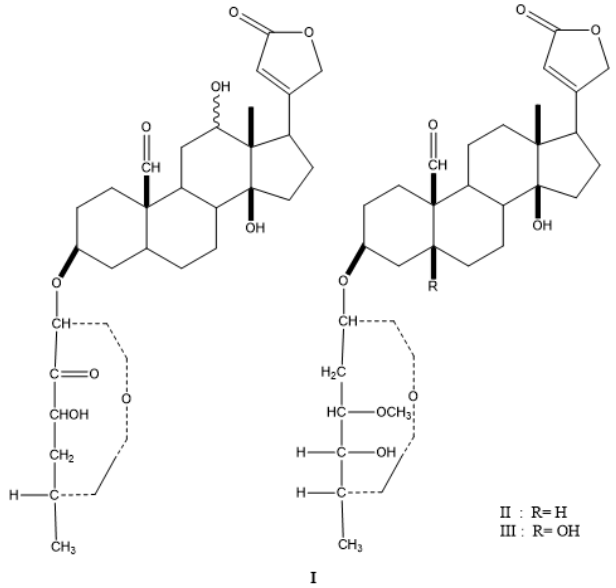

Figure 4: Calotropin.

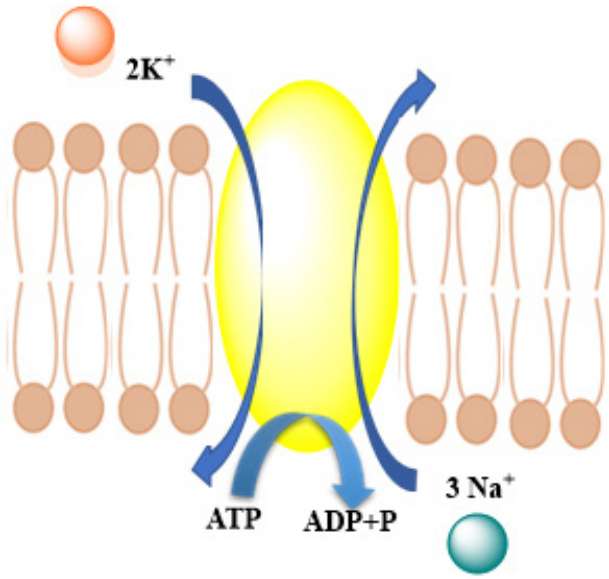

Figure 5: An illustration of the sodium-potassium pump.

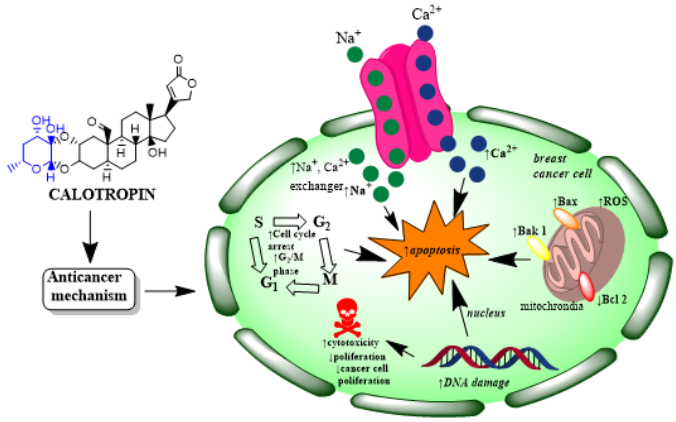

Figure 6: Breast cancerous tumors and the possibility of anticancer effects of calotropin: Tumors By using the Na^+/Ca^{2+} exchanger, calotropin suppresses Ca^{2+} outflow. Apoptosis can be induced by an increase in intracellular Na^+ and Ca^{2+} levels. By destroying DNA and stopping the cell cycle in the G_2/M phase, calotropin also causes apoptosis and cell death in tumor cells. This Bax/Bcl-2 ratio plays a crucial role in deciding apoptosis since it also increases Bax/Bakl expression while drastically reducing Bcl-2 expression. Symbols for ↑increase, ↓decrease.

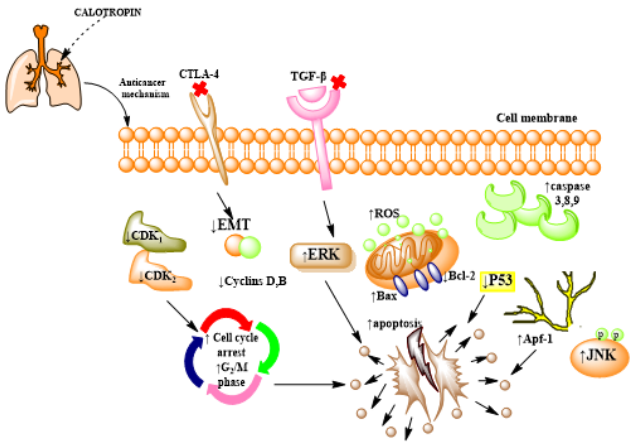

Figure 7: Representation of Calotropin's cytotoxic actions in lung cancer. Abbreviations and symbols: ↑ increase, ↓decrease, Epithelial-Mesenchymal Transition (EMT), Reactive Oxygen Species (ROS), Mitogen-Activated Protein (MAP) kinases and cJun NH2-terminal kinase (JNK), Transforming Growth Factor β (TGF-β), Cytotoxic T-Lymphocyte-Associated antigen 4 (CTLA-4).

