Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1s,
January-June 2024: P.36-53

Print version ISSN 0970 0765
Online version ISSN 2320 3188

Original Research Article *Content Available online at: https://www.bpasjournals.com/*

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

How to cite this article: H.V. Girish, N.A. Reshi, E.G. Ramkishore, S.K. Wagh (2024). Revisiting Chlorophyll and Chlorophyllin – A Way to Drug Discovery. *Bulletin of Pure and Applied Sciences-Zoology*, 43B (1s), 36-53.

1. 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).

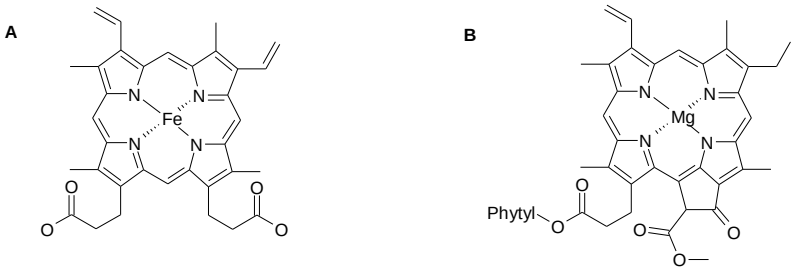

Figure 1. Chemical structures: heme (A), and chlorophyll 'a' (B).

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.

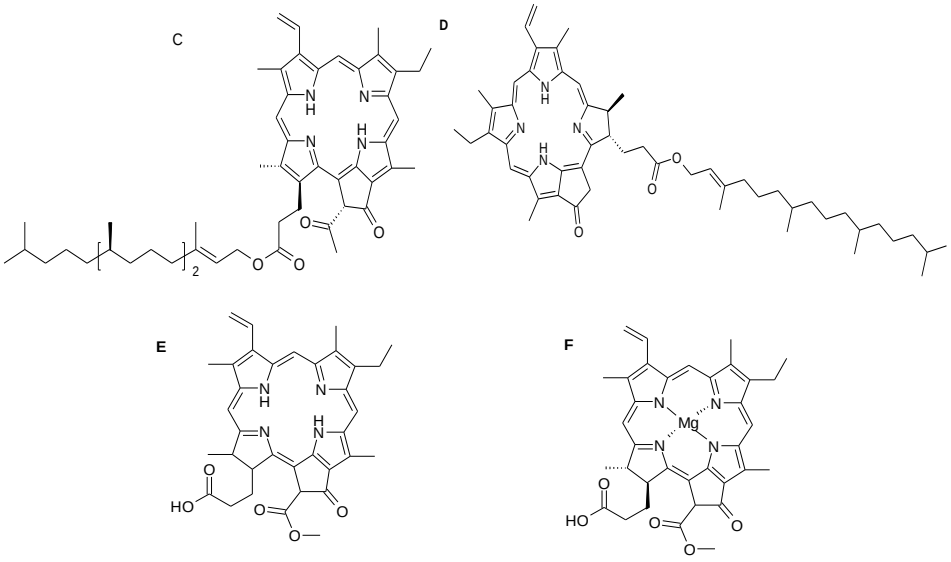

Figure 2. Chemical structures: pheophytin (C), pyropheophytin (D), pheophorbide (E), and chlorophyllide (F).

