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# Medicinal Uses of Chlorophyll: A Critical Overview

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## Abstract

Reports on traditional medicinal uses of chlorophyll in alternative forms of medicine are known since ages. Now-a-days chlorophyll has been used in the field of medicine as remedy and diagnostics. Chlorophyll molecules are used in pharmacy as photosensitizer for cancer therapy. Their roles as modifier of genotoxic effects are becoming increasingly important, besides these it being known to have multiple medicinal uses. Chlorophyll has its place in modern medicine. Here, we present a review of recent developments in medicinal uses of chlorophyll. This article enumerates therapeutic claims of chlorophyll as drugs based on investigative findings of modern science. A brief overview of research and developments of medicinal uses of chlorophyll will be presented in this review along with challenges of potential applications of chlorophyll and its derivatives as chemotherapeutic agents

**Keywords:** Chlorophyll, medicine, genotoxicity, photosensitizer

## Abbreviations:

CHL: Chlorophyllin

ROS: Reactive oxygen species

PDT: Photodynamic therapy

PSMA: Prostate-specific membrane antigen

ALA: Aminolevulinic acid

CDK: Cyclin dependent kinase

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## **1. Introduction**

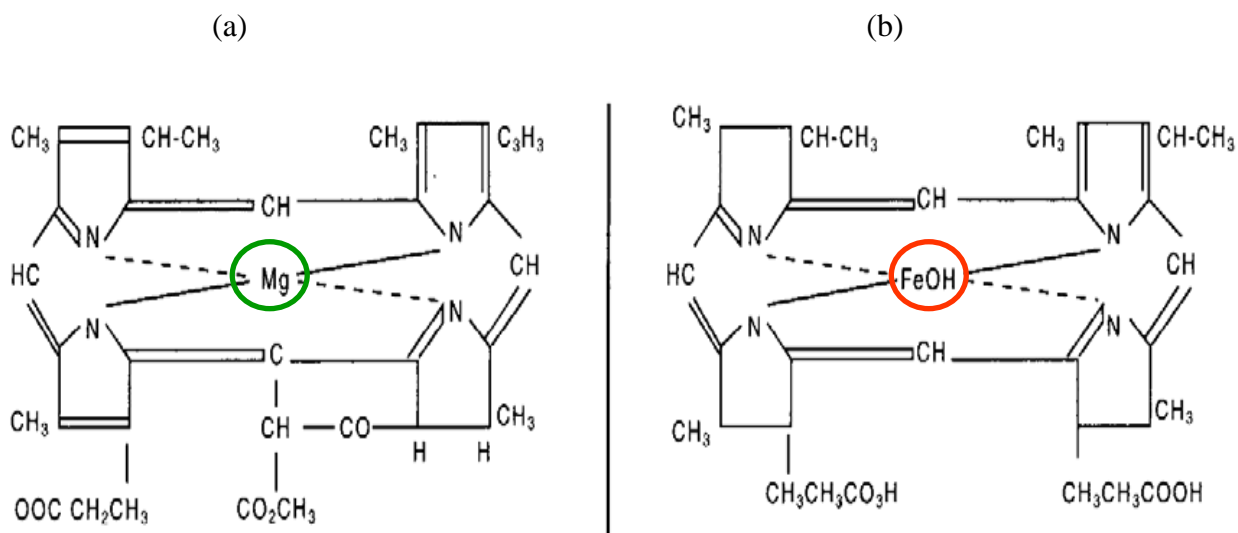
Natural products have been the most important source of drugs. Throughout history, these products have been used as important source of anticancer and chemopreventive agents. Many natural products from our daily consumption of fruits, vegetables, tea beverages whose active ingredients have potential health benefits. Recently, their uses are becoming increasingly popular as evident from the sales of food supplements/functional foods which is growing at an amazing proportion, \$4.59 billion for 2006 and \$4.79 billion for 2007 (Knasmüller et al., 2008). Despite, growing body of epidemiological and investigative findings supporting health claims of dietary supplements, there is urgent need to ensure consumer concern about their efficacy and potentiality as medicine. Among several dietary phytochemicals, chlorophyll being most ubiquitous natural pigments with physiological effects to cure of chronic diseases, such as some forms of cancer.

