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Revisiting Chlorophyll and Chlorophyllin – A Way to Drug Discovery

H.V. Girish[1], N.A. Reshi[*2], E.G. Ramkishore[4], S.K. Wagh[2]

Author's Affiliation:
[1]DOS in Botany, University of Mysore, Manasagagngotri, Mysuru, Karnataka, India
[2]Department of Biological Sciences, School of Science, Sandip University Nashik, Maharashtra, India
[3]Pharma Genica Healthcare Pvt. Ltd, Bangaluru, India

[*]**Corresponding Author**
Dr. Nissar A. Reshi
Associate Professor
Department of Biological Sciences
School of Science, Sandip University Nashik
Maharashtra India – 422 213
Email: nissarreshi@gmail.com

ABSTRACT:
Historically plants have served as a backbone in alleviating various human ailments. Plants are known as a source of diverse biologically active chemicals, essential for maintaining human health and useful for treating and preventing various diseases. Chlorophyll is considered as one of most abundant molecules on the planet and has been used since ages in folklore medicine for the treatment of various human ailments like cancer, inflammation, diabetes, Fungal and bacterial infections etc. Since last few decades various scientific investigations have discovered and validated the potential health benefits of green plant pigments (Chlorophyll) and their derivatives, which have been clinically proved to prevent various diseases and improve general health. In the present article, a comprehensive study is presented detailing and summarising the various earlier scientific investigations on therapeutic applications of chlorophyll and its water soluble derivative; chlorophyllin.

Keywords:
Phytoconstituents, Chlorophyll, Chlorophyllin, Alternative Medicine, Therapeutics

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Introduction

Although minimal scientific literature is available about the health benefits of chlorophyll and its derivatives, the authors have compiled the available data with the aim of directing scientific world to further validate its health benefits, which could prove to be highly beneficial to the mankind. The development of herbal products as therapeutic agents would result in identifying their potential benefits for further development of plant based therapeutics and provide data to understand the molecular mechanisms. Inverse relationships between the consumption of fresh leafy vegetables and human gastrointestinal cancer have been reported (Sarkar et al., 1994). Increased consumption of green vegetables protects from genotoxic agents which otherwise, could lead to carcinogenic, mutagenic and clastogenic (chromosome breaking) effects (Sarkar et al., 1996).Today a substantial number of drugs e.g., digoxin as cardiotonic, camptothecin as anticancerous, reserpine as antihypertensive and tranquilizer and many more are developed from plants (Verma and

Singh, 2008). The majority of drug precursor molecules are isolated from a particular medicinal plant and are further modified chemically. For example topotecan is a semisynthetic anticancer drug derived from camptothecin, from Camptotheca acuminata. A semi-synthetic analogue of plant-derived compounds could typically be a useful pharmaceutical product. It has been reported that 87 approved anticancer drugs are of natural derived / modified compounds based on natural product parents (Cragg et al., 1997). Most of thesynthetic drugs available in the market have more adverse effects than their natural counterparts specifically inducing hepatotoxicity, nephrotoxicity and gastrointestinal toxicity. Drugs discovered from herbs will give good therapeutic medicine with fewer side effects at lower cost. (Pandey et al., 2011). Several pharmacological studies have demonstrated the value of medicinal plants as potential source of bioactive compounds for therapeutic use against various infectious diseases (Prusti et al., 2008). Medicinal plants serve as a rich source of novel druggable compounds that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds. The World Health Organization (WHO) pointed out that about 25% of modern medicines are developed from plants that were first used traditionally (http://www.who.int/mediacentre/news/releases/release38/en/). Many other drugs are synthetic analogues built on parent compounds isolated from plants. Almost, 70% of modern medicines that are available in India are derived from natural products. Also, WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care needs (Verma and Singh, 2008; Pandey et al., 2011).

In the modern life style everyone is exposed to more refined foods, air pollutants, water pollutants and chemicals (Kelishadi, 2012). From household cleaners to the food consumed, everyday toxic elements are introduced into our systems and toxicity is a greater concern today. In the last 50 years some 80,000 chemicals have been developed and introduced into the environment (National Institute of Environmental Health Sciences (NIEHS), (http://www.niehs.nih.gov/), with the increase of toxic elements in the environment, there has also been an increase in occurrence of toxicity ailments. Cancer, cardiovascular disease, arthritis, allergies, headaches, gastrointestinal problems, fatigue, and immune weakness to name a few, can all be related to toxicity (Kelishadi, 2012). The human body handles toxins by neutralizing, transforming and eliminating them. Toxicity occurs in the body when intake is more than that can be utilized, processed, or eliminated. Many herbs and natural nutrients have demonstrated their beneficial effects on the elimination of toxic compounds (Bhat et al., 2010). Studies have shown that green tomato extracts inhibits the growth of human cervical and lung cancer cells (Choi et al., 2010). Leaves of Solanum xanthocarpum exhibit antihyperglycemic and antioxidant effects (Poongothai et al., 2011). The potential health benefit of diet rich in chlorophylls have been indicated in recent studies, though quite a few in numbers reporting their role as therapeutic agent. (Mishra et al., 2012)

