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Review Article

The Study on the Effect of Cantharidine and Its Derivates on Tumor Cells

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Abstract

Millions of people worldwide die from cancer each year, making it the greatest danger to the survival of the human race. Historically, natural substances have been seen as viable cancer therapeutic options. Traditional medicine has utilised the terpenoid cantharidin (CTD), which was extracted from blister beetles, extensively. Chinese drugs to treat cancer and other diseases. CTD has been shown to inhibit both the heat shock transcription factor 1 (HSF-1) and protein phosphatase 2A (PP2A), which are possible targets for its anticancer effect. Even though it contains certain toxins, CTD's tremendous anticancer potential cannot be understated since cancer-specific delivery of the drug might mitigate its fatal consequences. Additionally, a number of compounds have been created to lessen its toxicity. The anticancer action of CTD is shown in both in light of substantial studies, both in vitro and in vivo cancer models exist. Additionally effective when used in conjunction with radiation and chemotherapy, CTD can also target some cancer cells that have developed a resistance to certain drugs. The goal of this mini-review is to analyse and condense current findings about the molecular underpinnings and anticancer potential of CTD. CTD's relevant anticancer properties might be used to create a potent anticarcinogenic medication.

Keywords: Cantharidin; blister beetles; anticancer; molecular mechanism; cancer.

Introduction

The perception of cancer as a harmful health condition is universal [1]. According to current data, it ranks top for deaths in high-income nations while being the second most common cause of death globally [2]. According to the American Cancer Society, by 2020, there will be around 1.8 million new cases of cancer and 606,520 fatalities in the US alone [3]. Cancer is a diverse condition made up of many different types of cells that are connected to support the affected cells' aberrant development and proliferation. The main indicators of cancer include increased proliferative signals, increased angiogenesis, metastatic invasion, aneuploidy, and immunological dysfunction, which cause the afflicted cells to become immortal and ultimately cause the patient's death [4]. The increased heterogeneity of the cells inside the cancer microenvironment has made this illness one of the most difficult to treat. Cancer cells that have been isolated, even from the same place, vary greatly [5]. As time has gone on, researchers have suggested a variety of treatments for this fatal condition. Conventional medication, chemotherapy, radiation, surgery, targeted treatment, and immunotherapy are a few methods [6, 7].

Unfortunately, these obstructions come with certain adverse effects, including dermatological toxicities, vomiting, nausea, anaemia, lack of appetite, exhaustion, hypersensitivity, and neurotoxicity, which impair patient quality of life and reduce organ functioning [8,9]. Given these issues, there is a pressing need to research naturally occurring bioactive molecules to combat this lethal ailment, since natural toxins have specific therapeutic effects on a variety of diseases and are an important source for the development of contemporary drugs. A remarkable antimalarial and antiviral substance against multidrug-resistant malarial strains, HBV, HCV, and HCMV is artemisinin from the Artemisia annua plant [10,11]. The use of resveratrol, another naturally occurring phenolic chemical found in red grapes and berries, can help individuals with inflammatory bowel disease have a better prognosis [12]. The venom from insects like Apis dorsata, Nasonia vitripennis, and Bracon hebetor can be used as anti-inflammatory agents in mammalian cell lines, for example, and several natural compounds from insects have also been described as possible therapeutic agents to treat various medical conditions. model mice [13]. Cantharidin (CTD), which is isolated for use in medicine from male blister beetles of the meloid family (Mylabris phalerata, Mylabris cichorii), is one of these effective biotoxins [14,15].

About 40 years ago, in 1980, Chen et al. published the first study highlighting the anticarcinogenic effectiveness of this terpenoid toxin [16]. It was discovered that its anticancer action is mostly caused by the suppression of protein phosphatase 1 (PP1) and protein phosphatase type 2A (PP2A) after further study was undertaken to clarify its antitumor effects [17]. Additionally, it has been demonstrated that CTD can lower the levels of HSP70 and BAG3 protein production by blocking heat shock transcription factor 1 (HSF-1) from attaching to the protein's promoter [18,19].

considerable investigation revealed that it was discovered that CTD might inhibit breast, bladder, colon, pancreatic, and liver cancer. Additionally, reports of its antitumor activity against leukaemia and oral cancer have been made [20]. This study summarises current knowledge on CTD's antineoplastic activities in several cancer cell lines and tries to emphasise the molecular processes behind its anticarcinogenic potential.