The chlorophyll and its derivatives have long history in traditional medicine ((Esterl and Dannin, 1950; Kephart, 1955), and also various therapeutic uses including wound healing (Dashwood, 1997), anti-inflammatory agent (Bower, 1947; Larato et al., 1970), internal deodorant (Young et al., 1980). Although these applications illustrate various medicinal uses of chlorophyll but interestingly recent research works are more focused on its role as potent anti-mutagen and anti-carcinogen (Dashwood, 1997, 2002, Egner et al., 2001, 2003), and also as photosensitizer in photodynamic therapy (Henderson et al., 1997; Park, 1989; Li, et al., 2005). The intent of present article is aimed at providing better understanding of science based health claims of chlorophyll.

## **2. Chemotherapeutic Potential of Chlorophyll**

### **2.1. Chlorophyll and Its derivatives**

Chlorophyll has a porphyrin ring similar to that of heme in hemoglobin, although the central atom in chlorophyll is magnesium instead of iron (Figure 1). Chlorophyllin is a semi-synthetic mixture of sodium copper salts derived from chlorophyll. During the synthesis of chlorophyllin, the magnesium atom at the center of the ring is replaced with copper and the phytol tail is lost. Unlike natural chlorophyll, chlorophyllin is water-soluble. Although the content of different chlorophyllin mixtures may vary, two compounds commonly found in commercial chlorophyllin mixtures are trisodium copper chlorin  $e_6$  and disodium copper chlorin  $e_4$  (Figure 2).