Chlorophyll

Chlorophyll is the pigment present in all green leafy vegetables; like heme, having a planar porphyrin backbone. It gives color to vegetables and several fruits, where it plays a key role in photosynthesis. The dietary sources of chlorophyll include dark green leafy vegetables, algae, spirulina, chlorella, wheat grass and barley grass etc. The chlorophyll containing green leafy vegetables are also a rich source of vitamins (A, E & C), nutrients (protein, biotin, folic acid and pantothenic acid) and minerals (calcium, chromium, phosphorus, silicon, selenium, potassium, manganese, magnesium iron & zinc) (Baruah

et al., 2009). Chlorophyll is called the green blood of plants because its molecular structure is very similar to the structure of hemoglobin molecule present in red blood cells. Chlorophyll is different from heme by having the nonreactive magnesium instead of highly reactive transition metal iron in the center of porphyrin. In addition, chlorophyll has an esterified phytol tail instead of a propionic side chain (Fig. 1A, 1B).

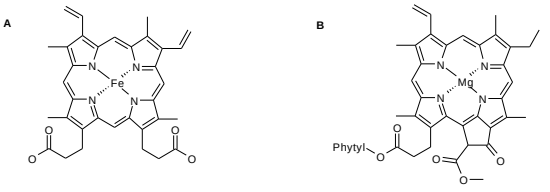

Figure 1. Chemical structures: heme (A), and chlorophyll 'a' (B).

Generally chlorophyll 'a' predominates over chlorophyll 'b' by a 3:1 margin (Yin and Cheng, 1998). Chlorophylls cannot be synthesized by animal tissues, though animal cells can chemically modify them for assimilation. Thus, these molecules must be obtained from plants. Degradation of chlorophylls during food processing of green fruits and vegetables has been thoroughly studied and is the subject of a number of reviews (Simpson, 1985). Chlorophylls are considerably sensitive to physical and chemical changes during food processing. These changes result in discoloration of vegetable tissue from green to olive brown that occur during thermal processing and / or acidification. The change in color is predominantly a result of replacement of the centrally chelated magnesium atom by two atoms of hydrogen, producing metal-free pheophytin derivatives (Schwartz and Lorenzo, 1990; Levent, 2011). Enzymatic removal of the esterified phytol by chlorophyllase results in the formation of water-soluble chlorophyllide derivatives (Fig. 2) (Schwartz and Lorenzo, 1990). Chlorophyll, however, is known to be converted into pheophytin, pyropheophytin, and pheophorbide (Fig. 2) in processed vegetable food and following ingestion by humans (Ferruzzi and Schwartz, 2001). Major chlorophyll degradation and derivatization reactions occurring during food processing operations are shown in Fig. 3.

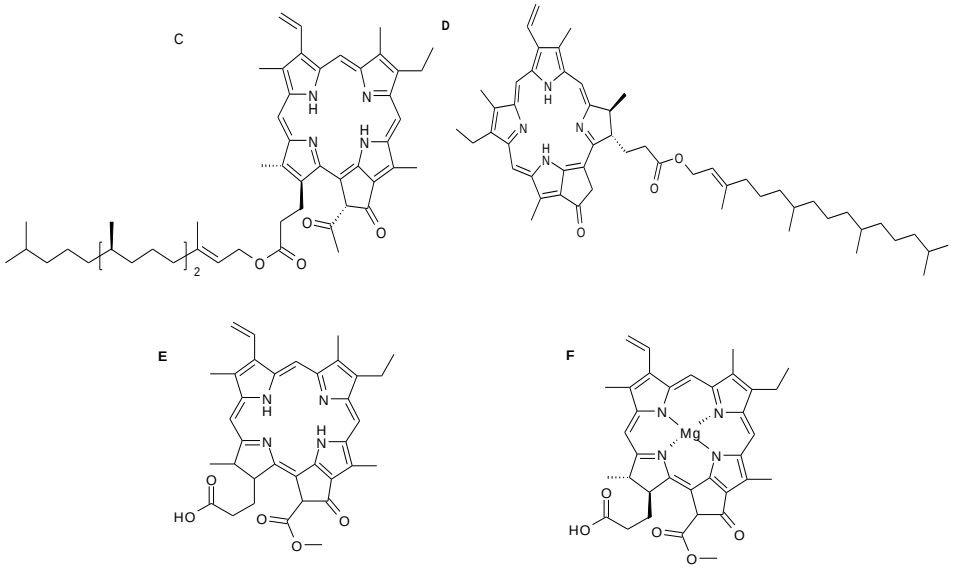

Figure 2. Chemical structures: pheophytin (C), pyropheophytin (D), pheophorbide (E), and chlorophyllide (F).