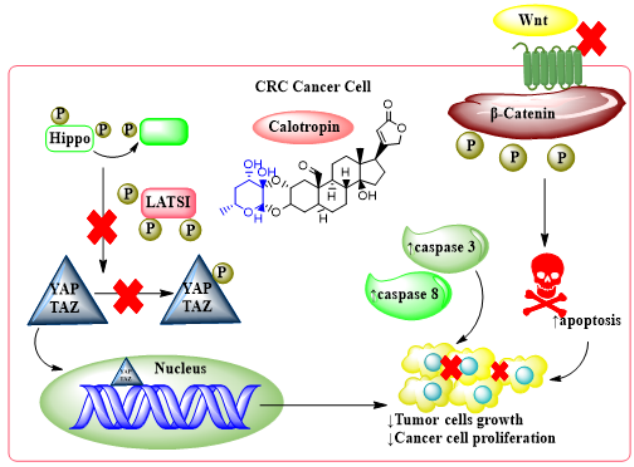

Figure 8: Calotropin's anticancer mechanisms in colorectal cancer are shown schematically.

SYNERGISTIC EFFECTS OF CALOTROPIN WITH CHEMOTHERAPEUTIC AGENTS

A recent study found that a combination of low-concentration doxorubicin and calotropin-induced apoptosis is accompanied by suppressed Adenosine triphosphatase production in hepatocarcinoma cell line (HepG2) cells, revealing the role of cardenolides as a potential anticancer agent and arising as a potential adjunctive therapy to anticancer therapeutics.[66] During this therapy, there was a decrease in adenosine triphosphatase synthesis, which was linked to a reduction in glucose consumption. In contrast to a high dose of 5-Fluorouracil (5-FU), we combined modest quantities of 5-FU with calotropin, decreased cell viability, and eliminated resistance to this joint chemotherapeutic agent in HCT116 colorectal cancer cells.[67] Despite calotropin

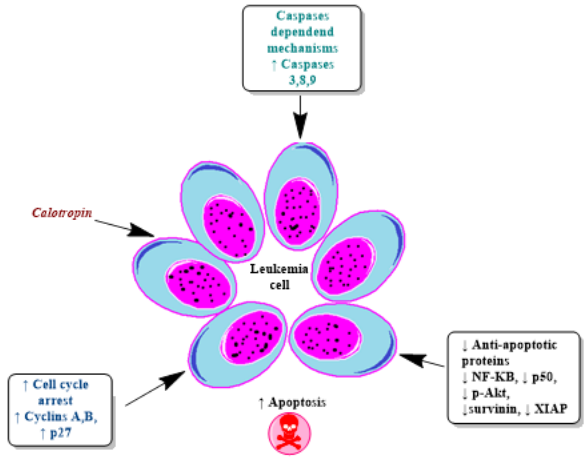

Figure 9: Demonstration showing calotropin's anti-leukemic mechanisms. Symbols: ↑increase, ↓decrease.

treatment, autophagy-related genes may express at a lower level or even not in cancer cells.[50,68,69]

CONCLUSION

With an extensive history of use, improvement, and effect, traditional treatments from the Near East, China, and India have enormously impacted the subsequent evolution of a comprehensive range of recognized medications and additives. Nevertheless, their widespread usage and the entire mechanism of action for most traditionally utilized herbs still need to be discovered. Calotropin has cytotoxic and antitumor impacts, with cancers of the breast, colon, lung, and leukemia malignancies exhibiting the most significant effects. The biggest drawback of calotropin is that, while its anticancer activity has been demonstrated *in vitro*, there needs to be more evidence from *in vivo* investigations; this is especially true for studies involving humans; no clinical trials have looked into calotropin. There is less proof of anticancer efficacy for substances derived from plants than for calotropin. Nevertheless, it is evident that animal *in vivo* tests still need improvement. Thus, calotropin research should move on to those.

ACKNOWLEDGEMENT

We are thankful to GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India for providing the necessary facilities to carry out this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT AND AUTHORSHIP

This study was supported by GITAM School of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

ABBREVIATIONS

HBD: Hydrogen bond donor; **HBC:** Hydrogen bond acceptor; **OH:** Hydroxyl group; **ATP:** P-type adenosine triphosphatase (ATPase); **IC_{50}:** Inhibitory Concentration 50; **BT_{549}:** *Bacillus thuringiensis* 549; **BT_{459}:** *Bacillus thuringiensis*-459; **TNBC:** Triple-negative breast cancer; **Na^+:** Sodium ion; **K^+:** Potassium ion; **Ca^{2+}:** Calcium ion; **NSCLC:** Non-small cell lung cancer; **TGF-β:** Transforming growth factor-β; **ERK:** Extracellular signal-regulated protein kinases; **EMT:** Epithelial-mesenchymal transition; **ROS:** Reactive oxygen species; **MAP:** Mitogen-activated protein; **JNK:** cJun NH_2-terminal kinase; **CTLA-4:** Cytotoxic T-lymphocyte-associated antigen 4; **μm:** Micrometer; **HCC:** Hepatocellular carcinoma; **CRC:** Colorectal cancer; **YAP:** Yes-associated protein; **XIAP:** X-linked inhibitor of apoptosis protein; **ATL:** Adult T-cell leukemia; **5-FU:** 5-Fluorouracil.

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Cite this article: Nag S, Paul R, Mondal S, Panigrahi N, Roy P. Calotropin: Natural Phytomolecules for Cutting-edge Features. Pharmacog Res. 2023;16(1):19-25.