Adlay (薏苡 yì yǐ; "soft-shelled job's tears"; the seeds of Coix lachryma-jobi L. var. ma-yuen Stapf) is a Potential Cancer Chemopreventive Agent toward Multistage Carcinogenesis Processes

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ABSTRACT

Adlay (薏苡 yì yǐ; "soft-shelled job’s tears", the seeds of Coix lachryma-jobi L. var. ma-yuen Stapf) is a grass crop that has long been used in traditional Chinese medicine (TCM) and as a nourishing food in China for the treatment of warts, chapped skin, rheumatism, neuralgia, inflammatory, and neoplastic diseases. In addition, adlay also has been said to have stomachic, diuretic, antiphlogistic, anodynic, and antispasmodic effects. Carcinogenesis is a multistage process that begins with exposure of viruses or chemicals that are found in the environment. Chemoprevention refers to the use of natural or synthetic, non-toxic chemical substances to reverse, repress, or prevent carcinogenesis. In this review, we summarize recent research attempting to study the chemopreventive blocking and suppressing potential of adlay and its active components in scavenging electrophiles and reactive oxygen species, antimutagenicity, enhancing Nrf2-mediated detoxification and antioxidant effect, altering carcinogen metabolism, suppressing proliferation, decreasing inflammation, and enhancing antitumor immunity. In addition, several active components with diverse chemopreventive properties have been also mentioned in this review article.

Keywords: Adlay, Traditional Chinese medicine, Cancer chemoprevention, Blocking agent, Suppressing agent

Adlay (Coix lachryma-jobi L. var. ma-yuen Stapf.)

Adlay (薏苡 yì yǐ; the seeds of Coix lachryma-jobi L. var. ma-yuen Stapf.), also named coix seeds, Chinese pearl barley, pearl barley, semen coicus, yokuinin, 薏苡仁 (yì yǐ rén), and 薏米 (yì mǐ), belongs to the family Gramineae. It is an annual or perennial herb, 100 ~ 180 cm high, flowering from July to September and fruiting from September to October. The adlay seed consists of four parts from outside to inside including the hull, testa, bran, and endosperm (polished adlay) (Figure 1). Adlay is widely planted in Taiwan, China, and Japan, where it is considered a healthy food supplement.

According to the first pharmaceutical monograph in ancient China, The Divine Husbandman’s Herbal Foundation Canon (神農本草經 shén nóng běn cǎo jīng), adlay is considered as a top grade (上品 shàng pǐn) in traditional Chinese medicine.
pǐn) of Traditional Chinese Medicine (TCM). People received adlay for a long period can nourish life (養命 yǎng mìng), boost qi (益氣 yì qì), rejuvenate the body (輕身 qīng shēn), and extend life (增年 zēng nái). Since adlay can make body healthy and prolong life with nontoxic properties, it has been thought to be the longevity of monarch herb (不老長生之君藥), and generally form the basis of dietary functional foods. Adlay traditionally has long been used in China for the treatment of warts, chapped skin, rheumatism, neuralgia, and inflammatory diseases. In addition, adlay also has been said to have stomachic, diuretic, antiphlogistic, anodynic, antispasmodic, and antitumor effects.