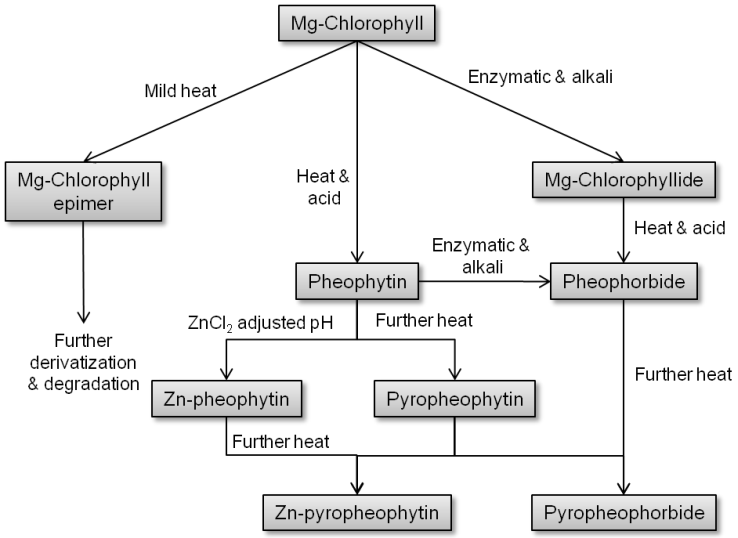

Figure 3. Chlorophyll degradation products during food processing.

Chlorophyllin

Chlorophyllins or chlorophyllides (Fig. 4) are food-grade molecules derived from chlorophyll that are studied for cancer prevention in vitro and in vivo because they might mimic the effects of chlorophyll (Breinholt et al., 1995).

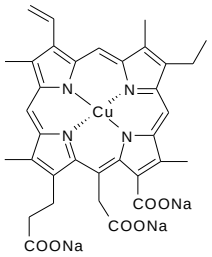

Figure 4. Chemical structure: sodium copper chlorophyllin.

They are hydrophilic, due to hydrolysis of the phytol tail and magnesium in the center of porphyrin ring is removed or replaced by another metal usually copper and are charged at neutral pH thereby appear green in solution (Guoet al., 2011). Derivatives of chlorophyll 'a' were found to be more effective radical squenchers than those of chlorophyll 'b'. Also, metal-free derivatives such as pheophytins, pyropheophytins and chlorins exhibited significant lower antiradical capacity than metallo-derivatives such as Mg-chlorophylls, Zn-pheophytins, Zn-pyropheophytins, Cu-pheophytin and Cu-chlorophyllins (Ferruzziet al., 2002). Metal-free as well as metallo-chlorophyll derivatives demonstrated similar dose-dependent inhibitory activity against mutagenesis (Bowers, 1947)

Isolation of Chlorophyll & Chlorophyllin

Chlorophyll in the leaves is extracted using acetone, followed by addition of hexane & water, the mixture is shaken thoroughly and centrifuged. The top hexane layer containing the pigment is pipetted out and treated with sodium sulfate. Finally, chlorophyll from the hexane layer is further isolated by column chromatography using alumina. Sodium copper chlorophyllin is stabilized chlorophyll and is prepared from chlorophyll by saponification and replacement of magnesium atom with copper (Pavia et al., 1999).

Health Benefits of Chlorophyll & Chlorophyllin

Chlorophyll

Anticancer property

The change of a cell from a normal to cancerous cell can be caused by a simple point mutation in the DNA sequence. Each of the 3-billion nucleotide combinations in the human genome can experience mutation. The types of mutations (e.g., frameshifts, translocations, inversions, deletions or insertional activations) and the location of mutations in the

chromosomes have been determined to be key in the onset and progression of cancer. Preclinical studies have shown that chlorophyll can be a powerful therapeutic agent for cancer, chemo-prevention and chemo-therapeutics (McQuistan et al., 2012; Hayatsu et al., 1993). The antiproliferative effect of chlorophyll has been studied in Hep3B hepatoma cells wherein the cell cycle was shown to be arrested in $G0/G_1$ phase (Tsai et al., 2010). The ability of chlorophyll to conduct gene splicing on DNA molecules with wrong gene sequencing is being discovered. The research implies that chlorophyll may reduce the error formation in DNA, utilizing an error-prone repair system on damaged DNA (Whong et al., 1988). Dashwood et al., (1991) demonstrated 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. Inhibiting the activity of the enzyme that attacks the DNA sequence significantly reduces the incidence of mutagenicity and reduces the onset of cancer. Chlorophyll inhibits the carcinogenic enzymes or the enzymes involved in phase 1 metabolism through a noncompetitive inhibition mechanism (Breinholt et al., 1995; Dashwood et al., 1991). Chlorophyll has been shown to take an active part in controlling the enzymes that are involved in mitosis (Gruskin B, 1940; Hayatsu +et al., 1993). It controls the rapid cell division and significantly reduces the tumor development, potentially giving the cell its ability to repair (Oda et al., 1971). Chlorophyll has shown to inhibit gut related tumors (Breinholt et al., 1995) and protects benzo [a] pyrene-initiated mouse skin tumorigenesis (Park et al., 1994). Oral intake of chlorophyll has been studied and shown its safety for daily dosage of 100-300 mg. (Chernomorsky and Segelman, 1988; Dashwood et al., 1991). High level of oral ingestion of chlorophyll has shown to be effective for chemo-therapeutic effects (Breinholt et al., 1995). Dietary inhibitors of mutagenesis and carcinogenesis are of interest because they may be used for human cancer prevention and treatment. A few studies have shown that chlorophyll prevents the detrimental, cytotoxic and hyperproliferative colonic effects of dietary heme. Diets high in red meat are associated with increased colon cancer risk (Balder et al., 2006). This association might be partly due to the haem content of red meat. In rats, dietary haem is metabolized in the gut to a cytotoxic factor (cytotoxic haem metabolite), the exact nature of which needs further investigation (de Vogel et al., 2005). This factor increases colonic cytotoxicity and epithelial proliferation. Researchers found that heme increased cytotoxicity of the colonic contents approximately 8-fold and proliferation of the colonocytes almost 2-fold. Spinach or an equimolar amount of chlorophyll supplement in heme diet inhibited these haem effects completely (de Vogel et al., 2005). Pheophorbide 'a' the catabolic product of chlorophyll, was shown to possess anticancer properties. The growth inhibitory activities of pheophorbide 'a' using MTT assay and phase-contrast microscopy by using various human cancer cell lines have been demonstrated (Cieckiewicz et al., 2012).