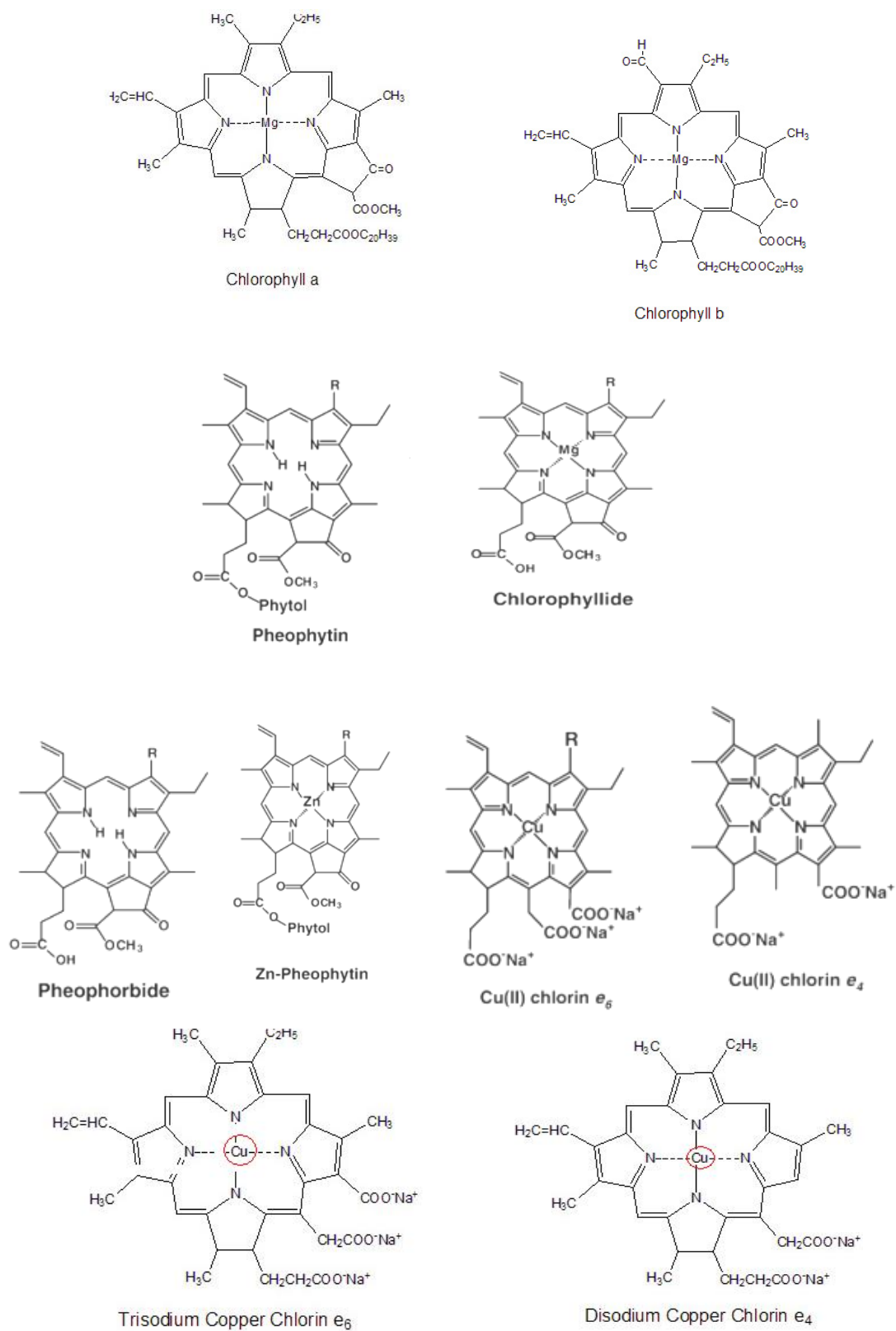


**Figure 1.** Molecular structure of (a) chlorophyll and (b) red blood cell

An excellent account of structure of chlorophyll and its derivatives, stability, bioavailability and their cancer preventing activity has been reviewed by Ferruzzi and Blakesle (2007). Chlorophyllin as been extensively studied for its effect in animal/human, and also utilized as food grade colorant in Europe, Asia and to a more limited and growing extent in United States (Ferruzzi and Blakesle, 2007). Some of the important chlorophyll and its derivatives are listed in Table 1.

**Table 1.** Chlorophyll and its derivatives used in medicine

Natural chlorophyll	Chlorophyll a, b, c, d, e
Metal free chlorophyll derivatives	Pheophytin, Pyropheophytin
Metallochlorophyll derivatives	Zn-Pheophytin
	Zn-pyropheophytin
	Chlorophyllide
	Pheophorbide
	Cu(II)chlorin e 4
	Cu-chlorin e 6
	Cu-chlorin e 4 ethyl ester



**Figure 2.** Structure of chlorophyll and its derivatives

## **2.2. Potential Mechanism of action of Chlorophyll**

Chlorophyll derivatives after release from the plant food matrix, natural chlorophyll (CHL) derivatives are exposed to the acidity of the gastric digestion resulting in conversion to respective metal-free pheophytins (PHE). Following digestive degradation of commercial chlorophyll derivatives, they are absorbed by intestinal cells and finally passes into blood circulation (Egner, 2000, Ferruzzi et al., 2002). Chlorophyll and its derivatives act through variety of mechanisms which include: (i) antioxidant activity; (ii) modifier of genotoxic effect; (iii) inhibition of cytochrome P450 enzymes; (iv) induction of phase II enzymes; (v) increased level of glutathione S-transferase; (v) cell differentiation, cell arrest and apoptosis.