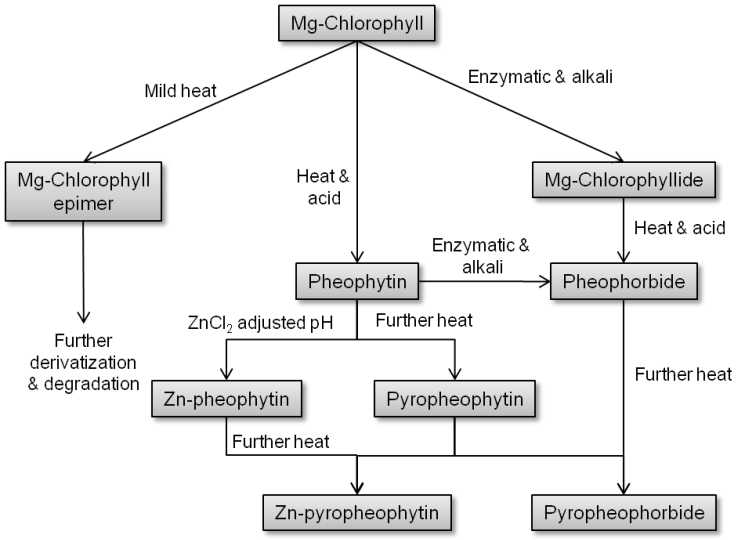

Figure 3. Chlorophyll degradation products during food processing.

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).

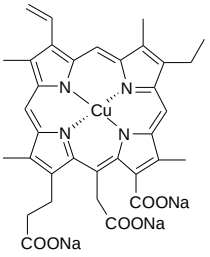

Figure 4.Chemical structure: sodium copper chlorophyllin.

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.

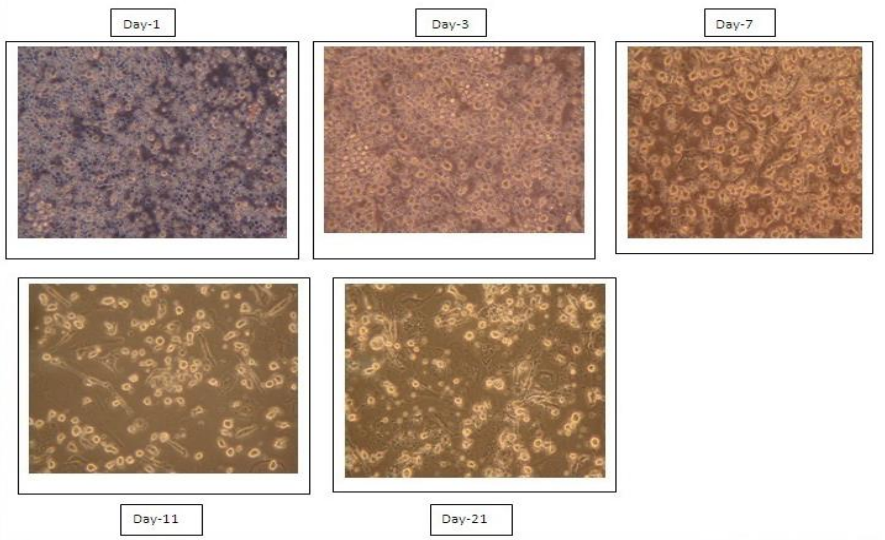

Figure 5.Pictures representing liquid chlorophyll treated cord blood cells, Day 21 representing fully differentiated stem cells.

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; Breinholt*et 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 (Ong*et al*., 1986).Its antioxidant activity may have accounted for this effect.

2. 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.

Acknowledgement

The authors wish to acknowledge the support from Sandip University Nashik,Pharma Genica Healthcare Pvt. Ltd., Bangalore and University of Mysore.

References

[1] Balder, H. F., Vogel, J., Jansen, M. C., Weijenberg, M. P., van den Brandt, P. A., Westenbrink, S., van der Meer, R., and Goldbohm, R. A. (2006). Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev*. **15**(4)**:** 717-725.

[2] Baruah, A. M., and Borah, S. (2009). An investigation on sources of potential minerals found in traditional vegetables of north-east India.Int J Food SciNutr.4: 111-115.

[3] Benati, F. J., Lauretti, F., Faccin, L. C., Nodari, B., Ferri, D. V., Mantovani, M. S., Linhares, R. E., and Nozawa, C. (2009). Effects of chlorophyllin on replication of poliovirus and bovine herpes virus in vitro.LettApplMicrobiol. 49(6): 791-795.

[4] Bezpalov, V. G., Barash, N., Ivanova, O. A., Semenov, I. I., Aleksandrov, V. A., and Semiglazov, V. F. (2005). Investigation of the drug "Mamoclam" for the treatment of patients with fibroadenomatosis of the breast.VoprOnkol.51(2): 236-241.