Antioxidant property

Chlorophyll is a good source of antioxidant nutrients (Rudolph, 1930) and its antioxidant properties are well studied (Hoshina et al., 1998; Chernomorsky et al., 1988). Reports suggest that chlorophyll exhibits antioxidant activity when assayed using DPPH [2, 2 diphenyl-1-picrylhydrazyl] and ABTS [2, 2 azino-bis ethylbenzthiazoline-6-sulfonic acid] methods (Shanab et al., 2011).

Antidiabetic property

Chlorophyll may have health benefits on diabetes and its metabolite phytanic acid is used for treatment and prevention of diabetes (McCarty, 2001; Schluter, 2002). The chlorophyll metabolite phytanic acid has been shown to be a natural ligand for retinoid X receptor (RXR), active in concentrations near

its physiological levels (McCarty, 2001). Further, phytanic acid was also shown to be a ligand of 9-cis-retinoic acid receptor and peroxisome proliferator-activated receptor (PPAR) (Hellgren, 2010). PPAR agonists are widely used in the treatment of type 2 diabetes. Phytanic acid is also found to act via different PPAR isoforms to modulate expression of genes involved in glucose metabolism (Heim et al., 2002).

Antiviral property

It was shown that IC50 value of chlorophyll for anti-HIV activity was 1.5µg / mL and a concentration of up to 20µg/mL was completely devoid of toxicity (Zhang et al., 2003). Two chlorophyll derivatives, pheophorbide 'a' and pyropheophorbide 'a' isolated from the stem of Opuntiaficus-indica exhibited potent virucidal effects on HSV-2 (herpes simplex virus type 2) and IFV-A (influenza A virus). The virucidal effects could be due to recognition of specific glycoproteins of enveloped viruses, precluding their binding to host cell receptors (Bouslama et al., 2011).

Other benefits

Chlorophyll 'a' inhibited bacterial lipopolysaccharide-induced TNF-α (a pro-inflammatory cytokine) gene expression in HEK293 cells. Study indicated that chlorophyll 'a' and its degradation products serve to be anti-inflammatory agents, thereby paving the way for development of phytomedicine or conventional medicine to treat inflammation and related diseases (Subramoniam et al., 2012). Chlorophyll and its derivatives were used for gastrointestinal problems, such as constipation and to stimulate blood cell formation in anemia. Chlorophyll was used as a breath freshener (Niccolini, 1952), to get rid of bad smells and to heal the infected wounds. Besides that chlorophyll was used to increase lactation during breast feeding. Chlorophyll has a positive effect on cardio-vascular, respiratory, digestive and endocrine systems. The therapeutic effect of chlorophyll 'a' in the treatment of patients with chronic pancreatitis is well studied. The disgusting abdominal pain due to pancreatitis disappeared in a week or so with infusion of 5-20 mg of chlorophyll 'a' per day for 1-2 weeks with no unfavourable adverse-effects, such as allergy, photosensitivity, or hepatotoxicity (Yoshida et al., 1980). Chlorophyll provides an overall restorative effect, increases host defences and increase oxygen levels in blood. Chlorophyll improves bowel function, and is a wonderful product for prophylaxis of kidney stone formation (Suzuki et al., 1987). It clears up the skin and is used in photodynamic therapy for acne and plays an important role in cosmetics (Kim et al., 2012). Chlorophyll along with other phytoconstituents is known to participate in the synthesis of prostaglandin and phospholipid components of cell membrane thus enhancing skin protection mechanisms (Butnariu and Giuchici, 2011).

It has been demonstrated that chlorophyll-related compounds, promote neurite outgrowth and stimulate differentiation in PC12 cells through enhancement of the mitogen-activated protein kinase signal transduction pathway (Ina and Kamei, 2006). Similarly, our in vitro data (Fig. 5) represents the novelty of the concept of supporting liquid chlorophyll in differentiating cord blood cells into stem cells. Human cord blood CD34+ cells were maintained at 37o C in a humidified atmosphere of 5% CO2 in DMEM medium supplemented with Fetal Bovine Serum (FBS), penicillin & streptomycin. Cells were seeded into well plate and were treated with liquid chlorophyll & observed for proliferation & differentiation.