### **2.2.1. Antioxidant Effect**

The major source of reactive oxygen species (ROS) is electron leakage from the mitochondrial electron transport chain, which then reacts with molecular oxygen forming ROS. ROS includes free radical such as superoxide ( $O_2^{\cdot-}$ ) and hydroxyl radical ( $OH^{\cdot}$ ) and non-radical species such as hydrogen peroxide ( $H_2O_2$ ). These free radicals set chain reaction of free radical formation when they interact with another molecule. High concentration of ROS causes oxidative damage to bio-molecules such as lipids, proteins and nucleic acids, leakage of electrolytes via lipid peroxidation, which results in the disruption of the cellular metabolism. Antioxidants act as an electron sink that neutralizes free radicals either through preventing free radical formation (preventive antioxidants) or preventing free radical chain propagation. Free radicals have been implicated to play an important role in development several diseases (Yoshikawa et al., 2000; Devasagayam et al., 2004; Knasmüller et al. 2008 ), which include some forms of cancer, neurological disorders, inflammatory diseases, dermatitis, tissue damage and sepsis, cardiovascular ailments (Elahi and Matata, 2006; Lefer and Granger 2000), and rheumatoid arthritis, idiopathic infertility (Agarwal et al., 2006; Pasqualotto et al., 2001), decreased immune function, several diseases of ageing (Von et al., 2004). There are contradictory views about ROS and cancer-one suggesting increased level of ROS causes cancer formation and proliferation while other opined that ROS may kill cancer cells (Schumacker, 2006).

Dietary chlorophyll derivative has ability to scavenge long lived free radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (Ferruzzi et al., 2002; Lanfer-Marquez et al., 2005). Natural chlorophyll a and b exhibited lower antioxidant activity than metal-free derivative (chlorins, pheophytins, and pyropheophytins), however

metallo-derivatives (Mg-chlorophylls, Zn-pheophytins, Zn-pyropheophytins, Cu-pheophytins, and Cu-chlorophyllins) have highest antioxidant activity (Lanfer-Marquez et al., 2005). Chlorophyll and derivatives have potent antioxidant and radioprotective effects *in vitro* and *in vivo*. They inhibit lipid peroxidation (Sato et al., 1983, 1984, 1985), protein oxidation, DNA damage, membrane damage (Kamat et al., 2000; Kumar et al., 2001). A burst of free radical formation is demonstrated during cerebral ischaemia and reperfusion induced injury. Chlorophyll salt and the aqueous extract of *Baccopa monneria* and *Valeriana wallichii* exerts neuroprotective effects (Rehni et al., 2007).

### **2.2.2. Modifier of genotoxic effect**

Hartman and Shankel (1990) reviewed inhibitors that directly interact with mutagen and carcinogen and sequester so that they may not have any harmful effect on body. These inhibitors act as interceptor molecules against mutagen and carcinogen. Interceptors are proficient in binding to, or reacting with, mutagenic chemicals and free radicals, and serves as a first line of defense against mutagens and carcinogens (Hartman and Shankel, 1990). Following interception, the defense mechanism may either involve induction of detoxification enzyme or inhibition of carcinogen activating enzyme. Data on activity profiles of antimutagens has been reviewed *in vitro* and *in vivo* data by Waters et al. (1996). Among the various inhibitors reviewed, chlorophyllin (CHL) was identified as almost uniformly protective against a broad range of direct- and indirect-acting mutagens, including aflatoxins, polycyclic aromatic hydrocarbons, heterocyclic amines, alkylating agents and several miscellaneous compounds (Arimoto et al., 1993; Breinholt et al., 1995; Tachino et al., 1994; Negishi et al., 1997; Dashwood et al., 1992, 1996, 1998, Dashwood, 2000).