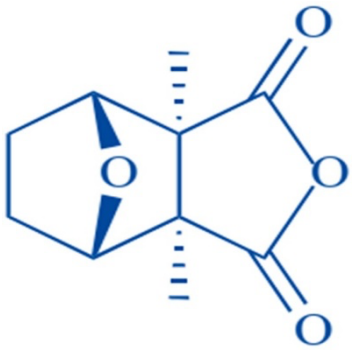
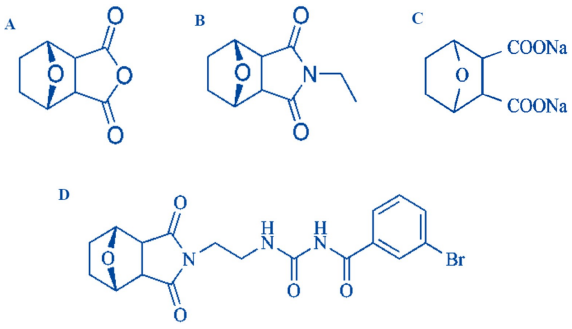


Figure 1:- Chemical Structure of Cantharidine (21)

Sources of CTD and its Derivatives

Meloid insects produce CTD as a defence mechanism against predators. Additionally, it is a key component of mating since male beetles create it as a sexual attractant, which is then given to female beetles during copulation [22,23]. Even though it is solely generated by male beetles, certain female species nonetheless have a 5-6% greater concentration [24]. With the exception of New Zealand and Antarctica, meloid species of insects are found everywhere across the planet [25]. Around 3000 species and 125 genera are found in this family [26], most of which are found in temperate and arid regions as well as subtropical and tropical savannas [27]. Although CTD is toxic by nature and has several toxic side effects, such as dysphagia, liver congestion, and renal toxicity, its anticancer potential cannot be denied. be discontinued since it has several anticancer effects on malignant cells, including the suppression of PP1 and PP2A, activation of apoptosis, and modification of protein synthesis [28]. The

delivery of CTD must be cancer-specific in order to prevent its deadly adverse effects. Furthermore, since it specifically modifies phosphatidylethanolamine (PE)-associated processes, ethanolamine (ETA) can act as a potent antidote to reverse CTD cytotoxicity [29]. To combat its toxicity, many of its compounds have also been created, some of which show remarkable anticancer properties [30]. Norcantharidin, norcantharimide, cantharidinamides, sodium cantharidate, anhydride-modified derivatives, and N-hydroxycantharidimide are a few significant derivatives of CTD (Figure 2) [31].



FIGURE;- (A) Norcantharidin, (B) norcantharimide, (C) sodium cantharidin, (D) cantharidinamides

Anticancer Attributes of CTD:-

Cancerous cell growth and proliferation are restrained:-

Chemotherapeutic chemicals known as cytotoxic medicines are frequently used to treat cancer. These prevent the development and division of cancer cells by inducing DNA damage [32]. The CTD's mechanism propelled Thomas E. et al. reported DNA damage in cancer cells in 2005. In a leukaemia cell line (CCRF-CEM), their research shown that CTD may cause both double-strand and single-strand DNA breaks [33]. Following that, numerous researchers looked at the cytotoxic effects of CTD on a variety of human cancer cell lines, including skin cancer A431 , A375.S2 [34], bladder cancer T24, RT4 [35], non-small cell lung cancer (NSCLC) NCI-H460 [36,37], A549 [38], H358 [39], colorectal cancer colo 205 [40], hepatocellular carcinoma HepG2, Hep3B, arrest the development and spread of certain human malignancies (Figure 3). One of the biggest obstacles to treating chronic myeloid leukaemia (CML) is imatinib resistance, which affects 20–30% of patients. By reducing BCR-ABL transcription, CTD can effectively stop the proliferation of CML K562 and CML resistant cells k562R [41]. Additionally, CTD can inhibit the growth of the triple-negative breast cancer cell lines MDA-MB-231 [42] and MDA-MB-468, which are the most challenging to treat because they are resistant to the standard treatments for the disease, such as endocrine therapies and HER-2 targeted therapy . Together, our findings show that CTD is a potent cytotoxic drug that may inhibit the development and proliferation of carcinoma cells in a dose- and time-dependent manner[43,44].

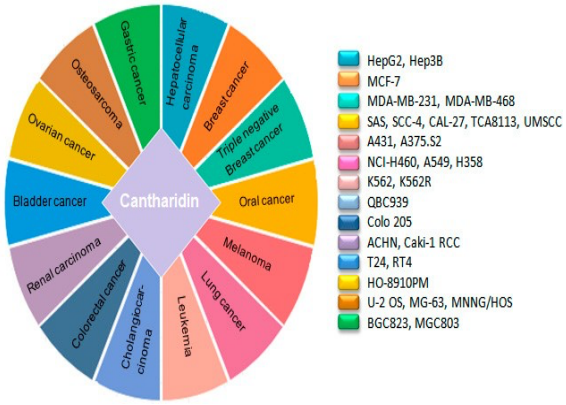


Figure 3. Anticancer profile of cantharidin in different cancer cell lines

Effect of CTD on Cancerous Cell DNA Damaging and Repair Associated Proteins

Since DNA damage may be repaired or bridged, DNA repair mechanisms are thought to be essential for the survival of both healthy and cancerous cells [45]. As a result, medications that stop DNA repair are seen as favourable for eliminating tumours [46]. Due to its ability to cause DNA fragmentation in lung H460, colon colo 205, and skin A431 cells, CTD has

