Potential Of Mangosteen Xanthones As Anti-Oral Cancer Agents By Induction Of Apoptosis

Potensi *Xanthones* Kulit Buah Manggis Sebagai Anti Kanker Rongga Mulut Melalui Mekanisme Apoptosis

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Abstract

The pericarp of mangosteen (Garcinia mangostana L.) is rich in various xanthones that are known to possess unique biological activities. The aim of this study was to determine of anti-proliferative and cytotoxic activities of mangosteen xanthones. The researchers found that all tested xanthones inhibited the growth of the cancer cells. They observed cytotoxic properties against three human cancer cell lines, epidermoid carcinoma of the mouth, breast cancer, and small-cell lung cancer. The specific xanthones include alpha-mangostins, showed that mangosteen xanthones not only inhibit the proliferation of target cells but also induce their death by apoptosis that involves the activation of the caspase cascade. To establish a more precise mechanism of action, a cell free biochemical kinase assay against multiple cyclins/CDKs involved in cell cycle progression; the most significant inhibition in the cell free based assays was CDK4, a critical component of the G1 phase.Through molecular modeling α-mangostin against the ATP binding pocket of CDK4, and propose three possible orientations that may result in CDK4 inhibition. Histopathological evaluation and biochemical analysis of tumors that received mangosteen xanthones indicate the induction of apoptosis in tumors, which resulted in the repression of their growth and the reduction of their sizes. The study concluded that mangosteen xanthones potentially as an agent for cancer prevention and the combination therapy with anti-cancer drugs.

Keywords: xanthones, mangosteen, anticancer, apoptosis

Abstrak

Kulit buah manggis (Garcinia mangostana L.) diketahui banyak mengandung senyawa xanthones yang mempunyai beragam aktivitas biologi yang bermanfaat. Tujuan dari penulisan ini adalah mengulas tentang aktivitas sitotoksik dan anti-proliferatif xanthones mangosteen, terutama dalam menghambat pertumbuhan sel cancer. Beberapa peneliti mengobservasi sitotoksisitas human cancer cell lines, yaitu: epidermoid carcinoma rongga mulut, kanker payudara dan kanker paru. Hasilnya menunjukkan bahwa dalam alphamangostins tidak hanya menghambat proliferasi sel target namun juga menginduksi kematian sel melalui apoptosis, yang melibatkan aktivasi caspase cascade. Untuk mengetahui meknisme kerjanya, metode a cell free biochemical kinase assay terhadap multiple cyclins/CDKs yang terlibat dalam progresi siklus sel. Proses inhibisi yang paling signifikan dalam cell free based assays adalah CDK4, dimana merupakan komponen penting (critical component) pada fase G1. Melalui mekanisme molekular α-mangostin pada ATP yang berikatan dengan pocket CDK4, akan menghasilkan 3 kemungkinan orientasi yang akan menyebabkan inhibisi CDK4. Pada evaluasi histopathological dan analisis biokimia dari jaringan tumor diketahui bahwa xanthone mngosteen dapat

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menginduksi apoptosis pada tumor, dengan adanya penekanan pertumbuhan dan penurunan ukuran dari jaringan tumor. Dari hasil review dapat disimpulkan bahwa xanthone mangosteen berpotensi sebagai agen pencegahan terhadap kanker dan sebagai terapi kombinasi bersamaan dengan obat-obatan anti kanker.

Kata kunci: xanthones, kulit buah manggis, anti-kanker, apoptosis

Introduction

Oral cancer is one of the 10 most frequently occurring cancers world-wide. The 5-year survival rate of less than 50% has not substantially improved over the past several decades, since many oral carcinomas respond poorly to chemotherapy approaches and their responses to radiation therapy have been highly variable. [1,2]

Oral squamous cell carcinoma is a type of cancer, that usually develops on the squamous or epithelial cells, that cover the lips and the oral cavity. The malignant or cancerous cells are usually found on the floor of the mouth or on the surface of the tongue. These cancerous cells also originate on the lower lips and palate or the tonsillar area of the oral cavity. The squamous cell carcinoma is believed to develop from the keratinizing or malpighian epithelial cells, as the presence of keratin has been observed in the malignant cells. It is one of the most prevalent types of oral and pharyngeal cancers. [3]