Although medicinal functionality of adlay has been around for thousands of years, recently, it is interesting to note that integration exists between traditional medicine and modern medicine because a number of biological activities of adlay have been elucidated by scientific investigation, including antioxidant/free radical scavenging (Kuo et al., 2001; Kuo et al., 2002; Yao et al., 2011; Yu et al., 2011; Chen et al., 2012a; Wang et al., 2012), anti-inflammatory (Otsuka et al., 1988; Huang et al., 2009a; Huang et al., 2009b), anti-mutagenic (Huang and Chiang, 1999; Chen et al., 2011), anti-tumor (Tanimura, 1961; Numata et al., 1994; Kuo et al., 2001; Chang et al., 2003; Hung and Chang, 2003; Shih et al., 2004; Lee et al., 2008; Chung et al., 2010; Chung et al., 2011b; Li et al., 2011), anti-allergic (Hsu et al., 2003; Chen et al., 2010; Chen et al., 2012b; Chen et al., 2012c), hypolipidemic (Kim et al., 2004; Yu et al., 2004; Huang et al., 2005; Yeh et al., 2006; Son et al., 2008; Yu et al., 2011), hypocholesterolemic (Wang et al., 2012), hypoglycemic (Takahashi et al., 1986; Huang et al., 2005; Yeh et al., 2006; Lin et al., 2010), anti-obesity (Kim et al., 2004; Huang et al., 2005; Kim et al., 2007), anti-ulcer (Chung et al., 2011a), prebiotic activity (Chiang et al., 2000), abortifacient (Tzeng et al., 2005), hormonal modulating (Kondo et al., 1988; Chang et al., 2006; Hsia et al., 2006; Hsia et al., 2007; Hsia et al., 2008; Hsia et al., 2009), osteoporosis preventing (Yang et al., 2008), and antimicrobial (Ishiguro et al., 1993) effect. Since cancer mortality and incidence worldwide has increased rapidly in recent decades, thus, this review article focused on chemopreventive role of adlay in blocking carcinogenesis progress, preventing cancer formation and progression.

**Multidrug Carcinogenesis**

Carcinogenesis is a multistage process that begins with exposure of viruses or chemicals that are found in the environment. This process involves various molecular and cellular events that transform a normal cell to a malignant neoplastic cell. It can be conceptually identified as tumor initiation, tumor promotion, and tumor progression (Figure 2) (Panayiotidis, 2012).
Tumor initiation is a step where the normal cell undergoes irreversible genetic and epigenetic alterations to generate an initiated cell (Lengauer et al., 1998). Oxidative stress plays a major role in the initiation of carcinogenesis (Khansari et al., 2009). Oxidative stress can be induced through a number of endogenous sources, such as the production of free radical by mitochondrial oxidative phosphorylation, and exogenous sources, including ionizing radiations, ultraviolet exposure and environmental carcinogens. In fact, chemical carcinogens cannot damage DNA until they are metabolized by cytochrome P-450 in cells and converted to reactive electrophiles. Reactive oxygen species (ROS) can react with DNA as well as chromatin proteins, resulting in several types of DNA damage, including depurination, depyrimidination, single- and double-strand breaks, base modifications, and DNA-protein cross linkages (Barzilai and Yamamoto, 2004; Fruehauf and Meyskens, 2007). In addition, carcinogen-DNA adduct formation is an important molecular mechanism that contributes to chemical carcinogenesis (Windmill et al., 1997). ROS-mediated DNA damage contributes to the initiation of carcinogenesis through increasing a cell’s mutation rate, influencing the activation of protooncogene and tumor-suppressor gene, and promoting the oncogenic phenotype (Campos et al., 2007). Tumor promotion involves the clonal expansion of initiated cells to form an actively premalignant, proliferating lesion. Direct DNA interaction and damage is not involved in this process. Tumor promoters generally alter gene expression resulting in decrease of apoptosis cell death and increase of cell proliferation (Klaunig and Kamendulis, 2004). Tumor progression involved in the expression of the malignant, aggressive, invasive, and metastatic phenotype. During this process, further genetic and epigenetic alterations may occur resulting in the formation of neoplasms.

**Cancer Chemoprevention**

The prominent and effective strategy to inhibit cancer formation is elimination, reverse or delay of genetic and epigenetic alterations induced by chemical carcinogens (Hail et al., 2008). Chemoprevention refers to the use of natural or synthetic, non-toxic chemical substances to reverse, repress, or prevent carcinogenesis. These chemopreventive agents can be broadly categorized into two types: blocking agents and suppressing agents (Figure 2) (Wattenberg, 1985; Manson et al., 2000). Blocking agents generally inhibit the initiation of carcinogenesis. Blocking agents...
alter carcinogen metabolism and enhance carcinogen detoxification, resulting in elimination of carcinogens reaching their target site to induce DNA damage (Surh, 2003). In addition, blocking agents effectively scavenge electrophiles and ROS to prevent DNA alterations. Furthermore, blocking agents induce DNA repair machinery to reduce DNA damage as well as genetic mutations. Suppressing agents inhibit pre-malignant or malignant conversion of initiated cells during the stages of tumor promotion and progression. They efficiently modulate gene expression, including oncogenes and tumor-suppressor genes, resulting in suppressing cell proliferation, encouraging apoptosis, and inhibiting angiogenesis (Hayes and McMahon, 2001).