[5] Bhat, S. D., Ashok, B. K., and Acharya, R. (2010).Critical analysis of herbs acting on Mutravahasrotas.Ayu. 31(2): 167-169.

[6] Bouslama, L., K. Hayashi, J. B.Lee,A. Ghorbeland T. Hayashi, (2011).Potent virucidal effect of pheophorbide ‘a’ and pyropheophorbide ‘a’ on enveloped viruses.J Nat Med.65(1): 229-233.

[7] Bowers, W. F.,(1947). Chlorophyll in wound healing and suppurative disease.Am J Surg.73: 37-50.

[8] Breinholt, V., M. Schimerlik, R. Dashwoodand G. Bailey, (1995). Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1: complex formation with the carcinogen. Chem. Res. Toxicol. 8: 506–514.

[9] Butnariu, M. V., and C.V. Giuchici,(2011). The use of some nanoemulsions based on aqueous propolis and lycopene extract in the skin's protective mechanisms against UVA radiation. J Nanobiotechnology.9: 3.

[10] Carter, O., G. S. Bailey and R. H. Dashwood,(2004). The dietary phytochemical chlorophyllin alters E-cadherin and beta-catenin expression in human colon cancer cells. J Nutr.134: 3441-3444.

[11] Chernomorsky, S. A., and A. B. Segelman, (1988). Review article: Biological activities of chlorophyll derivatives. N. Engl. J. Med.85: 669-673.

[12] Chimploy, K., G. D. Díaz, Q. Li, O. Carter, W. M.Dashwood, C. K.Mathews, D. E. Williams, G.

S.Baileyand R. H.Dashwood, 2009. E2F4 and ribonucleotidereductase mediate S-phase arrest in colon cancer cells treated with chlorophyllin. Int J Cancer. 125(9): 2086-2094.

[13] Chiu, L. C., C. K.Kongand V. E.Ooi, 2005. The chlorophyllin-induced cell cycle arrest and apoptosis in human breast cancer MCF-7 cells is associated with ERK deactivation and Cyclin D1 depletion. Int J Mol Med.16(4): 735-740.

[14] Choi, S. H., S. H. Lee, H. J. Kim, I. S.Lee,N.Kozukue, C. E.Levinand M. Friedman, 2010. Changes in free amino acid, phenolic, chlorophyll, carotenoid, and glycoalkaloid contents in tomatoes during 11 stages of growth and inhibition of cervical and lung human cancer cells by green tomato extracts. J Agric Food Chem.58(13): 7547-7556.

[15] Cieckiewicz, E., L. Angenot, T. Gras, R. Kissand M. Frederich,2012. Potential anticancer activity of young Carpinusbetulus leaves. Phytomedicine.19 (3-4): 278-283.

[16] Cragg, G. M., D. J.Newman and K. M.Snader, 1997.Natural products in drug discovery and development.J. Nat. Prod.60: 52-60.

[17] Dashwood, R. H., V. Breinholtand G.S. Bailey, 1991. Chemo preventative properties of chlorophyllin: inhibition of aflatoxin-B- DNA binding in vivo and antimutagenic activity against AFB and two heterocyclic amines in the Salmonella mutagenicity assay. Carcinogenesis.12: 939-942.

[18] Dashwood, R., T. Negishi, H. Hayatsu, V. Breinholt, J. Hendricks and G. Bailey, 1998.Chemopreventive properties of chlorophylls towards aflatoxin B1: a review of the antimutagenicity and anticarcinogenicity data in rainbow trout. MutationResearch/Fundamental and Molecular Mechanismsof Mutagenesis.399 (20): 245-253.

[19] DeCamp, D. L., L. M. Babé, R. Salto, J. L. Lucich, M.-S. Koo, S. B. Kahl and C. S. Craik, 1992.Specific inhibition of HIV-1 protease by boronatedporphyrins.J. Med. Chem. 35:3426-3428

[20] De Vogel J.,D.S.M.L. Jonker-Termont, E.M.M .vanLieshout,M.B. Katanand Roelof van der Meer, 2005. Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis.26: 387-393.