The pericarp of mangosteen (Garcinia mangostana L.) is rich in various xanthones that are known to possess unique biological activities. The researchers found that all tested xanthones inhibited the growth of the cancer cells. They observed cytotoxic properties against three human cancer cell lines, epidermoid carcinoma of the mouth, breast cancer, and small-cell lung cancer. The specific xanthones include alphama ngostins, showed that mangosteen xanthones not only inhibit the proliferation of target cells but also induce their death by apoptosis that involves the activation of the caspase cascade.[4]

Some research for anti cancer agents from plant sources, all the polyphenols and terpenoids tested which exhibited an anti-proliferative effect, were observed to induce apoptosis by targeting mitochondria with a decreased membrane potential, leading to the activation of the intrinsic apoptotic signal transduction. In some cases, the early responsive signaling cascades including protein kinases MAPK and Akt referring to growth and survival, respectively, were down regulated. reports indicated a potent anti-proliferative activity of 4 xanthones (α-mangostin, β-mangostin, γ-mangostin, and methoxy β-mangostin) from the pericarps of mangosteen against human leukemia HL60 cells. Interestingly, α-mangostin was observed to induce mitochondrial dysfunction. Moreover, it induced cell-cycle arrest and apop-tosis in human colon cancer DLD-1 cells.[5] In this review, we discuss the mecha nism of anti-cancer effect of xanthones and the potentiality of chemopreventive agents for oral cancer.

Mangosteen Xanthones

The mangosteen (Garcinia Mangostana) is a tropical fruit considered to be one of the finest tasting fruits in the world and has earned the popular title "the Queen of Fruit." The mangosteen tree is found predominantly in Southeast Asia in countries like Cambodia, China, Indonesia, Malaysia, Singapore, Taiwan and Thailand.

Xanthones are a class of plant derived nutrients or "phytonutrients." They have been demonstrated in numerous scientific studies to hold tremendous nutritional value. Found to exhibit strong antioxidant activity xanthones disarm free radicals in the body and enhance and support your body's immune system. Although xanthones exist in small amounts throughout nature, it is found in concentrated amounts in the Pericarp of the mangosteen fruit. [5]

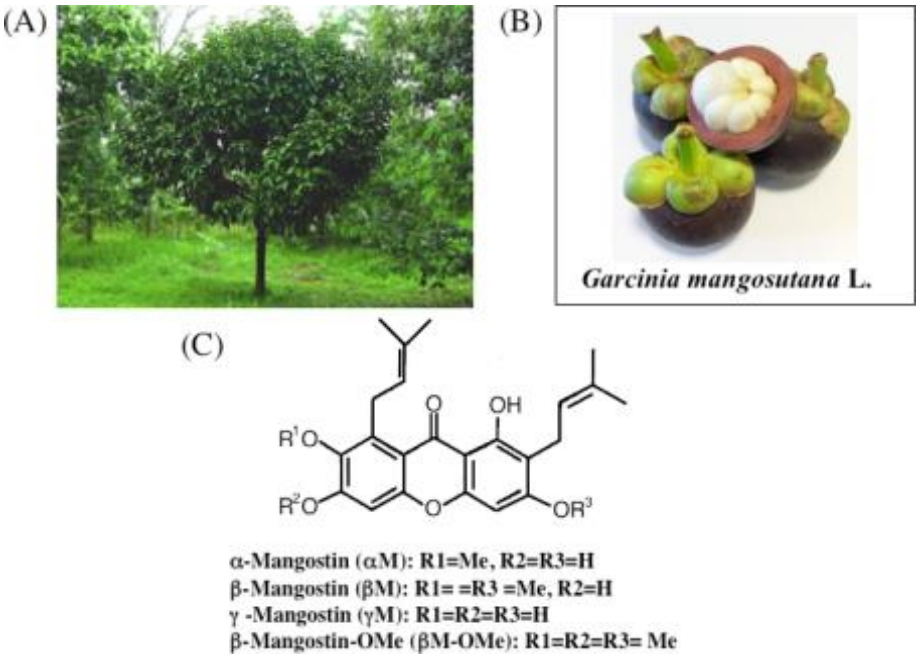

Figure 1. The *Garcinia mangostana* Linn tree (A), the appearance of mangosteen fruit (B) and the chemical structures of xanthones included in the pericarps (C)