**Adlay Acts as a Blocking Agent for Cancer Chemoprevention**

Scavenge Electrophiles and Reactive Oxygen Species

Reactive oxygen species (ROS) are induced through a variety of endogenous and exogenous sources. Overwhelming of antioxidant and DNA repair mechanisms in a cell by ROS may result in oxidative stress and oxidative damage to a cell. Oxidative damage resulting from ROS generation can participate in all stages of the carcinogenesis process (Klaunig et al., 2011). Therefore, elimination of ROS or electrophiles by chemopreventive blocking agents is an important strategy to prevent the malignant development. Kuo et al. have demonstrated that the methanol extract of adlay hull (AHM) is a highly effective ROS scavenger that inhibits free radical-generating enzymes, blocks tumor promoter-generated oxidative processes in neutrophile-like leukocytes, and exhibits a cytoprotective effect on cultured cells exposed to tert-butyl hydroperoxide (Kuo et al., 2001). Furthermore, active component that represent strong antioxidant activity were isolated from the methanol extract of adlay hull to be coniferyl alcohol, syringic acid, ferulic acid, syringaresinol, 4-ketopinoresinol, and mayuenolide (Kuo et al., 2002).

Antimutagenicity

It is well-documented that cancer begins after a mutational episode in a single cell and then it progressively transforms to malignancy in multiple stages through sequential acquisition of additional mutations (Khan and Pelengaris, 2006). The Ames Salmonella typhimurium mutagenicity assay (Ames test) is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage leading to gene mutations (Mortelmans and Zeiger, 2000). Several lines of evidence showed that the compound prevents mutagenesis caused by known carcinogens in the Ames test indicates a possible role in chemoprevention (Weisburger, 2001). Notably, Huang and Chiang previously investigated the antimutagenic activity of different portions of adlay, including adlay hull, adlay testa, adlay bran, dehulled adlay, and polished adlay. Among them, the acetone extract of adlay hull and adlay bran possesses most potent antimutagenic activity against Benzo(a)pyrene (B[a]P), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 4-nitroquinoline -N-oxide (NQNO) induced mutagenesis in Salmonella typhimurium TA98 (Huang and Chiang, 1999). Recently, Chen et al. further identified the antimutagenic constituents from adlay hull by used Ames antimutagenic activity-guide isolation procedures, and found that p-hydroxybenzaldehyde, vanillin, syringaldehyde, trans-coniferylaldehyde, sinapaldehyde, and coixol exert great antimutagenic activity in suppressing IQ-induced mutagenecity in Salmonella typhimurium TA98 (Chen et al., 2011). In addition to antimutagenic activity, trans-coniferylaldehyde and sinapaldehyde also exhibit potent effect in scavenging of 2,2’-diphenyl-1-picrylhydrazyl (DPPH) radicals and inhibiting 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulated superoxide anion generation in neutrophil-like leukocytes (Chen et al., 2011).