[21] De Vogel J, D. S. Jonker-Termont,M. B. Katanand R. van der Meer, 2005. Natural chlorophyll but not chlorophyllin prevents heme-induced cytotoxic and hyperproliferative effects in rat colon. J Nutr.135(8): 1995-2000.

[22] Ding, X. W., X. L. Ding, S. Zheng and H. J.Yang, 2004. CHL prevent colon neoplasms in mice and its selective inhibition on COX-2. Ai Zheng.23(11):1409-1413.

[23] Dutta, A. K., and J. C. Chakravarty, 1963.Role of Chlorophyllin in chronic ulcer.Indian J Dermatol.9:1-6.

[24] Egner, P. A., K. H. Stansbury,.,E. P Snyder,., M. E.Rogers, , P. A Hintz,., and T. W Kensler,. (2000). Identification and characterization of chlorin e (4) ethyl ester in sera of individuals participating in the chlorophyllin chemoprevention trial.Chem.Res.Toxicol. 13: 900-906.

[25] Egner, P. A.,Wang,J.B ., Zhu Y.R., Zhang B.C., WuY.,et al. (2001). Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. Proc. Natl. Acad. Sci. U. S. A. 98: 14601–14606.

[26] Egner, P. A., A Munoz,., and T. W Kensler,.2003). Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin.Mutat. Res.523–524: 209–216.

[27] Ferruzzi, M. G., and , S. J Schwartz. (2001). Current Protocols in Food Analytical Chemistry F4.1.1-F4.1.9

[28] Ferruzzi, M. G., V.Bohm, ,P. D Courtney,., and S. J.Schwartz, (2002).Antioxidant and Antimutagenic Activity of Dietary Chlorophyll Derivatives Determined by Radical

Scavenging and Bacterial Reverse Mutagenesis Assays. Journal of Food Science.67:2589–2595.

[29] Fu, J., Y. G., Jiang, X. N Bin,., and X. M. Chen, (2006). Study on E-cadherin, Cyclin D1 and Cyclin E expression in anti-malignant transformation by chlorophyllin. Wei Sheng Yan Jiu.35(6): 678-681.

[30] Gao, F., and X. F. Hu, (2005). Analysis of the therapeutic effect of sodium copper chlorophyllin tablet in treating 60 cases of leukopenia.Chin J Integr Med.11(4): 279-282.

[31] Gogel, H. K., D.Tandberg, , and R. G Strickland,. (1989). Substances that interfere with guaiac card tests: implications for gastric aspirate testing. Am J Emerg Med.7(5): 474-480.

[32] Gruskin B. (1940). Chlorophyll-its therapeutic place in acute and suppurative disease.Am J Surg.49: 49-56.

[33] Guo, H., X. Pan, , R Mao,., X Zhang,., L Wang,., X Lu,., J.Chang, , T. J Guo,., S Passic,., F. C., Krebs, Wigdahl, B., Warren, T. K., Retterer, C. J., Bavari, S., Xu, X., Cuconati, A., and Block, T. M. (2011). Alkylated Porphyrins Have Broad Antiviral Activity against Hepadnaviruses, Flaviviruses, Filoviruses, and Arenaviruses. Antimicrobial Agents and Chemotheraphy.55(2): 478-486.

[34] Harttig, U., and G. S Bailey,. (1998). Chemoprotection by natural chlorophylls in vivo: inhibition of dibenzo [a, l] pyrene-DNA adducts in rainbow trout liver. Carcinogenesis19: 1323–1326.

[35] Hayatsu, H., T.Negishi, ,S Arimoto,., (1993). Porphyrins as potential inhibitors against exposure to carcinogens and mutagens.Mutat Res.290: 79-85.

[36] Heim M, J Johnson, F Boess, I Bendik, P Weber, W Hunziker, B Fluhmann (2002).Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. FASEB J,16(7):718-720.