been shown to disrupt genomic integrity in a variety of cancer cells [47,48,49]. CTD treatment of NCI-H460 cells changed the expression of genes related to DNA repair and damage because it decreased the levels of BRCA-1, ATM, 14-3-3, MGMT, DNA-PK, and MDC1 proteins. On the other hand, there was an increase in the expression levels and cytoplasm to nucleus translocation of phosphorylated p53, MDC1, and H2A.X [50]. . Another research found that CTD increased the levels of the DNA damage-related genes GADD45A (2.60-fold) and DNIT3 (2.26-fold) while decreasing the levels of DdiT4 (3.14-fold) genes [51]. By increasing DNA damage and decreasing DNA repair-related genes such UBE2T, RM1, RPA1, XRCC1, GTF2HH5, RAD51B, RAD50, RAD51B, PRKDC, LIG1, FANC1, DMC1, POLD3, and FAAP100 through JNK, ERK, p38, PKC, and NF-B pathways, CTD made pancreatic cancer cells more sensitive to radiation [52]. It has been discovered that CTD causes DNA damage in CML cells by increasing H2AX, a marker for DNA double-strand breaks [53]. Similar to how it caused DNA damage and condensation in osteosarcoma cells, it also increased the activity of PARP, p-ATR, p-ATM, and DNA-PK [54]. CTD was found to have inhibitory effects on promyeloid cells by Zhang et al. DNA polymerase delta, FANCG, ERcc2, hMSH6, and RuvB-like DNA helicase Tip49b were among the genes related with DNA replication and repair that were downregulated in HL-60 cells after CTD treatment [55]. As bladder cancer cells formed a DNA comet tail and DNA condensation in response to CTD treatment, the DNA-damaging reaction of CTD was also observed in these cells. By lowering the levels of PARP, BRCA-1, DNA-PK, MDC1, MGMT, ATR, and phosphohistone H2A.X and boosting the accumulation of p-p53, this effect was achieved . Additionally, the cDNA microarray demonstrated a 4.75-fold increase in DDIT3 gene expression [56]. According to the findings summarised in Table 1 (Figure 4), CTD is a potential drug for causing DNA damage and inhibiting its repair process in tumour cells.

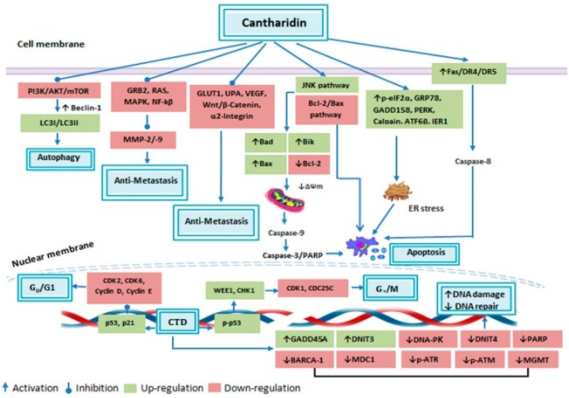


Figure 4. Anticancer attributes of cantharidin and its molecular targets.

Cancerous Cell Cycle Arrest Induced

Targeting expression of cell cycle regulatory genes to arrest cells cycle—a state where the cell is no longer able to duplicate and divide [57]—is considered an alluring approach to stop unchecked growth of the cancerous cells [58]. This is because abnormal proliferation of malignant cells leads to divergent activity of several cell cycle regulatory genes. The cell cycle is a tightly regulated process in higher organisms, governed by a number of processes. In order to guarantee proper cell cycle progression within a cell, a collection of connate proteins called cyclins, cyclin-dependent kinases (CDKs), and CDKs inhibitors (CDKi) work together [59]. Examples of negative cell cycle regulators are p21 and p53 [60]. By upregulating p21, CTD stopped bladder cancer cells at the G0/G1 phase reducing the amount of p53 gene translation, as well as through suppressing Cyclin E and CDC25C [61]. It was discovered that 43.31% of control cells and 52.14% of cells treated with CTD were in the G0/G1 phase, respectively [61]. Mitotic arrest was caused by CTD treatment of CML cells K562 and imatinib-resistant cells K562R, and was mediated by activation of the cyclin B1/Cdc2 complex and downregulation of cyclin D1. After 24 hours of treatment, 10.8-13% of K562R cells and 19.2-24.5% of K562 cells (control