There are over 20 known forms of xanthones naturally occurring in the Pericarp, the two most widely studied are Alpha Mangostin and Gamma Mangostin. Accor ding to the research, the most concentrated source of xanthones found in nature is in the Pericarp (rind) of the mangosteen fruit. The majority of clinical studies on xanthones specify that the xanthones used were from the mangosteen Pericarp. Phytochemical studies have shown that they contain a variety of secondary metabolites, such as oxygenated and prenylated xanthones. Recent studies revealed that these xanthones exhibited a variety of biological activities containing anti-inflammatory, anti-bacterial, and anti-cancer effects.[7]

Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma is a type of cancer, that usually develops on the squamous or epithelial cells, that cover the lips and the oral cavity. The malignant or cancerous cells are usually found on the floor of the mouth or on the surface of the tongue. These cancerous cells also originate on the lower lips and palate or the tonsillar area of the oral cavity. The squamous cell carcinoma is believed to develop from the keratinizing or malpighian epithelial cells, as the presence of keratin has been observed in the malignant cells. It is one of the most prevalent types of oral and pharyngeal cancers.

The most important risk factors of oral squamous cell carcinoma are tobacco smoking and alcohol consumption. Many studies have shown that smoking and excessive intake of alcohol are the leading causes of this disease. More than 90% of the people affected by this disease have been found to smoke and/or drink alcohol. In addition, squamous cell carcinoma of the tongue may be caused by chronic dental caries, chewing tobacco and betel quid. Besides this, oral human papilloma virus (HPV) can also be responsible for causing oral squamous cell carcinoma.

The common symptom of oral squamous cell carcinoma is the appearance of scaly or ulcerated plaque or lesions in the oral cavity. Sometimes, a red patch of lesions, known as erythroplakia, can be observed. Appearance of leukoplakia, a patch of white tissue on the mucous membrane of the mouth is also very common. Besides these, a sore on the lips or gums, a lump on the lips or the gums, a white or red patch on the gum, tongue and tonsils

and swelling of the jaw are some other symptoms of this cancer. A sore throat can also be a symptom of squamous cell carcinoma of the tonsils.

Oral lesions or ulcers are usually detected during a physical examination of the lips and oral cavity. But all these lesions and plaque are not necessarily malignant or cancerous, which necessitates further tests, to ensure a proper diagnosis of the disease. One of the most important tests, is a biopsy of the affected area. In addition to this, laryngoscopy, bronchoscopy and esophagoscopy are also carried out to detect and exclude cancers of the larynx, bronchial tubes and esophagus. In addition to these, chest X-ray and CT scan of the head, chest and neck are performed to properly diagnose the stages of the disease.

The commonly used treatment options for oral squamous cell carcinoma are surgery and radiation therapy. Surgery is usually carried out in the early stage of the disease. Sometimes, chemotherapy is used, particularly if the disease spreads to other areas like the lungs, bones, pericardium and heart. Squamous cell carcinoma of the lips and tongue are treated by surgically removing the affected area. However, surgical reconstruction of lips is required to enable individuals to carry out normal oral activities.

Oral squamous cell carcinoma can significantly increase the risk of both head and neck cancer, accounting for almost 90% of all head and neck cancers. Almost 30,000 people are affected each year by this disease in the United States. As it has been observed that this cancer largely affects those individuals who indulge in excessive smoking and alcohol consumption, so controlling these risk factors can play a significant role in preventing the occurrence and reducing the severity of the disease[3].

Mechanism apoptosis of squamous cell carcinoma

The apoptotic potential of cancer cells in correlation to their proliferative dynamics profoundly affects malignant phenotypes, and it appears that pathways governing cell proliferation and cell death are interconnected. Failure to enter apoptosis allows transformed cells to enter further cell divisions and acquire further mutations. In the present review, we focus on genetic alterations of caspases and their regulators, underlining the role of these molecules in cancer development.