[37] Hellgren, L. I. (2010). Phytanic acid--an overlooked bioactive fatty acid in dairy fat?Ann N Y Acad Sci. 1190: 42-49.

[38] Hendler, S. S., and D. R., Rorvik, eds. (2008).PDR for Nutritional Supplements. 2nd ed. Montvale: Physicians' Desk Reference, Inc.

[39] Hoshina, C., K Tomita,., and Y Shioi,. (1998). Antioxidant activity of chlorophylls: its structure–activity relationship. Photosynthesis: Mechanisms Effects 4: 3281-3284.

[40] Imai, K., T Aimoto,.,M Sato,., K Watanabe,., R Kimura,., and T Murata,. (1986). Effect of sodium metallochlorophyllins on the activity and components of the microsomal drug-metabolizing enzyme system in the rat liver.Chem. Pharm. Bull. 34: 4287-4293.

[41] Ina, A., and Y Kamei,. (2006). Vitamin B (12), a chlorophyll-related analog to pheophytin 'a' from marine brown algae, promotes neurite outgrowth and stimulates differentiation in PC12 cells. Cytotechnology.52(3): 181-187.

[42] Kamat, J. P.,K. K Boloor,., and T. P Devasagayam,. (2000). Chlorophyllin as an effective antioxidant against membrane damage in vitro and ex vivo.BiochemBiophysActa.1487(2-3): 113-127.

[43] Kelishadi, R. (2012). Environmental pollution: health effects and operational implications for pollutants removal. J Environ Public Health.2012: 341637.

[44] Kensler, T. W., P. A Egner,.,J. B Wang,., Y. R Zhu,., B. C., Zhang, G. S Qian,.,S. YKuang,., S. J Gange,., L. P Jacobson,., A Munoz,., and J. D. Groopman, (2002). Strategies for chemoprevention of liver cancer.Eur J Cancer Prev.2: 58-64.

[45] Kephart, J. C. (1955). Chlorophyll derivatives-their chemistry, commercial preparation, and uses.Econ. Botany 9: 3-38.

[46] Kim, J. E., J. I Hwang,.,J. I Lee,.,B. K., Cho, and H. J Park,. (2012). Pilot study on photodynamic therapy for acne using chlorophyll: evaluator-blinded, split-face study. J Dermatolog Treat.23(1): 35-36.

[47] Kochneva, E. V., E. V Filonenko,.,E. G Vakulovskaya,., E. G Scherbakova,., O. V., Seliverstov, N. A Markichev,., and A. V Reshetnickov,. (2010). Photosensitizer Radachlorin®: Skin cancer PDT phase II clinical trials. PhotodiagnosisPhotodynTher.7(4): 258-267.

[48] Kumar, S. S., B Shankar,., and K. B Sainis,. (2004). Effect of chlorophyllin against oxidative stress in splenic lymphocytes in vitro and in vivo.BiochemBiophysActa.1672(2): 100-111.

[49] Kumar, S. S., T. P Devasagayam,.,BBhushan,., and N. C Verma,. (2001). Scavenging of reactive oxygen species by chlorophyllin: an ESR study. Free Radic Res.35(5): 563-574.

[50] Kumar, M., V Verma,.,R Nagpal,., A.Kumar, , P. V Behare,., B Singh,., and P. K Aggarwal,. (2012). Anticarcinogenic effect of probiotic fermented milk and chlorophyllin on aflatoxin-B1-induced liver carcinogenesis in rats. Br J Nutr.107(7): 1006-1016.

[51] Larato, D. C., and F. R Pfau,.(1970). Effects of a water-soluble chlorophyllin ointment on gingival inflammation.N Y State Dent J.36(5): 291-293.

[52] Levent, A. (2011).Chlorophyll: Structural Properties, Health Benefits and Its Occurrence in Virgin Olive Oils. Academic Food Journal.9(2): 26-32

[53] Lozovskaia, M. E. (2005). Effectiveness of using the biologically active additive to food from Laminaria in adolescents during complex treatment of the pulmonary tuberculosis.VoprPitan.74(1): 40-43.