From the observation it seemed that liquid chlorophyll may act as a growth factor for stimulation of cord blood cells into stem cells.

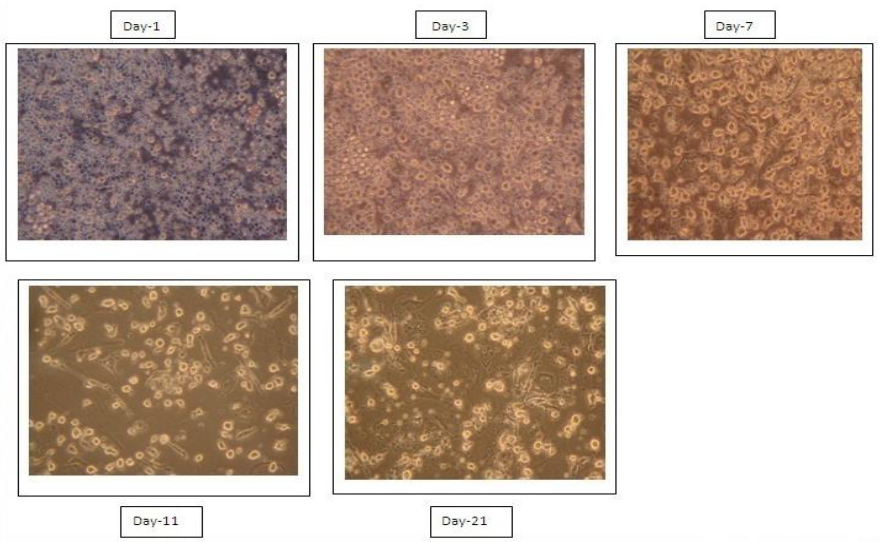

Figure 5. Pictures representing liquid chlorophyll treated cord blood cells, Day 21 representing fully differentiated stem cells.

Chlorophyllin

Anticancer property

In contrast to the limited in vivo studies with natural chlorophyll (Harttig and Bailey, 1998), several studies indicate that chlorophyllins may have anticarcinogenic effects (Shaughnessy et al., 2011). Chlorophyllin treatment in human colon cancer cells resulted in reduced level of ribonucleotide reductase, a pivotal enzyme for DNA synthesis and repair, at the mRNA and protein level and the enzymatic activity was inhibited in a concentration-dependent manner both in vitro and in vivo. It potentiates the anticancer activity in combination with currently available cancer therapeutic agents (Chimploy et al., 2009; Nagini et al., 2015). Chlorophyllin induces E-cadherin expression in human colon cancer cells which was localized primarily in the plasma membrane. The concomitant increase in β-catenin at the plasma membrane, coupled with lower nuclear β-catenin expression, suggested that β-catenin may be redistributed away from the nucleus and into the cytosolic pool, where it is subsequently transported to the plasma membrane via β-catenin–E-cadherin complexes. This redistribution pathway represents a novel mechanism for cancer chemoprevention and chemotherapy in the colon (Carter et al., 2004). The effects of chlorophyllin on malignant transformed human bronchial epithelial cell line 16HBE by trans-benzo[a]pyrene-trans-7, 8-dihydrodiol-9, 10-epoxide (trans-BPDE) was studied. The results indicated that the expression levels of Cyclin D1 and Cyclin E were enhanced in malignant transformed cell line while those were inhibited significantly in the anti-transformed cells treated with 100pmol/L chlorophyllin. The loss of E-cadherin expression was found after being transformed by trans - BPDE, while its expression existed normally after being anti-transformed by chlorophyllin (Fu et al., 2006). There exists evidence that chlorophyllin deactivates extracellular signal-regulated kinases (ERKs) to inhibit the breast cancer cell proliferation (Chiu et al., 2005). Chlorophyllin was also found to prevent colon neoplasms in mice induced by dimethylhydrazine (DMH) by selective inhibition of COX-2 (Ding et al., 2004). Chlorophyll and chlorophyllin can form complexes with certain carcinogens such as aflatoxin-B1 present in spices, herbs, higher plants, heterocyclic amines found in cooked meat, polycyclic aromatic hydrocarbons found in tobacco smoke (Breinholt et al., 1995). The formation of these complex structures may interfere with gastrointestinal absorption of potential carcinogens, and the amounts of

