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Review on Different Aspects of the Photodynamic Product Pheophorbide

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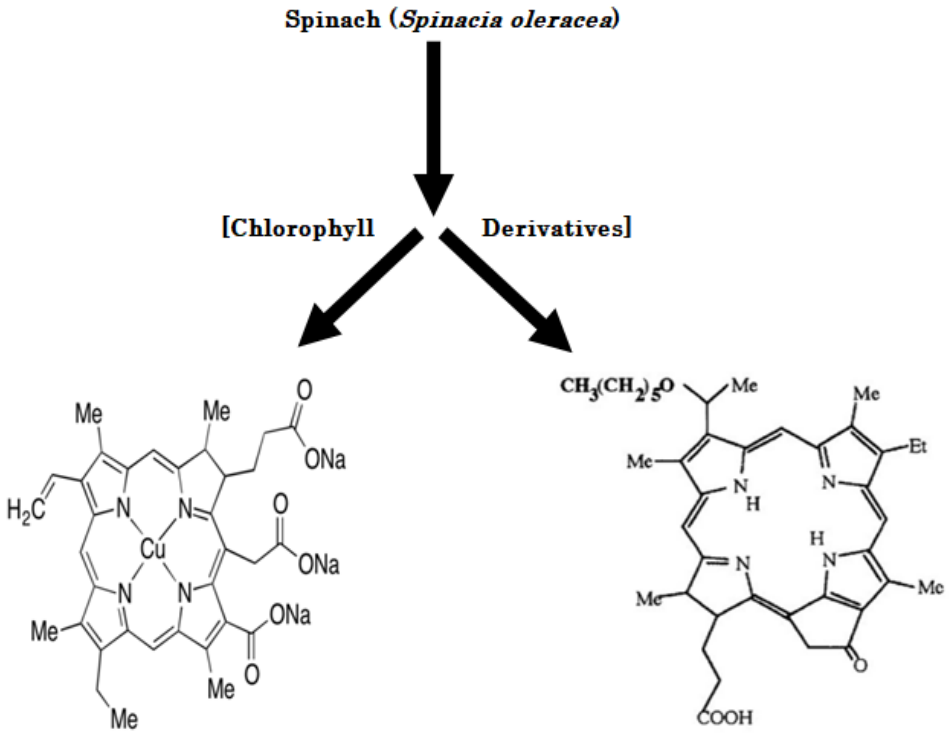
Abstract: *Chlorophyll derivatives such as pheophorbide and chlorophyllin is used to photodynamically destroy snails, Fasciola and mosquito larvae. These photodynamic products are formed from natural chlorophyll extracted from spinach and any other greens plants. Chlorophyll is insoluble in water so by using chemical modification it can be transformed into water soluble chlorophyllin. The acidification of this chlorophyllin transformed into pheophorbide. As a result these substances are applied very easily in aquatic medium due to their solubility in water. This method implicates significantly low concentrations of pheophorbide. Upon illumination it starts reacting and leading to apoptosis as well as necrosis of the cells in the intestine resulting in the death of the snails and larvae. In developing counties to control fasciolosis and other water borne diseases the use of photodynamic product is inexpensive and therefore a promising approach. An outline on the basics of the photodynamic reactions and about the results obtained thus far using photodynamic properties of pheophorbide in order to control the vectors of parasitic diseases and parasites.*

Keywords: *Fasciolosis, Spinach, Photodynamic Pheophorbide, Phytochemicals, Pharmacological aspects.*

INTRODUCTION

Spinach (*Spinacia oleracea*)

Spinach (***Spinacia oleracea***) is one of astonishing green-leafy vegetable often recognized as one of the functional foods for its nutritional, antioxidants and anti-cancer composition (Machowsky, 2013). Botanically, it belongs to the *Amaranthacae* family, and its scientific name: *Spinacia oleracea*. Spinacia plant grows to about 1 foot in height. While it can be grown year round, its fresh greens are best available soon after the winter season from March from beginning to end May in the Northern hemisphere, and from September until November in the South of the equatorial line As a minimum, two varieties of spinach are cultivated for their edible leaves with dark-green (wrinkled) leaves and flat-leaf type with smooth surfaced leaves (Machowsky, 2013).



a. Chlorophyllin **b. Pheophorbide**

Advantages of Spinach:

- Spinach is store house for many phyto-nutrients that have health promotional and disease prevention properties.
- Fresh spinach contains about 25% of daily intake of iron one of the richest among green leafy vegetables. Iron is an important trace element required by the human body for red blood cell production and as a co-factor for oxidation-reduction enzyme, **cytochrome-oxidase** during the cellular metabolism.
- In addition, vitamin A is required for maintaining healthy mucus membranes and skin and is essential for normal eyesight. Consumption of natural vegetables and fruits rich in vitamin A and flavonoids also known to help the body protect from lung and oral cavity cancers.
- Spinach leaves are an excellent source of **vitamin K** and fresh greens provides daily vitamin-K requirements. Vitamin K plays a vital role in strengthening the bone mass by promoting bone building (osteotrophic) activity in the bone. And it also plays an important role in patients with Alzheimer's disease by limiting neuronal damage in the brain.
- Its leaves also contain a good amount of minerals like potassium, manganese, magnesium, copper and zinc. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Manganese and copper are used by the body as a co-factor for the antioxidant enzyme, superoxide. Copper is required in the production of red blood cells. Zinc is a co-factor for many enzymes that regulate growth and development, sperm generation, digestion and nucleic acid synthesis.

Chlorophyll:

In 1817 chlorophyll was first isolated and named by Joseph Pelletier and Pierre Joseph Caventou (Pelletier and Caventou, 1817). It is a chlorine pigment which is structurally similar and produced through the same metabolic pathway as other porphyrin pigments such as heme. A Mg^{2+} ion present at the centre of chlorine ring and first time Mg^{2+} had been detected in living tissue in 1906 (Wilstatter, 1906; Motilva, 2008). Initial

work is completed by a German chemist Richard Willstatter (1905 to 1915). Chlorophyll obtains in every green plant and it can easily be extracted with ethanol or methanol (Wohllebe et al., 2009) processed and offers an inexpensive photodynamic treatment with solar radiation for controlling vector of parasites (Henderson et al., 1992). The amount of chlorophyll in water is usually highest in summer and lowest in winter because of course it is not easy for plants to grow in winter. There are many human activities that affect chlorophyll in water, such as sewage inputs and destruction of lake and river shorelines (Horne and Goldman, 1994).

Pheophorbide as plant product:

Pheophorbide is a derivative of chlorophyll, is the most active compound which was firstly isolated from *Scutellaria barbata* by bioassay-guided method (Chan et al., 2006). Wohllebe et al. (2009) reported that water soluble pheophorbide produced from chlorophyllin by acidification, when used as low concentrations and added to the water body, were able to kill mosquito larvae and other small organisms within a few hours under exposure of solar radiation.

Pheophorbide ($C_{35}H_{36}N_4O_5$, M.W. 592.69) from a Chinese herbal medicine *Scutellaria barbata* and *Silkworm excreta* has been proved to be potential photosensitizer (Liu et al., 2011). Photodynamic therapy is a specific antitumor protocol that is interested in a number of medical and surgical aspects, and it has been applied on dermatology, gastroenterology, ophthalmology, cardiology, neonatology as well as the anti-virus routinely or in research purpose nowadays (Meisel and Kocher, 2005). PDT requires the presence of two non-toxic elements, photosensitizer and light irradiation, a rapid intracellular stress is generated in target tissues when they are applied at the same time. Reactive oxygen species (ROS) will be produced after photosensitizer receives light energy during illumination in an oxygen-rich environment, which eventually will initiate apoptosis or necrosis in the treated cells (Dalla and Marciani, 2001). Wohllebe et al. (2009) determined EC_{50} in *Chaoborus* at pheophorbide concentration was less than 2mg/l. Pheophorbide was also toxic for the larvae in darkness. Erzinger et al. (2011) determined that about 3-4 h incubation time is necessary before the accumulation of pheophorbide reaches a plateau. Toxicity of pheophorbide was also observed in mouse myeloma cell cultures (Chernomorsky et al., 1999). Earlier, toxicity of pheophorbide against fresh water snail *Lymnaea acuminata* was observed in winter and summer season in laboratory as well as in sunlight condition (Singh and Singh, 2016b). Very recently Singh et al. (2017) studied the photomediated larvicidal activity of pheophorbide a against cercaria larvae of *Fasciola gigantica* in different wave length of light and in sunlight.