[54] McCarty, M. F. (2001). The chlorophyll metabolite phytanic acid is a natural rexinoid--potential for treatment and prevention of diabetes. Med Hypotheses.56(2): 217-219.

[55] McQuistan, T. J., M. T Simonich,., , M. M Pratt., C. B Pereira,., J. D Hendricks,., R. H.,Dashwood, D. E.,Williams, and G. S.Bailey, (2012). Cancer chemoprevention by dietary chlorophylls: a 12,000-animal dose-dose matrix biomarker and tumor study. Food ChemToxicol.50(2): 341-352.

[56] Mishra, V. K., R. K. Bacheti,., and A .Husen,. (2012). "Medicinal Uses of Chlorophyll: a critical overview" Chlorophyll: Structure, Function and Medicinal Uses. Ed. Hua Le and Elisa Salcedo. Nova Science Publishers, Inc., Hauppauge, NY 11788, 177-196.

[57] Nagayama, J., T Takasuga,.,H Tsuji,., and T Iwasaki,. (2005). Promotive excretion of causative agents of Yusho by one year intake of FBRA in Japanese people.Fukuoka IgakuZasshi.96(5): 241-248.

[58] Nagini S, Palitti F&Adayapalam T. Natarajan (2015) Chemopreventive Potential of Chlorophyllin: A Review of the Mechanisms of Action and Molecular Targets, Nutrition and Cancer, 67(2), 203–211

[59] Niccolini, P. (1952). Contribution to the study of the deodorizing effects of chlorophyll.Boll SocItalBiolSper.28(5): 978-979.

[60] Oda, T., O Yokono,.,A Yosida,., KMiyake,., and Iino, S. (1971). On the successful treatment of pancreatitis.Gastroenterol.6: 49-54.

[61] Ong, T. M., W. Z Whong,.,J Stewart,., and H. E Brockman,. (1986). Chlorophyllin: a potent antimutagen against environmental and dietary complex mixtures. Mutat Res.173(2): 111-115.

[62] Osowski, A., M Pietrzak,.,Z Wieczorek,., and J Wieczorek. (2010). Natural compounds in the human diet and their ability to bind mutagens prevent DNA-

mutagen intercalation. J Toxicol Environ Health A. 73(17-18): 1141-1149.

[63] Pandey, M., M Debnath,.,S.Gupta, , and S. K Chikara,. (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. Journal of Pharmacognosy and Phytotherapy.3(3): 27-37.

[64] Park, K. K., Y. J Surh,.,B. C Stewart,., and J. A Miller,. (1994). Chemoprotective activities of chlorophyllin: Inhibition of mutagenicity and covalent binding of various ultimate carcinogens, Proc. Am. Assoc. Cancer Res. 35: 139.

[65] Park, K. K., J. H Park,.,Y. J Jung,., and W. Y Chung,. (2003). Inhibitory effects of chlorophyllin, hemin and tetrakis (4-benzoic acid) porphyrin on oxidative DNA damage and mouse skin inflammation induced by 12-O-tetradecanoylphorbol-13-acetate as a possible anti-tumor promoting mechanism. Mutat Res.542(1-2): 89-97.

[66] Pavia, D. L., G. M Lampman,.,G. S Kriz,., and R. G Engel,. (l999). Introduction to Organic Laboratory Techniques: A Microscale Approach 3rd EditionSaunders College Publishing: New York, NY.

[67] Poongothai, K., P Ponmurugan,.,K. S Ahmed,., B. S Kumar,., and S. A Sheriff. (2011). Antihyperglycemic and antioxidant effects of Solanumxanthocarpum leaves (field grown &in vitro raised) extracts on alloxan induced diabetic rats. Asian Pac J Trop Med.4(10): 778-785.

[68] Prusti, A., Mishra, S.R.,Sahoo, S and Mishra, S.K. (2008)."Antibacterial Activity of Some Indian Medicinal Plants," EthnobotanicalLeaflets.Vol. 2008: Iss. 1, Article 27.