1.6% each) were arrested in the mitotic phase, respectively [62]. By halting cells during the G0/G1 phase, CTD dramatically inhibited the proliferation of skin cancer cells [63]. It did this by increasing the expression of p21 and decreasing that of cyclin D, cyclin E, and CDK6. CTD stopped CDK1 activity, which led to the arrest of colon cancer cells in the G2/M phase [64]. However, to halt pancreatic cells in the G2/M phase, it vitalized the APC complex by inhibiting PP2A and downregulating CDK1 [65]. Additionally, CTD caused G2/M phase arrest in osteosarcoma cells by upregulating the expression of CHK1, phosphorylated p53, and WEE1, while downregulating the expression of CDC25C and CDK1 [66]. Additionally, it was able to initiate the G2/M phase in the CD133+ stem cells from hepatocellular carcinoma [67], the MDA-MB-231 breast cancer cells [68], the HCT-116 colorectal cancer cells, and the ACHN and Caki-1 RCC renal cancer cells [69]. As a result, it is likely that CTD can stop the malignant cell cycle by controlling the expression levels of several cell cycle-associated proteins, as shown in Table 1 (Figure 4).

Cancer Cell Metastasis Inhibition

Around 90% of cancer patients are deadly due to neoplastic metastasis, which is a hallmark of an advanced stage of malignancy [70]. The invasion and migration of tumour cells to nearby tissues or organs is recognised to be a multiphase phenomenon called metastasis [71]. The successful treatment of cancer patients is thought to be seriously hampered by cancer metastasis . As a result, the development of anti-metastasis drugs is receiving increased attention [72]. The extracellular matrix (ECM) is degraded by cancer cells, allowing them to penetrate healthy tissues. MMPs (matrix metalloproteinases) are essential for the breakdown of the ECM . There are already more than 20 MMPs known, and MMP-2 and MMP-9 are regarded as intrinsic for cancer metastasis [73]. Through the expression of several metastasis-associated proteins being downregulated, CTD dramatically prevents the ability of various cancer cells to metastasize. By downregulating CCAT1, CTD prevented migration and invasion of gastric cancer cells via the PI3 K/AKT signalling pathway [74]. By changing the p38 and JNK1/2 MAPK signalling pathway to down-regulate MMP-2 and MMP-9 mRNA, protein level, and enzymatic activity, CTD concentration-dependently stopped the adhesion, migration, and invasion of bladder cancer cells TSGH-8301 [75]. By inhibiting the MAPK signalling pathway in NCI-H460 cells by lowering NF-kB p56 and AKT, CTD prevented migration, invasion, and adhesion as well as the enzymatic activity of MMP-2 and MMP-9 [65]. The anti-metastatic impact of CTD was different in the lung cancer cell line A549 since it only prevented the MMP-2, but not MMP-9, had a gelatinous effectiveness while neither MMP-2 nor MMP-9's expression level had altered. Not the MAPK signalling route, but the PI3 K/AKT signalling pathway was shown to be responsible for this impact [76]. Similar to another work, CTD therapy prevented the migration of A549 cells by suppressing the PIK3/Akt/mTOR pathway [77].

Conclusion

The anticancer properties of CTD in several cancer cell lines are exemplified in this review paper. Given the evidence provided above, it is evident that CTD is a highly effective anticancer substance that inhibits PP2A and HSF-1. Cancerous cells' growth, proliferation, and migration may be inhibited by CTD. Additionally, it might cause apoptosis, cell cycle arrest, and autophagy, as well as lessen the activity of several proteins linked to DNA damage and repair in cancerous cells. However, it is necessary to explain how CTD affects the differentiating of malignant cells. Cancer must be treated with a multi-target medicine since it is understood to be a

multifaceted illness that arises from several abnormalities. A number of cell signalling pathways can be impacted by CTD, although MAPK, Bcl2/Bax, JNK, NF-B, ERK, PKC, -catenin, Wnt/-catenin, PI3/AKT, and PIk3/ATK/mTOR are the most often affected. identified possible molecular targets for it. The anticancer potential of CTD has also been demonstrated in mouse xenograft models, although more research in various cancer models is required. Furthermore, it was discovered to be antiangiogenic in the breast cancer mouse model, but it boosted neoplastic cell development as a result of higher angiogenesis in the lung, pancreatic, and colorectal cancer model. Therefore, it is necessary to determine if it promotes or inhibits angiogenesis in other cancer cells. As CTD can significantly lessen chemotherapy side effects and makes cells more sensitive to radiation, it has also been demonstrated to be helpful when combined with chemotherapy and radiotherapy. However, its effectiveness in clinical patients has to be more thoroughly examined in a large sample size. Furthermore, there is less information accessible on when combined with other natural substances, its synergistic anticancer properties.

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