carcinogenic substances exposure to susceptible tissues may be reduced. The chemoprotective properties of chlorophyllin in several animal models coupled with lack of reported toxicities provided a justification for evaluation of the efficacy of chlorophyllin in individuals exposed to aflatoxin B1 (Kumar et al., 2012). A number of studies on cancer preventative effects of chlorophyll derivatives have been done (Wu et al., 1994; Breinholtet al., 1995; Egner et al., 2001; Kensler et al., 2002; Egner et al., 2003). Chlorophyllin has previously been shown to inhibit the mutagenic activity of a variety of xenobiotics by mechanisms that are likely to include: (a) direct antioxidant activity, and (b) formation of complexes with mutagens/carcinogens via strong stacking interactions thereby facilitating the excretion of these carcinogens (Dashwood et al., 1998). Data indicated that in order to bind 50% of the mutagen in a complex, less than twice the concentration of chlorophyllin was needed (Osowski et al., 2010). Chlorophyllin and related tetrapyrroles are inducers of transferases (phase 2 enzymes) involved in detoxification. Notably, chlorophyll itself is a potent inducer of detoxification enzymes (World Cancer Research Fund, 1997). Chlorophyllin is 10-fold more potent as a phase 2 enzyme inducer than chlorophyll, and it has other detoxification properties. It can be used as dietary supplement or a pharmaceutical ingredient as a chemoprotective agent (Egner et al., 2000). The chemoprotective properties of chlorophyllin may also be due to antioxidative activities or the nonspecific inhibition of cytochrome P450 enzymes involved in the bioactivation of carcinogens (Imai et al., 1986). Dietary administration of chlorophyllin (4 mg/kg bw) suppressed the development of DMBA (7, 12-dimethylbenz [a] anthracene) induced hamster buccal pouch carcinomas by regulating IKKβ and reducing the expression of nuclear NF-κB. Inactivation of NF-κB signaling by chlorophyllin was associated with the induction of intrinsic apoptosis and chlorophyllin may serve as novel candidates for cancer chemoprevention (Thiyagarajan et al., 2012). Yunos et al., (2011) indicate that combination of oxaliplatin and phytochemicals like chlorophyllin have produced synergistic effects in cisplatin resistant and non-resistant ovarian cancer cell lines.

Antioxidant property

Chlorophyllin induces antioxidant enzymes and protects against oxidative damage. Chlorophyllin induces heme oxygenase-1(HO-1) and quinone acceptor oxidoreductase 1(NQO1) expression in human umbilical vein endothelial cell (HUVEC) in a time- and dose-dependent manner and protects them against hydrogen peroxide induced oxidative damage (Zhang et al., 2008). The induction of HO-1 and NQO1 by chlorophyllin was accompanied with the accumulation of transcription factor Nrf2 in nucleus and the activation of PI3K/Aktsignalling pathway (Zhang et al., 2008). Iron-containing chlorophyllin is a promising cytoprotective against oxidative stress-mediated cellular toxicity (Yu et al., 2010). In vitro studies of chlorophyllin inhibited lipid peroxidation induced by 2, 2′-azobis (2-propionimidinedihydrochloride) (AAPH) in lymphocytes (Kumar et al., 2004). Results have also shown that chlorophyllin is highly effective in protecting mitochondria at a lower concentrations tested and the equimolar concentration was more than that observed with ascorbic acid, glutathione, mannitol and tert-butanol (Kamat et al., 2000). Chlorophyllin is able to scavenge the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and inhibits the formation of 5, 5-dimethyl-1-pyrroline-N-oxide adduct with hydroxyl radical (DMPO-.OH adduct) generated by gamma-radiation in a dose-dependent manner (Kumar et al., 2001). These studies suggest that chlorophyllin may mediate antioxidant ability involving scavenging of various physiologically important reactive oxygen species (ROS).

Kidney stone prevention

Chlorophyllin inhibits the growth of calcium oxalate dihydrate formation; being considered to be a primary phase in calcium oxalate stone formation (Tomazic and Nancollas, 1980). The inhibitory effect of sodium copper chlorophyllin on the formation, growth and aggregation of calcium oxalate crystals in vitro has been studied (Suzuki et al., 1987). The growth of calcium oxalate dihydrate crystals (weddellite) in simulated urine and its transformation into the more stable monohydrate (whewellite) was studied. Results have indicated that sodium copper chlorophyllin in a concentration of 100µg/mL inhibited the growth of calcium oxalate dihydrate crystals in simulated urine. The size distribution parameters of the dihydrate crystals in the presence and absence of chlorophyllin suggest that soluble chlorophyllin could be of clinical significance in calcium oxalate urolithiasis (Tawashi et al., 1980).

Antiviral property

The chlorophyllides have been known to have antiviral activity for more than 40 years and have been shown to have activity against HIV-1 (DeCamp et al., 1992) and it appears to disrupt the viral envelope particles and result in the destruction of the viral nucleic acid. Oral administration of chlorophyllides at 300mg / day for 4 months, found to be safe for human consumption (Egner et al., 2001). Chlorophyllin inhibited bovine herpes virus (BoHV-1) and (poliovirus) PV-1 infection and the IC50 against BoHV-1 and PV-1 were 8.6 and 19.8µg/mL respectively (Benati et al., 2009). Chlorine 6, a metal-free chlorophyllide-like molecule, showed the strongest antiviralactivity againsthepatitis B virus (HBV)as well as antiviral effects on other enveloped viruses, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), dengue virus (DENV), Marburg virus (MARV), Tacaribe virus (TCRV) and Junin viruses (JUNV) (Guo et al., 2011).