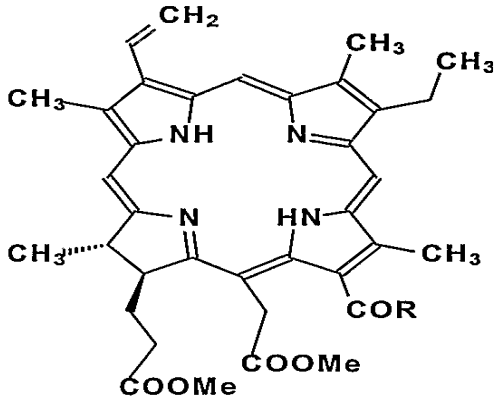


Figure 1: Structure of Pheophorbide

Pharmacological Aspects of Pheophorbide:

Pheophorbide as anti- tumor agent

The photodynamic therapy is a specific anti-tumor practice that is involved in a number of medical and surgical aspects and applied on dermatology, ophthalmology, gastroenterology, cardiology and as well as the

antivirus or in the research purpose nowadays (Meisel and Kocher, 2005). Pheophorbide has been widely applied as anti-inflammatory and anti-tumor agents to human uterine leiomyoma, mammalian and ovarian cancer (Lee et al., 2004). (*Scutellaria barbata*) is photodynamic product and has been reported to inhibit proliferation of a number of cancers including; Jurkat leukemia, pigmented melanoma, pancreatic carcinoma and colonic cancer in the presence of light (Hajri et al., 2002; Lim et al., 2004). Ranjit et al. (2012) studied the potential of pheophorbide a for photodynamic therapy of human epithelial cervix carcinoma (HeLa) and human breast cancer (T47D) cell lines.

Pheophorbide as antioxidant

Among the derivative of pheophorbide (pheophorbide a, b) and pheophytin (pheophytin a, b) the pheophorbide b is strongest antioxidant because of the importance of aldehyde group for functionality (Marquez et al., 2005). Yoon, et al. (2011) studied the photodynamic and antioxidant activities of divalent transition metal complexes of methyl pheophorbide a by DPPH (donating potency of hydrogen) method. Together, these compounds help act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a healing role in aging and various disease processes (Machowsky, 2013, Yoon et al., 2011).

Pheophorbide in therapeutic effects

Photodynamic therapy is an alternative therapy for cancer treatment and it requires the presence of both a photosensitizer and light of an appropriate wavelength, which are non-cytotoxic individually (Parvaiz, 2001). The activation of the photosensitizer by light irradiation, reactive oxygen species (ROS) including singlet oxygen radical are generated. These species induces cellular apoptosis and damage the cellular component such as lipids, proteins and DNA (Parvaiz, 2001). New studies established that pheophorbide is photodynamic in nature, and light possibly will activate pheophorbide to deactivate liver cancer cells (Tang et al., 2006).

It induces inhibitory effects on human hepatocellular carcinoma Hep3B cell as photosensitize with the approach of PDT (Chan et al., 2006; Tang et al., 2006).

Development of a new product:

In 2009, Erzinger and Hader developed and patented at INPI (National institute of intellectual property) a new Bioinsecticide nontoxic biodegradable from a new semi-synthetic derivative of chlorophyll and in conjunction with a formulation system was able to get a product with high stability front light and maintained the same lethal power of chlorophyll and chlorophyllin for mosquito larvae. Currently is being done to study the possible environmental impacts of the continued use of this new method. Synthetic molluscicides have been widely used for the effective control of harmful snails, but it has now been realized that these molluscicides cause serious environmental hazards (Wohllebe et al., 2012). Consequently, more researches are now being focused on molluscicides of plant origin (Wohllebe et al., 2012) as these are easily biodegradable and therefore, safer to use than their synthetic counterparts (Srivastava and Singh, 2005; Kumar and Singh, 2006). Very recently, the molluscicidal activity of different plant products have been reported by numerous workers (Hanif and Singh, 2013; Kumar et al., 2014; Singh et al., 2014; Singh and Singh, 2015a; Singh and Singh, 2016a,b). The data emerging from this research work for preventing the neglected tropical disease fasciolosis/Schistosomiasis.