Deregulation of caspase expression and/or activity could be a result of various factors, including genetic alterations, promoter methylation, alternative splicing and post-translational modifications. We show examples that different mutation could have profound effects on caspases activity. Caspase-3 mutation was investigated in squamous cell carcinoma of the head and neck (SCCHN).[8,9,10]

The majority of currently available anticancer drugs act at least in part through induction of apoptosis, therefore, a defect in the apoptotic propensity of tumours affects their response to treatment. As described above, a number of anticancer therapies are being tested that influence the expression and/or activity of factors that regulate apoptosis. Targeting caspases and apoptotic machinery will play an increasingly important role in future modern cancer therapy, and approaches are being developed that allow "on demand" activation of expression. This will be achieved using siRNA technology, the small molecule inhibitors, as well as peptides and peptidomimetics. These approaches may eventually replace the traditional chemo and radiation therapies, and result in more efficient cancer treatments that are devoid of side effects.[11]

Mechanism of α-Mangostin-inducing Apoptosis

In our previous study, it was demonstrated that α-mangostin activated caspase-9 and -3 but not -8 in HL60 cells, indicating that α-mangostin may mediate the mitochondrial pathway in the apoptotic process. Parameters of mitochondrial dysfunctions such as swelling, loss of membrane potential, decrease in

intracellular ATP, ROS accumulation, and cytochrome c/AIF release, Matsumoto was observed within 1 or 2 h after the treatment, indicating that α-mangostin preferentially targets mitochondria in the early phase. Interestingly, replacement of hydroxyl group by methoxy group remarkably decreased the potency to cause mitochondrial dysfunction. It was also shown that the cytotoxicity was correlated with the decrease in the mitochondrial membrane potential. Furthermore, we demonstrated that α-mangostin induced a cell cycle arrest at G1/S and the subsequent apoptosis via the intrinsic pathway in DLD-1 cells, while a cell cycle arrest by γ-mangostin was at S phase. The changes in expression of cell cycle regulatory proteins were shown in. α-Mangostin induced apoptosis was mediated by a caspase independent pathway via mitochondria with the release of Endo-G, a known 30-kD nuclease residing in mitochondria, is able to induce nucleosomal DNA fragmentation. [12,13]