[69] Rudolph, C. (1930). The therapeutic value of chlorophyll.Clin Med Surg37: 119-121.

[70] Sarkar, D., A Sharma,., and G Talukder. (1994). Chlorophyll and chlorophyllin as modifiers of genotoxic effects.Mutat Res.318(3): 239-247.

[71] Sarkar, D., A Sharma,., and G Talukder,. (1996). Chlorophyll and chromosome breakage.Mutat Res.360(3): 187-191.

[72] Schluter, A., P Yubero,.,R Iglesias,., M Giralt,., and F.Villarroya, (2002). The chlorophyll-derived metabolite phytanic acid induces white adipocyte differentiation. International Journal of Obesity.26: 1277–1280

[73] Schwartz, S. J., and T. V. Lorenzo, (1990).Chlorophylls in foods.Critical Reviews in Food Science and Nutrition.29: 1-17.

[74] Shanab, S. M., E. A Shalaby,., and E. A El-Fayoumy,. (2011). Enteromorphacompressa exhibits potent antioxidant activity. J Biomed Biotechnol.2011: 726405.

[75] Shaughnessy, D. T., L. M Gangarosa,., B Schliebe,.,D. MUmbach,., Z Xu,., B MacIntosh,., M. G Knize,., P. P Matthews,., A. E Swank,., R. S Sandler,., D. M DeMarini,., and J. A. Taylor, (2011). Inhibition of fried meat-induced colorectal DNA damage and altered systemic genotoxicity in humans by crucifera, chlorophyllin, and yogurt.PLoS One.6(4): e18707.

[76] Simpson, K. L. (1985). Chemical changes in natural food pigments. In Chemical Changes in Food during Processing (T. Richerson and J.W. Finley, eds.) AVI Publishing, Westport, Conn.

[77] Simonich, M. T. Egner P.A., Roebuck B.D., Orner G.A., Jubert C., Pereira, C., et al. (2007). Natural chlorophyll inhibits aflatoxin B1-induced multi-organ carcinogenesis in the rat. Carcinogenesis 28(6): 1294-1302.

[78] Simvolokov, S. I., A. V Nikitin,., and L. G Iakovleva,. (1989). Clinico-immunologic effectiveness of chlorophyllypt in the treatment of acute destructive pneumonia.Klin Med (Mosk).67(2): 108-112.

[79] Smith, L. W. (1955). The present status of topical chlorophyll therapy.N Y State J Med.55(14): 2041-2050.

[80] Subramoniam, A., V. V Asha,.,S. A Nair,.,S. P Sasidharan,., P. K Sureshkumar,., K. N., Rajendran, D.Karunagaran, , and K Ramalingam,. (2012). Chlorophyll Revisited: Anti-inflammatory Activities of Chlorophyll 'a' and Inhibition of Expression of TNF-α Gene by the Same. Inflammation.35(3): 959-966.

[81] Suzuki, K., C Yamaguchi,.,K Miyazawa,., T Taniguchi,., A Ben,., and R Tsugawa,. (1987). Inhibitory effect of sodium copper chlorophyllin on the formation, growth and aggregation of calcium oxalate crystals in vitro.Nihon HinyokikaGakkaiZasshi.78(8): 1306-1310.

[82] Tawashi R, M Cousineau, M Sharkawi.(1980). Effect of sodium copper chlorophyllin on the formation of calcium oxalate crystals in rat kidney.Invest Urol.18(2):90-92.

[83] Telgenhoff, D., K Lam,.,S Ramsay,., V Vasquez,., K Villareal,., P Slusarewicz,., P Attar,., and B Shroot,. (2007). Influence of papain urea copper chlorophyllin on wound matrix remodeling.Wound Repair Regen.15(5): 727-735.