Other benefits

Chlorophyllin is used as a water-soluble ointment for gingival inflammation (Larato and Pfau, 1970). Sodium copper chlorophyllin has been used in the treatment of leucopenia, characterized by abnormal reduction of circulating white blood cells (Gao and Hu, 2005). Studies have shown that chlorophyllin inhibited IL-1beta production and its mRNA expression in a lipopolysaccharide (LPS)-stimulated murine macrophage cell-line, RAW 264.7 indicating its role in controlling inflammation and cell proliferation (Yun et al., 2006). Chlorophyllin is used as a food additive and in alternative medicine. As a food-coloring agent, chlorophyllin is known as natural green 3 and has the E number E141 (Wood et al., 2004). Commercial formulations of chlorophyllins which are widely available for control of body, fecal, and urinary odor in geriatric and osteomy patients, as wound-healing accelerators (Telgenhoff et al., 2007) especially in treating chronic ulcers, and as food colorants (Dutta and Chakravarty, 1963; PDR for Nutritional Supplements, 2001) may have more robust and broad-based chemoprotective activity than previously suggested (Egner et al., 2003)

Toxicity studies

No major adverse events such as toxicity, skin sensitization or other serious side effects have been reported from the use of chlorophyllin. Chlorophyllin may cause green discoloration of urine or feces, or yellow or black discoloration of the tongue (Hendler and Rorvik, 2008). There have also been occasional reports of diarrhea related to oral chlorophyllin use. When applied topically to wounds, chlorophyllin has been reported to cause mild burning or itching in some cases (Smith, 1955). Oral chlorophyllin may result in false positive results on guaiac card tests for occult blood (Gogel et al., 1989).

Clinical studies

Both natural chlorophyll and its water soluble derivative sodium copper chlorophyllin have been extensively studied for a variety of significant biological activities (Kephart, 1955). An attempt was made to replace antibiotics in the treatment of pneumonia by chlorophyllypt, which is an antibacterial Russian drug consisting of chlorophyll & other substances extracted from eucalyptus leaves and 0.25% chlorophyllypt was administered by intravenous. The clinical, laboratory and X-ray parameters in 22 patients normalized when treated by this drug compared to 19 patients who received the traditional antibiotic therapy. Chlorophyllypt was found to have the immune - corrective effect manifested by the normalization of the T-lymphocyte number and their theophylline-resistant subpopulation. No such an effect was achieved when broad-spectrum antibiotics were used (Simvolokov et al., 1989).A double-blinded, placebo-controlled intervention trial in Qidong, People's Republic of China, demonstrated that chlorophyll intervention can reduce aflatoxin-DNA adduct excretion among individuals in a population at high risk for liver cancer. Clinical trials with chlorophyllin have reduced aflatoxin-DNA adducts in individuals at high risk for liver cancer (Egner et al., 2003). In rats, dietary fiber and chlorophyll have been shown to promote the fecal excretion of dioxins and to reduce their levels in rat liver. A japanese study in humans was undertaken to ascertain whether such kinds of effect were also observed by FBRA, which was the health food and relatively rich with dietary fiber and chlorophyll. The study revealed that the amounts of excretion of dibenzofurans and dioxins in the FBRA-intake group were 1.81 and 1.74 times, respectively, greater than those in the non-intake group (Nagayama et al., 2005).Several in vitro clinical studies revealed the ability of chlorophyll to neutralize free radicals (Kumar et al., 2001). Prophylactic interventions with chlorophyllin or supplementation of diets with foods rich in chlorophylls may represent practical means to prevent the development of hepatocellular carcinoma or other environmentally induced cancers (Egner et al., 2001). The clinical trial of a drug "mamoclam" containing chlorophyll derivatives, omega-3 polyunsaturated fatty acids and iodine was carried out in patients with benign breast disease. The study was conducted in 33 patients (mean age 42.5 +/- 1.1 yrs). Clinical examination included evaluation of symptoms of mastopathy and dysalgomenorrhea, breast sonography and mammography. Post treatment there was reduced mastalgia, premenopausal syndrome, dysmenorrheal, algomenorrhea and breast cyst regression as well as attenuated pain associated with benign breast disease and palpation. Positive response was reported in 94% of the cases indicating that the drug may be recommended for the treatment of benign breast disease (Bezpalov et al., 2005). Copper derivatives of chlorophyll as biologically active constituents with tuberculosis chemotherapy of 48 adolescents has shown favourable results compared to 30 patients, receiving only chemotherapy (Lozovskaia, 2005). Daily consumption of 100-300 mg of chlorophyll has been shown to reduce the smell of urine and faeces in patients with faecal incontinence (Chernomorsky and Segelman, 1988; Young and Beregi, 1980). In a test group of 62 geriatric nursing home patients, the administration of chlorophyllin was found to be helpful in controlling body and fecal odors (Young and Beregi, 1980). Studies also reveal that chlorophyllin is safe and facilitates identification of retroperitoneal lymph nodes, allows more complete nodal excision and shortens the time of operation in patients undergoing radical hysterectomy with lymphadenectomy in malignant uterine tumors (Wang et al., 2001).The clinical study on patients suffering from trimethylaminuria, accompanied by repulsive smell, showed that after 3 weeks of oral intake of chlorophyllin (60 mg 3 times a day) the level of trimethylamine in the urine of the patients was markedly reduced (Yamazaki et al., 2004).