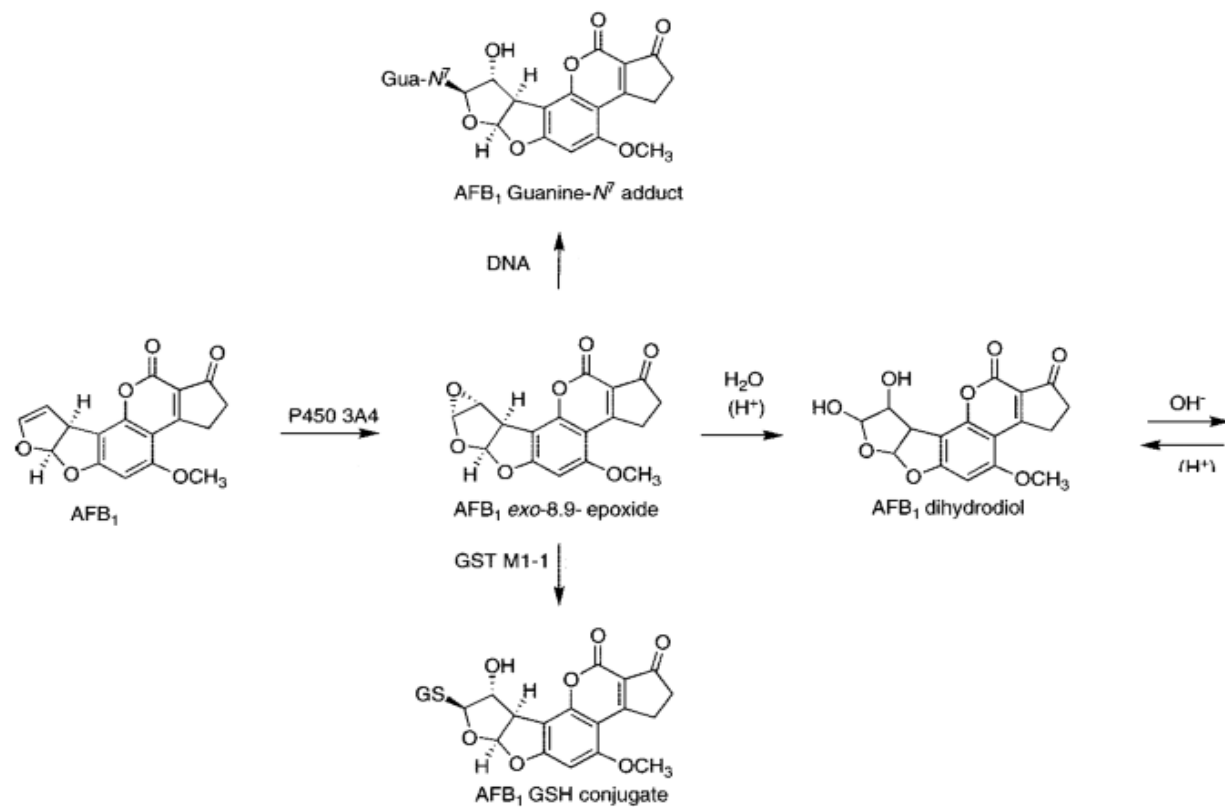
Although chlorophyll and its compounds has potential to act anti- mutagens *in vitro* (Negishi et al., 1989, Dashwood et al., 1995) however they have shown chemopreventive properties *in vivo* such as chemoprevention of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) hepatocellular carcinoma (HCC) in rainbow trout model (Breinholt et al., 1995, Dashwood et al., 1998; Reddy et al., 1999 Pratt et al., 2006; Simonich et al., 2008; Castro et al., 2009) and in rodent model (Guo et al., 1995; Hasegawa et al., 1995, Simonich et al., 2007) and human intervention (Yu, 1995; Egner et al., 2001). Chlorophyllin has strong binding capacity to acridine, more effectively than resveratrol and xanthenes (Osowski et al., 2010), which prevents DNA-mutagen intercalation.

### 2.2.3. Inhibition of Cytochrome P450 Enzymes

Cytochrome P450 enzymes are involved in the removal of carcinogenic compounds from the body. However, in some cases they can also activate compounds consumed in food, converting procarcinogens to carcinogens. Aflatoxin B1 is not carcinogenic until converted to the electrophilic 8,9-epoxide, which can form adduct with DNA. The metabolic activation of AFB1 is mediated by cytochrome p450 (Tachino et al., 1994; Yun et al., 1995). Dietary supplementation of chlorophyllin has significantly reduced AFB-1 induced DNA damage in the liver of rainbow trout and rats (Breinholt et al., 1995). The major pathway in metabolism of aflatoxin B1 in human is presented in Figure 3 (Guengerich et al., 2002, Guengerich, 2008).