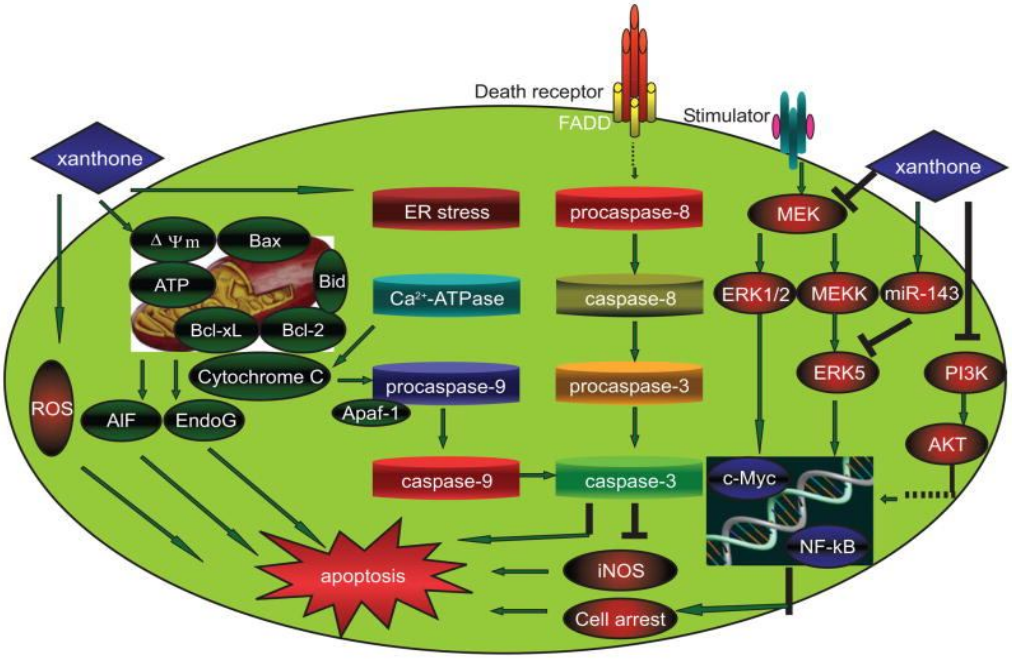

Figure 2. Schematic diagram shows the possible effect of xanthones on the apoptosis pathways. Xanthones induce apoptosis occurrence, preferentially activate the mitochondrial pathway, support intracellular ATP decrease, cytochrome c/AIF release, caspase-9 and caspase-3 activation, endonuclease-G release. Furthermore, xanthones also influence cancer cells apoptosis via miR-143/ERK5/c-Myc pathway, NO inhibition, cell-cycle arrest, sarcoendoplasmic reticulum Ca2+-ATPase inhibition, and intracellular ROS accumulation.14

Mitogen activated protein kinases (MAPKs) and Akt kinase are key regulatory proteins in cells. MAPKs are a widely conserved family of serine/threonine protein kinases involved in many cellular processes such as cell proliferation, differentiation, motility, and death. Akt, another serine / threonine protein kinase, is associated with cell survival, growth, and glycogen metabolism. Various phytochemicals have been shown to modulate the signaling pathways of MAPKs and/or Akt, leading to growth inhibition and cell death.

Erk1/2 may play a dual role, acting first as a cellular adaptive response at the initial phase and then as a cytotoxic response at the later stage. As reported, the decline in p-Erk1/2 after the later peak may be associated with the apoptotic machinery. On the other

hand, in the Akt signaling the level of p-Akt was markedly reduced at 6 h following α-mangostin treatment, coincident with the occurrence of apoptosis. Therefore, down-regulation of Akt signaling could participate in the mechanism of apoptosis induced by α-mangostin. [15]

The cell cycle is normally regulated by a number of proteins, including p53, p21waf, the cyclin-dependent kinases (cdks) and their activators, the cyclins. The dysregu lation of cell cycle machinery and checkpoint signaling pathways is a hallmark of malig nant cells. Thus, modulation of cell cycle progression is one of the major strategies for both chemoprevention and chemotherapy.

Treatment of mangosteen results in a direct inhibition of the proliferation and viability of various cancer cell types *in vitro*, as manifested by the significant arrest of cells at various phases of the cell cycle. by the inactivation of the signaling cascades involving Erk1/2 and Akt at 3 h-treatment. The cell cycle regulatory proteins cyclin D1 and cdc2 were also down-regulated at 3 h treatment. [14,15]