The investigations carried out in the 40-th of the last centuries, demonstrated that the consumption of chlorophyllin slows down the growth of anaerobic bacteria during the treatment of open wounds (in vitro), local use of chlorophyllin speeds up the healing process (animal trials), and highly effective for open wounds treatment in humans (Kephart, 1955). In the 1940's and 1950's, it was noticed that the local application of chlorophyll to the wounds with stinking odor had deodorizing effect. Since that time chlorophyll is being used as deodorant (Hayatsu et al., 1993). By the end of 1940's, beginning of 1950's, the series of studies showed that local use of chlorophyllin in patients with slow healing wounds, such as trophic ulcers and bedsores, is much more effective than widely used medications (Bowers, 1947). Chlorophyll and chlorophyllin are safe remedies that are demonstrated by several clinical applications (Kephart, 1955). The ability of chlorophyll to play a key role in cell's ability to repair damage was reported by Whong et al., 1988. Much attention has been given to the antigenotoxicity of chlorophyll and studies were conducted on the antimutagenic and tumoricidal potencies of these compounds (Shaughnessy et al., 2011). "Radachlorin" is used in the treatment of skin cancer, also known in the European Union as "Bremachlorin" containing 3 types of chlorophyll 'a' derivatives, was introduced into the Russian Pharmacopoeia. Phase II clinical studies involving photodynamic therapy (PDT) for "Radachlorin" was conducted. Safety study showed no side effects and a good tolerability of "Radachlorin"(®) by patients. The main part (98%) of the drug was excreted or metabolized in the first 48 hours. Having successfully passed clinical trials, "Radachlorin"(®) achieved marketing authorization in Russia in 2009 and a conditional approval in South Korea in 2008. It is a candidate for phase III clinical trials in the European Union and may be commercialized as a prospective second-generation photosensitizer in treating skin cancer (Kochneva et al., 2010). The animal studies suggest that additional chlorophyll intake can decrease the damage caused by free radicals, chemical carcinogens and radiation (Park et al., 2003). Preclinical studies in rats and rainbow trout suggested that chlorophyll acts by reducing bioavailability, genomic damage, and tumor induction caused by aflatoxins, heterocyclic amines and polycyclic aromatic hydrocarbons (Simonich et al., 2007). Chlorophyllin demonstrated significant inhibition of several mutagens including cigarette smoke, coal dust and diesel emission particles in an in vitro study (Onget al., 1986).Its antioxidant activity may have accounted for this effect.

Conclusions

Due to adverse effects of synthetic drugs, there has been an increasing interest in the natural product remedies. Throughout the history of mankind, many infectious diseases have been treated with herbals which have been the start point of many drugs used today. A number of scientific investigations have highlighted the importance and contribution of many plant families i.e. Asteraceae, Apocynaceae, Caesalpinaceae, Liliaceae, Piperaceae, Rutaceae, Solanaceae, Sapotaceae used as medicinal plants. Phytomedicine, in addition to their traditional values, also holds great public and medical interest worldwide as sources of nutraceuticals. Thus, these developments also indicate that the herbal remedy revolution has created new opportunities and has served to stimulate research in the field of human health care.

In this fast tracked era, toxins enter our body in various ways leading to several life threatening diseases and thus, have become a matter of great concern. Most of the available treatments involve use of several synthetic drugs to combat these diseases which in turn are associated with a high risk of adverse effects. In this context highly colored, conjugated polyenes that play central roles in photosynthesis, the plant chlorophylls play a vital role as phase 2 enzyme inducers. Chlorophyllins are other inducers of phase 2 enzymes. Since chlorophyllin is over 10-fold

more potent as a phase 2 enzyme inducer than chlorophyll, and since it has other detoxification properties because it is much more water-soluble than chlorophyll, its ultimate incorporation into either a dietary supplement or as a pharmaceutical component can be beneficial.Thus relying on natural products especially of plant origin such as chlorophyll and its derivative chlorophyllin helps to cleanse the body thereby prevents the development of diseases and also may act as a supportive in curing various ailments.

Chlorophyllin has gained considerable attention in recent years owing to its high safety and efficacy without any adverse side effects. Studies in a panel of human cancer cell lines and in a variety of experimental animal models have revealed that Chlorophyllin influences multiple molecules and pathways involved in the metabolism of carcinogens, antioxidant defenses, cell proliferation, apoptosis, invasion, and angiogenesis to exert its chemopreventive effects. Thus dietary phytochemicals such as Chlorophyllin that affect multiple signal transduction pathways involved in cancer initiation and progression hold promise as ideal candidates for cancer chemoprevention and therapy. However, caution is still warranted for clinical application, and extensive investigations on optimal dose, metabolism, bioavailability, tissue distribution, pharmacokinetics, interference with endogenous metabolic pathways, and crosstalk between signaling circuits are necessary before therapeutic utilization of Chlorophyllin.

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