[84] Thiyagarajan, P., M. R Senthil,.,K Kavitha,., P Anitha,., D Prathiba,., and S Nagini,. (2012). Dietary chlorophyllin inhibits the canonical NF-κB signaling pathway and induces intrinsic apoptosis in a hamster model of oral oncogenesis. Food ChemToxicol.50(3-4): 867-876.

[85] Tomazic, B., and G. H Nancollas,. (1980). Crystal growth of calcium hydrates: a comparative kinetics study. J. Colloid Interface Sci. 75: 149.

[86] Tsai, Y. C., W. B Wu,., and B. H Chen,. (2010). Preparation of carotenoids and chlorophylls from Gynostemmapentaphyllum (Thunb.) Makino and their antiproliferation effect on hepatoma cell.J Med Food.13(6): 1431-1442.

[87] Verma, S., and S.P Singh,. (2008). Current and future status of herbal medicines. Veterinary World.1(11): 347-350.

[88] Wang, H., Y Tan,.,X Wang,., and J Xie,. (2001). Identification of pelvic lymph nodes with chlorophyllin after injection into the uterine cervix: an experimental and clinical study. Lymphology.34(2): 69-76.

[89] Whong, W., J.Stewart, ,H. E Brockman,., and T Ong,. (1988). Comparative antimutagenicity of chlorophyllin and five other agents against aflatoxin B induce reversion in Salmonella typhimurium TA98.Teratog.Carcinog.Mutagen. 8: 215-224.

[90] Wood, R., L Foster,.,A Damant,., and P Key,. (2004). Analytical Methods for Food Additives, CRC Press, New York, USA, p24.

[91] Wu, Z. L., J. K Chen,.,T Ong,., H. E Brockman,., and W. Z Whong,. (1994). Antitransforming activity of chlorophyllin against selected carcinogens and complex mixtures.TeratogCarcinog Mutagen.14(2): 75-81.

[92] Yamazaki, H.,MFujieda,.,M Togashi,. et al. (2004). Effects of the dietary supplements, activated charcoal and copper chlorophyllin, on urinary excretion of trimethylamine in Japanese trimethylaminuria patients.Life Sci.74(22): 2739-2747.

[93] Yin, M. C., and W. S Cheng,. (1998). Inhibition of Aspergillusnigerand Aspergillusflavusby some herbs and spices. J Food Prot61: 123-125.

[94] Yoshida, A., O Yokono,., and T Oda,. (1980). Therapeutic effect of chlorophyll-a in the treatment of patients with chronic pancreatitis.GastroenterolJpn.15(1): 49-61.

[95] Young, R. W., and J. S. Beregi, (1980).Use of chlorophyllin in the care of geriatric patients.J Am Geriatr Soc.28(1): 46-47.

[96] Yu, J. W., R Yang,., and Y. S. Kim, (2010). Differential cytoprotective effect of copper- and iron-containing chlorophyllins against oxidative stress-mediated cell death.Free Radic Res.44(6): 655-667.

[97] Yun, C. H., Y. J Jeon,., Yang, Y., Ju, H. R., and Han, S. H. (2006). Chlorophyllin suppresses interleukin-1 beta expression in lipopolysaccharide-activated RAW 264.7 cells. IntImmunopharmacol. 6(2): 252-259.

[98] Yunos, N. M., P Beale,.,J. Q Yu,., and F Huq,. (2011). Synergism from the combination of oxaliplatin with selected phytochemicals in human ovarian cancer cell lines.Anticancer Res.31(12): 4283-4289.

[99] Zhang, H. J., G. T Tan,.,V. D Hoang,., N. V Hung,., N. M Cuong,., D. D Soejarto,., J. M Pezzuto,., and H. H Fong,. (2003). Natural anti-HIV agents. Part IV. Anti-HIV constituents from Vaticacinerea.J Nat Prod.66(2): 263-268.

[100] Zhang, Y., L Guan,.,X Wang,., T Wen,., J Xing,., and J Zhao,. (2008). Protection of chlorophyllin against oxidative damage by inducing HO-1 and NQO1 expression mediated by PI3K/Akt and Nrf2. Free Radic Res.42(4): 362-371.