CYP1B1 is also implicated in tobacco smoke-related cancers in several organs. Tobacco smoke contains several procarcinogens, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines and arylamines. PAHs can be activated into carcinogens by CYP1A1, CYP1A2 and CYP1B1. Benzo[a]pyrene (BP) is a potent pro-carcinogen and ubiquitous environmental pollutant. John et al. (2010) observed the induction and modulation of CYP1A1 and CYP1B1 and 10-(deoxyguanosin-N2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPdG) adduct formation in DNA from primary normal human mammary epithelial cell (NHMEC) strains. Maximum percent reductions of CYP1A1 and CYP1B1 gene expression and BPdG adduct formation were observed when cells were pre-dosed with chlorophyllin followed by administration of the carcinogen. Chlorophyllin is likely to be a good chemoprotective agent for a large proportion of the human population.





**Figure 3.** Metabolism of aflatoxin B1 in human (Guengerich et al., 2002, Guengerich, 2008)

#### **2.2.4. Induction of phase II Enzymes**

Induction of phase II response is recognized as an effective strategy for protecting cells against oxidants, electrophiles. Phase II enzymes include glutathione-S-transferase, UDP glucuronosyl transferase, sulfotransferase, and oxidoreductase. Phase II enzymes bind to oxygenated carcinogens making highly polar molecules that are excreted. Phase II enzymes decrease carcinogenicity by blocking carcinogen metabolic activation and enhancing carcinogen detoxification. Although the *Brassica* vegetables have long been known to contain potent inducers of mammalian phase 2 enzymes (Dinkova-Kostova et al., 2004), chlorophyllin may also increase the activity of the phase II enzyme, quinone reductase (Dingley et al., 2003). Chlorophylls, chlorophyllin and related tetrapyrroles are significant inducers of mammalian phase II cytoprotective genes, inducing the phase 2 enzyme NAD(P) H:quinone oxidoreductase 1 (NQO1) in murine hepatoma cells (Fahey et al., 2005). The drug-metabolizing enzyme comprises phase I (oxidation, reduction and hydrolysis). Physiological balance between Phase I and Phase II enzymes, and their level of expression and genetic polymorphism might dictate the sensitivity or risk of individual exposed to carcinogenic species (Kensler, 1997).

#### **2.2.5. Effect of Chlorophyll on Cell Differentiation, Cell arrest and Apoptosis of Cancer Cells**

Generally, growth rate of pre-neoplastic or neoplastic cells is fast than normal cell. Therefore, induction of apoptosis or cell cycle arrest can be an excellent approach to inhibit the promotion and progression of carcinogenesis. Distinct from apoptotic events in the normal physiological process, which are mainly mediated by interaction between death receptors and their relevant ligands (Jacks and Weinberg, 2002), many dietary supplements appear to induce apoptosis through the mitochondria-mediated pathways. The cytotoxic effects of chemotherapeutic compounds on neoplastic cells can be monitored by measuring their effect on mitochondria, caspases and other apoptosis-related proteins. Chlorophyllin induced apoptosis in HCT116 human colon cancer cells, via a cytochrome c-independent pathway (Diaz et al., 2003).

Progression through cell cycle is a sequential process that directs cells to pass through G1, S, G2 and M. There are G1-S/ or G2-M checkpoints that halt cell division whenever necessary. Cyclin-dependent kinase (CDKs) CDK inhibitors govern the progression of the cell cycle. Cell cycle arrest induced by chemopreventive compounds potentially affects and blocks the continuous proliferation of

tumorigenic cells. Lower doses of CHL also were observed to induce cell-cycle arrest and strongly altered markers of cell differentiation, such as E-cadherin (Carter et al., 2004). A recent study showed that human colon cancer cells undergo cell cycle arrest after treatment with chlorophyllin (Chimploy, 2009). The mechanism involved inhibition of ribonucleotide reductase activity. Ribonucleotide reductase plays a pivotal role in DNA synthesis and repair, and is a target of currently used cancer therapeutic agents, such as hydroxyurea (Chimploy et al., 2009).