Enhance Nrf2-mediated Detoxification and Antioxidant Effect

Recently, a potential chemopreventive strategy involving the induction of a battery of cytoprotective genes has represented a recent focus of attention in cancer research. NF-E2-related factor 2 (Nrf2), a member of the Cap’n’Collar (CNC) family of transcription factors that share a highly conserved basic region-leucine zipper (bZIP) structure mainly regulates transcriptional activation through antioxidant responsive element (ARE) (Huang et al., 2002). Nrf2-mediated cytoprotective genes are thought to protect cells against carcinogenesis and attenuate cancer development via neutralization of ROS or carcinogens (Klaunig et al., 2010; Kwak and Kensler, 2010). The gene families regulated by the Nrf2/ARE pathway include phase II metabolizing/detoxifying enzymes [e.g. aldo keto reductase (AKR), glutathione S-transferases (GST), UDP-glucuronoyltransferase (UGT), NADP(H) quinone oxidoreductase 1 (NQO1), heme oxygenase-
1(HO-1)), and antioxidants [e.g. superoxide dismutase (SOD), catalase, ferritin, s-glutamyl cysteine synthetase modifier subunit (GCLM), s-glutamyl cysteine synthetase catalytic subunit (GCLC), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin, thioredoxin reductase (TR), peroxiredoxins, metallothionein, etc.] (Zhang and Gordon, 2004; Giudice and Montella, 2006). Therefore, one of the most prominent strategies of cancer chemoprevention is to protect cells or tissues against various carcinogens and carcinogenic metabolites, both exogenous and endogenous, through the induction of metabolizing/detoxifying and antioxidant enzymes. We recently found that 4-ketopinoresinol, trans-coniferylaldehyde and sinapaldehyde, the active component isolated by free radical scavenge or antimutagenecity, significantly induced Nrf2/ARE-driven luciferase activity (Chen et al., 2011). Further study demonstrated that 4-ketopinoresinol strongly induces Nrf2-mediated detoxifying/antioxidant proteins, including HO-1, AKR1C1, AKR1C2, AKR1C3, GR, TR, GCLC, GCLM, etc., via the PI3K/AKT pathway, and at least in part, 4-ketopinoresinol -induced HO-1 expression contributes to a cellular defense mechanism against hydrogen peroxide-induced cell injury (Chen et al., 2012a). These results suggested that the cytoprotective effects induced by 4-ketopinoresinol may come from direct scavenging ROS directly (Kuo et al., 2002) and activating of PI3K/AKT/Nrf2/HO-1 axis (Chen et al., 2012a). Future studies evaluating the efficacy of 4-ketopinoresinol as a potential chemopreventive agent, using different carcinogenesis animal models, are warranted.

**Adlay Acts as a Suppressing Agents for Cancer Chemoprevention**

**Suppress Proliferation**

Dysregulated proliferation is a well-established hallmark of carcinogenesis (Hanhonen and Weinberg, 2000). The earliest antitumor study of adlay was demonstrated by Tanimura’s group at 1961. They reported that the growth of Ehrlich ascites sarcoma was inhibited by adlay (Tanimura, 1961; Ukita and Tanimura, 1961). Adlay is also applied as an adjuvant to treat lung, stomach, breast, colon, and cervical cancers (Chang et al., 2003; Hung and Chang, 2003; Woo et al., 2007; Hu et al., 2009). In addition to tumor transplantation experiment, Chang et al. used NNK-induced lung tumorigenesis model to investigate the chemopreventive potential of adlay seed. As the result, mice received the powder of adlay seed significantly reduced the number of surface lung tumors (Chang et al., 2003).

The active compound with anticancer property was first identified by Tanimura’s group to be a oil component, coixenolide (Tanimura, 1961; Ukita and Tanimura, 1961). Therefore, it is important to know the distribution of this compound in adlay seed. Huang and Chiang demonstrated that among different part of adlay seed, adlay bran contained the highest amount of coixenolide (473 ppm) (Huang and Chiang, 1999). In addition to coixenolide, other oil components, including palmmitic acid, stearic acid, oleic acid, and linoleic acid, also showed potent growth inhibitory activity on a transplantable mouse tumor (Numata et al., 1994). These results suggested that oil components of adlay play a critical role of anticancer efficacy. Recently, Kanglaite injection (KLT), an aqueous microemulsion of an oil extracted from adlay seed, have been developed using...
the latest and most complex modern technologies (Lu et al., 2008). KLT is a new biphasic extended-spectrum anticancer medicine, the food and drug administration (FDA) of United States also approved a phase II trial of KLT to test its efficacy in treating patients with stage IV of non-small-cell lung cancer or combination of KLT with gemcitabine to investigate the therapeutic efficacy in pancreatic cancer patients (http://www.clinicaltrial.gov). The mechanisms contributed to antitumor effect of neutral lipid components, at least in part, through cell cycle arrest and apoptosis induction involving upregulation of cyclin-dependent kinase inhibitor p21 and inhibition of antiapoptotic protein Bcl-2 expression (Bao et al., 2005).