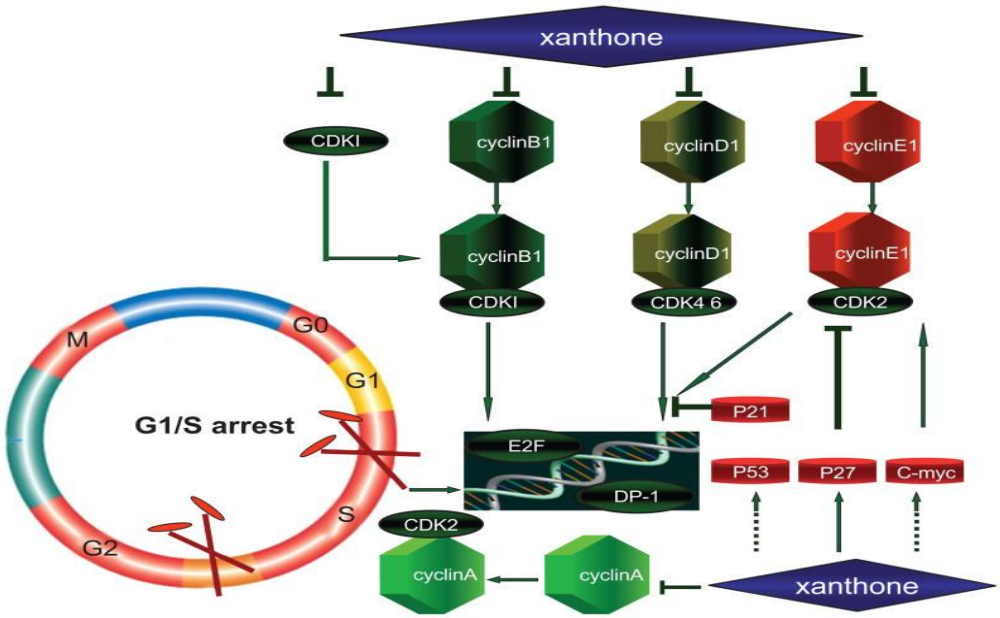

Figure 3: An overview: how xanthones induce cell-cycle arrest. Xanthones block the cell cycle by activation or inhibition of cyclins, cdks, inhibitor of cdks, transcription factors or oncoproteins in cancer cells. [14]

Discussion

Apoptotic cell death is controlled by proapoptotic caspases, proteases that are synthesized as inactive precursors and activated by proteolytic processing . The apoptotic cascade can be initiated *via* two major pathways, involving either the release of cytochrome C from the mitochondria (mitochondrial pathway) or activation of death receptors in response to ligand binding (death receptor pathway). Upon triggering of either pathway, caspases, the final executioners of apoptosis, are activated, causing degradation of cellular proteins and leading to typical morphological changes such as chromatin conden sation, nuclear shrinkage, and the formation of apoptotic bodies. Both pathways are differentially involved in the cellular response to diverse apoptotic stimuli. The majority of chemotherapeutic agents trigger the

mitochondrial pathway, but the death receptors have also been reported to be involved in chemotherapy-induced apoptosis. [11]

Death ligands such as TNF-α or CD95L recruit, *via* the adapter molecule FADD, cytoplasmic mono-meric initiator caspase-8 to their sur-face receptors, resulting in dimeri-zation and activation of caspase-8. Active caspase-8 cleaves and activates downstream effector caspases including caspase-3, -6 or -7, which degrade a broad range of cellular proteins and trigger the appearance of the apoptotic morphology . On the other hand, mitochondria are important regulatory sites of the apoptotic process. Defects in mitochondrial function result in release of cytochrome C, which can associate with Apaf-1 (apoptosis protease activating factor) and procaspase-9. The observation that chemical inhibition of caspase-9 blocks hypoxiainduced apoptosis points to a role of the complex in hypoxia-induced apoptosis. This activation complex results in autoprocessing of caspase-9 and further activation of down-stream caspases, such as caspase-3. Activation of caspase-3 has been linked to the proteolytic cleavage of cellular substrates including poly-ADP-ribose-polymerase (PARP), and is also necessary for the nuclear changes and chromatin condensation associated with apoptosis. [12]

The anti-proliferative effects of the xanthones were associated with cell-cycle arrest by affecting the expression of cyclins, cdc2, and p27; G1 arrest by α-mangostin and β-Mangostin, and S arrest by γ-mangostin. α-Mangostin found to induce apoptosis through the activation of intrinsic pathway following the downregulation of signaling cascades involving MAP kinases and the serine/threonine kinase Akt.

Conclusions

New strategies for cancer treatment are being developed, and one of the most promising treatment strategies involves the application of chemopreventive agents. The search for novel and effective cancer chemopreventive agents has led to the identification of various naturally occurring compounds. The potential chemopreventive and chemotherapeutic activities of xanthones have been demonstrated in different stages of carcinogenesis (initiation, promotion, and progression) and are known to control cell division and growth, apoptosis, inflammation, and metastasis. Carcinogenesis prevention is considered to be a promising alternative strategy for the treatment of cancer. Based on this information, this review presents compelling evidence for the use of mangosteen not only to prevent but also to treat cancer due to the similar molecular targets that affect tumor initiation, promotion, and progression. Taken together, these results support that mangosteen can modulate various molecular pathways involved in multiple processes of carcinogenesis including the inactivation of carcinogens, the induction of apoptosis, the initiation of cell cycle arrest, and the suppression of metastasis. Although there is compelling evidence to suggest that xanthones from mangosteen may be a remarkable candidate for chemopreventive and chemotherapeutic strategies due to its efficacy and pharmacological safety, further research must be conducted before the compounds can be employed as an agent for the chemoprevention/treatment of cancer.

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