### **3. Applications in Cancer Chemotherapy**

Cancer development is a long term process that involves initiation, promotion and progression that ultimately leads to spread from one area of the body to another during the late metastasis stage. Current clinical therapies which include surgery, radiotherapy and chemotherapy are limited to particularly during metastasis phase. However, there is increasing body of evidences from epidemiological and pathological studies that certain dietary substances may prevent or slow down progression of cancer. Because advance metastasis stage cancer are almost impossible to cure, therefore, cancer chemoprevention and containment at early stage is highly desirable. Dietary chemopreventive agents seems to have variety of cellular and molecular mechanism that may inhibit carcinogenesis (blocking agent) or suppress promotion and progression of carcinogenesis (suppressive agent) or function as both. Many dietary substances such as retinoic acid, sulforaphane, curcumin, EGCG, apigenin, quercetin, chrysin, silibinin, silymarin and resveratrol acts through induction of apoptosis. Potential mechanism underlying effectiveness of some of the dietary constituents is presented in Table 2. Many dietary compounds including chlorophyll possess cancer protective properties that include cellular detoxifying mechanism and antioxidant property that protects against cellular damage caused by environmental carcinogens or endogenously generated reactive oxygen species. These dietary substances can affect death signaling pathways which could prevent proliferation of tumor cells.

**Table 2:** Potential Mechanism of action of some of the dietary chemopreventive compounds (modified from Chen and Kong, 2005)

<b>Class</b>	<b>Function</b>	<b>Compounds</b>	<b>Source</b>
Cancer Blocking agent	Enhanced detoxification of chemicals	Indole-3-Carbinol	Cruciferous vegetables
		Chlorophyll and its derivatives	Green leafy vegetables
		Sulphoraphane	Cruciferous vegetables
		Curcumin	Turmeric
	Inhibit cytochrome P450	Isothiocyanates	Cruciferous vegetables
		Selenium	Nuts and meat
		Vitamin E	Vegetable oil
	Trap carcinogen	Flavonoids	Fruits and Vegetables
		Chlorophyllin	Commercial preparation from chlorophyll
Suppressive agents	Cell cycle disruption /or induce apoptosis	Chlorophyllin	Commercial preparation from chlorophyll
		EGCG	Green tea
		Quercetin	Onion and tomatoes
		Resveratrol	Grapes
		Curcumin	Turmeric
		Sulphoraphane	Cruciferous vegetables

Chlorophyll has a potential to act as chemopreventive agent. Clinical trials with chlorophyllin have reduced aflatoxin-DNA adducts in individuals at high risk for liver cancer (Kensler et al, 1998, Dingley et al., 2003). In another clinical trial on patient with fibroadenomatosis of breast cancer, the drug mamoclam- containing mega-3 polyunsaturated fatty acids, iodine and chlorophyll derivatives, produced from the brown sea alga laminaria, was effective in pain relief and breast cyst regression (Bezpalov et al., 2005 ).

Chlorophyll can assist with the effects of dietary and environmental exposure to carcinogens. Notable examples are the tobacco-related carcinogens (e.g., nitrosamines and polycyclic aromatic hydrocarbons-PAH), heterocyclic amines produced from sustained, high-temperature cooking of meats and the fungal food contaminants aflatoxins. Research indicates that chlorophyll reduces carcinogen binding to DNA in the target organ by inhibition of carcinogen activation enzyme or degradation of ultimate carcinogens with the target cells. *In vitro* and *in vivo* studies further substantiated medicinal cures offered by chlorophyll derivatives. Elucidation of the molecular mechanisms of chemical carcinogenesis provides insight into targets for chemoprevention. Microarray and proteomics analysis have shown alteration at the level gene expression and protein. Recently research investigations showing involvement of transcriptional factors and their intervention by chlorophyll and its derivative seems to be an attractive approach showing precise action at benefits of chlorophyll at cellular and molecular levels.