In addition to oil extract, Chiang’s group recently identified several active components from adlay bran with anticancer growth inhibitory ability, including: (i) caffeic acid and chlorogenic acid toward gastric cancer cell growth (Chung et al., 2011a); (ii) coixspirolactam D, coixspirolactam E, coixspirolactam A, coixspirolactam C, and coixlactam toward breast cancer cells growth (Chung et al., 2011b); and (iii) coixspirolactam A, coixspirolactam B, coixspirolactam C, coixlactam, and methyl dioxindole-3-acetate toward non-small cell lung and colorectal cancer cell growth (Lee et al., 2008). The mechanism underlying anticancer effect of these component merit for further investigation.

Decrease Inflammation

A causal association between inflammation and cancer has long been suspected. Multiple lines of compelling evidence from clinical, epidemiologic and laboratory studies support that inflammation plays a critical role in the promotion and progression stages of carcinogenesis (Fitzpatrick, 2001).

1. The Nuclear Factor kappa-B (NF-κB)

NF-κB comprises a family of transcription factors involved in the regulation of a wide variety of biological responses. NF-κB plays a well-known function in the regulation of immune responses and inflammation, but growing evidences support a major role in oncogenesis. NF-κB regulates the expression of genes involved in many processes that play a key role in the development and progression of cancer such as proliferation, migration and apoptosis (Wang and Cho, 2010). Thus, clinical trials with drugs that block NF-κB are currently in progress with promising result (http://www.clinicaltrial.gov). Woo et al. showed that anti-neoplastic activity of an adlay extract in breast MDA-MB-231 xenografts (Woo et al., 2007). Using gene array technology, adlay extract significantly inhibits NF-κB-dependent gene transcription, including downregulation of COX-2 and matrixmetalloproteinases (MMP), in these cells. In addition, adlay extract also inhibits activity of protein kinase C, a major mediator of signal transduction and activator of NF-κB (Woo et al., 2007). Likewise, treatment with KLT decreased the level of NF-κB in the nucleus in a dose-dependent manner, and KLT markedly decreased the expression of IkBα, IKK and EGFR in the cytoplasm of lung tumor cells (Pan et al., 2012).

2. Cyclooxygenase-2 (COX-2)

Aberrant upregulation of COX-2, a key player in inflammatory signaling, is frequently observed in various precancerous and malignant tissues. Therefore, the normalization of overamplified signaling cascades implicated in chronic inflammation-associated carcinogenesis by use of COX-2 specific inhibitors has been recognized as a rational and pragmatic strategy in molecular targeted cancer prevention (Surh and Kundu, 2007). Huang et al. demonstrated that the ethanolic extract of adlay hull (AHE) and adlay testa (ATE) possessed potent anti-inflammatory activity through inhibiting lipopolysaccharide (LPS)-induced COX-2 expression in RAW264.7 macrophage (Huang et al., 2009a; Huang et al., 2009b). The active COX-2 inhibiting components were identified to be ceramide and naringenin in AHE (Huang et al., 2009a), and gallic acid, caffeic acid, and ferulic acid in ATE (Huang et al., 2009b), respectively. In the in vivo investigation, rats received dehulled adlay significantly reduced the expression level of COX-2 and the number of preneoplastic aberrant crypt foci (ACF) in azoxymethane (AOM)-induced colon carcinogenesis animal model (Shih et al., 2004). Li et al. further showed that adlay bran and its ethanol extract and residue play an important role in suppressing ACF formation in an early stage of colon carcinogenesis (Li et al., 2011). In addition, Chung et al. demonstrated that the ethyl acetate fraction of ABE significantly suppresses DMH-induced preneoplastic lesion of the colon in F344 rats through an anti-inflammatory pathway, involved COX-2 inhibition (Chung et al., 2010). Moreover, Hung and Chang also suggested that the methanolic extract of adlay seed also inhibits cancer growth and prevents lung tumorigenesis through COX-2 suppression (Hung and Chang, 2003).

Enhance Antitumor Immunity

Cytotoxic T lymphocytes and natural killer cells are
essential effectors of anti-tumor immune responses in vivo (Seino et al., 2006). Miyai’s group have investigated the changes in lymphocyte subsets in seven volunteers before, during (four weeks) and after taking six adlay seed tablets. As the results, the level of CD3+CD56+ (MHC-non restricted cytotoxic T cells) markedly increased at four weeks. The level of CD16+CD57- (the mature, most active natural killer cells) also increased at three weeks (Hidaka et al., 1992; Kaneda et al., 1992). These results indicate that adlay seeds increase peripheral cytotoxic lymphocytes and may be effective not only to viral infection through the enhancement of cytotoxic activity but also to boost antitumor immunity. In addition, it is interesting to note that KLT boosted anticancer immunity in Lewis lung carcinoma-bearing C57BL/6 mice through stimulating T cell proliferation and IL-2 secretion (Pan et al., 2012).

**CONCLUSION REMARKS**

In summary, adlay is a potential chemopreventive TCM to block multistage carcinogenesis with anti-initiation, -promotion and -progression properties. The chemopreventive functionality of adlay involving in scavenging electrophiles and reactive oxygen species, antimutagenicity, enhancing Nrf2-mediated detoxification and antioxidant effect, altering carcinogen metabolism, suppressing proliferation, decreasing inflammation, and enhancing antitumor immunity. Oil extract, especially KLT, have been proved to test its therapeutic efficacy in clinical setting.

Otherwise, several active components with diverse chemopreventive properties have been identified, including coniferyl alcohol, syringic acid, ferulic acid, syringaresinol, 4-ketopinoresinol, and mayuenolide (scavenge electrophiles and reactive oxygen species); p-hydroxybenzaldehyde, vanillin, syringaldehyde, trans-coniferylaldehyde, sinapaldehyde, and coixol (antimutagenicity); 4-ketopinoresinol, trans-coniferylaldehyde and sinapaldehyde (enhance Nrf2-mediated detoxification and antioxidant effect); coixenolide, Kanglaite injection (KLT; neutral lipid components), caffeic acid, chlorogenic acid, coixspirolactam D, coixspirolactam E, coixspirolactam C, coixlactam, and methyl dioxygenolne-3-acetate (suppressing proliferation); ceramide and naringenin, gallic acid, caffeic acid, and ferulic acid (decrease inflammation); and KLT (enhance antitumor immunity). The underlying mechanism of these active components is merit for further investigation.

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**ABBREVIATIONS**

| ABE | the ethanolic extract of adlay bran |
| ACF | aberrant crypt foci |
| AHE | the ethanolic extract of adlay hull |
| AHM | the methanol extract of adlay hull |
| AKR | aldo keto reductase |
| AOM | azoxymethane |
| ATE | the ethanolic extract of adlay testa |
| Ames test | the Ames Salmonella typhimurium mutagenicity assay |
| ARE | antioxidant responsive element |
| B[α]P | Benzo[α]pyrene |
| COX-2 | Cyclooxygenase-2 |
| CYP | cytochrome P-450 |
| DMH | 1,2-dimethylhydrazine |
| DPPH radicals | 2,2'-diphenyl-1-picrylhydrazyl radicals |
| FDA | the food and drug administration |
| GCLC | g-glutamyl cysteine synthetase catalytic subunit |
| GCLM | g-glutamyl cysteine synthetase modifier subunit |
| GPx | glutathione peroxidase |
| GR | glutathione reductase |
| GST | glutathione S-transferases |
| HO-1 | heme oxygenase-1 |
| IQ | 2-amino-3-methylimidazo[4,5-f]quinoline |
| KLT | Kanglaite injection |
| LPS | lipopolysaccharide |
| NNK | 4-methylnitrosamino-1-(3-pyridyl)-1-butanone |
| NQNO | 4-nitroquinoline-N-oxide |
| NQO1 | NADPH quinone oxidoreductase 1 |
| Nrf2 | NF-E2-related factor 2 |
| ROS | reactive oxygen species |
| SOD | superoxide dismutase |
| TCM | Traditional Chinese Medicine |
| TPA | 12-O-tetradecanoylphorbol-13-acetate |
| TR | thioredoxin reductase |
| UGT | UDP-glucuronoyltransferase |

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