#### **4. Photosensitizer**

Photodynamic therapy (PDT) is increasingly becoming accepted as a treatment option for a variety of cancer which is usually based on the photosensitisation of tumour cells with subsequent light exposure leading to death of the malignant cells. The most commonly used photosensitisers, such as the haematoporphyrin derivatives have a number of drawbacks - poor selectivity in terms of tumour drug accumulation and low extinction coefficients so that relatively large amounts of drug and/or light are needed in order to obtain a satisfactory phototherapeutic response. These have led to the development of a further generation of photosensitisers based on chlorophyll derivatives, which are characterized by increased phototoxicity and strong absorption which allows deeper light penetration into tissues, rapid tissue clearance and minimal extravasation from the circulation (Rosenbach-Belkin et.al., 1996).

Aminolevulinic acid (ALA), a building block of tetrapyrroles, synthesized during chlorophyll biosynthesis, has shown photodynamic destruction of cancer cells (Reibeiz et al., 2002). It is available

as porphyric insecticides and show photodynamic property. It can be easily taken up by transformed cells, and is rapidly cleared from the circulatory stream within 48 hr of treatment (Reibeiz et al., 2002). Conjugating Cp 6 with histamine can help improve the effectiveness of PDT in oral cancer cells by enhancing its intracellular delivery (Parihar et al., 2010). "Radachlorin"(®), also known in the Bremachlorin, a composition of 3 chlorophyll a derivatives in an aqueous solution, was introduced into the Russian Pharmacopoeia. Iand may be commercialized as a prospective second-generation photosensitizer (Kochneva et al., 2010). Prostate-specific membrane antigen (PSMA), a validated biomarker for prostate cancer, has attracted considerable attention as a target for imaging and therapeutic applications for prostate cancer. PSMA inhibitor, i.e. conjugate of pyropheophorbide has been used for targeted PDT application and the mechanism of its mediated-cell death in prostate cancer: inducing apoptosis via activation of the caspase-8/-3 cascade pathway (Liu et al., 2010).

## **5. Contraindications and Safety**

Natural chlorophylls are not known to be toxic, and no toxic effects have been attributed to chlorophyllin despite more than 50 years of clinical use in humans. Although few contraindications have been reported by some investigators ((Chernomorsky, 1988; Gogel et al., 1989; Kephart , 1995; Egner et al., 2003 , Hendler et al., 2008) ) but much serious ill effects are less known. There is lack of reports about the safety of chlorophyll or chlorophyllin supplements in pregnant or lactating women. Although there is no major contraindication reported so far as but in order to be used in modern, pharmacological aspects the way these medicine interacts with human system needs to be further explored so as to recommend its safe, effective and widespread use of in medicine.

## **6. Challenges of potential application chlorophyll and its derivatives as chemotherapeutic agents**

Dietary chemopreventive compounds offer great potential in fight against cancer. The mechanistic insight into chemoprevention of carcinogenesis includes regulation of cell defensive and cell-death machineries. Though progress have been made in understating apoptosis, and cell cycle arrest in relation to chlorophyll and its derivatives, signaling pathways and gene expression events leading to pharmacological effects require further investigation. The ultimate goal is translation of the results of in vitro signaling and gene expression obtained in animal cell culture system /animal model to

beneficial pharmacological effects which have several challenges that need to be overcome. One of the concern is induction of detoxifying enzymes by chemotherapeutic agents varies within human population. Since the cancer development is a long term process, there is need to explore suitable potency indicators to assess the effect of chemopreventive agents. Dietary chemopreventive agents may not possess pharmacologically active properties to be used as drug. Studies on pharmacokinetics and toxicity profile of chemopreventive agents are important in drug development. Synergistic effect chemopreventive agent in association with other efficacious drug molecule might enhance the efficacy. Ultimately to convert a dietary chemopreventive agents into a viable drug, a clear understanding in this area will provide impetus for future developments.

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