Molluscicidal Activity of Pheophorbide:

The pheophorbide extracted from spinach has much potential to kill the fresh water snail *Lymnaea acuminata*. Pheophorbide 'a' is a derivative of chlorophyll 'a', is the most active compound which was firstly isolated from *Scutellaria barbata* by bioassay-guided method (Chan et al., 2006). Both extracted and pure pheophorbide are more effective in summer than winter. As in summer water temperature is higher and dissolve oxygen concentration is low, which pose higher mortality of snails and one of the main reasons in increasing the mortality of snail. At higher temperatures the dissolved oxygen concentration decreases, is reflected by higher mortality of the snails. In winter season the cause of less mortality of snail during this period is that in winter, water holds more oxygen (Waterwatch Australia, 2002). Dissolved oxygen is one of the major components which are used by snails during metabolic activity (Ishak and Mohamed, 1975). Consequently, at higher temperatures, the rate of metabolism in the snail body becomes increase (Berge et

al., 2006). Due to their water solubility, it is accumulated in the intestine of exposed mosquito larvae faster in summer season. The activation of the photosensitizer by light irradiation, reactive oxygen species (ROS) including singlet oxygen radical are generated. These species induces cellular apoptosis and damage the cellular component such as lipids, proteins and DNA (Parvaiz, 2001). Reactive oxygen substances damage the cell membranes and other structures of exposed cells, which immediately caused cell death (Kohen and Nyska 2002). Wohllebe et al., (2009) reported that water soluble pheophorbide produced from chlorophyllin by acidification, when used as low concentrations and added to the water body, were able to kill mosquito larvae and other small organisms within a few hours under exposure of solar radiation. Wohllebe et al., (2009) determined EC_{50} in *Chaoborus* at pheophorbide concentration was less than 2mg/l. Highest toxicity of pheophorbide in sunlight summer in comparison to winter is due to the production of toxic singlet oxygen by pheophorbide exposed to light. Toxicity of pheophorbide is time and concentration dependent, as evident from negative regression between exposure period and LC_{50} of pheophorbide.

Discussion

Spinach (*Spinacia oleracae*) is yearly plant but hardly ever biennial and native to central and western Asia and it is produced extensively throughout the world for its edible leaves (Harper, 2010). The leaves contain histamines (*N*-*N*- dimethylhistamine, trimethylhistamine and *N*-acetylhistamine), acetylcholine, and sterols (0.05- o.18%, dry basis), both free and esterified (mainly α-spinasterol, 7- stigmasterol and cholesterol also present). It is a good source of minerals, vitamin-B complex, ascorbic acid, carotene and also an important natural source of Vitamin K. The total content of folic acid in spinach is 0.12 mg/100 g and ascorbic acid (Anonymous, 1985). Pheophorbide is a derivative of chlorophyll is the most active compound which was firstly isolated from *Scutellaria barbata* by bioassay-guided method and pheophorbide is a photosensitizer molecules (Chan et al., 2006). A number of experiments with some other kind of photo sensitizers such as hematoporphyrin or rose Bengal, to fight water-borne parasites, demonstrated the suitability of the treatment but the high costs for these substances prevented their uses in large scale applications (Abdel-Kader and EL-Tayab, 2009). In contrast, chlorophyll can be extracted from all types of green plants such as *spinacaea oleracae* and some weeds at low expenses. Wohllebe et al. (2009) determined EC_{50} in *Chaoborus* at pheophorbide concentration was less than 2.0 mg/l. Pheophorbide is a chlorophyll catabolite that has been proposed as photosensitizer in photodynamic therapy (Rapozzi et al., 2013). It has been advocated by various scientists that chlorophyll derivatives like pheophorbide show more photo-mediated control of disease caused by mosquito and insect larvae (Richter et al., 2014; Erzinger et al., 2015; Azizullah et al., 2014; Wohllebe et al., 2011; Erzinger et al., 2013). The EC_{50} values exposed to pheophorbide for 3h to a light intensity of 147 W/m^2 were 8.44 mg/l and 1.05 mg/l against *Culex* and *Chaoborus* mosquito larvae, respectively (Wohllebe et al., 2011). Tang et al. (2009) reported that pheophorbide a based photodynamic therapy induces mitochondrial-mediated pathway in human uterine carcinoma. Erzinger et al. (2013) noted that illumination of pheophorbide and chlorophyllin, by visible light start reactions leading to apoptosis and necrosis of the cell in the gut, which ultimately caused death of exposed mosquito larvae. Wohllebe et al. (2009) noted that 24.18 mg/l chlorophyllin and 1.05 mg/l pheophorbide treatment against *Chaoborus* sp. larvae was effective in killing of the larvae.

Hence, the toxicity of pheophorbide was observed higher in sunlight condition than in laboratory condition in winter as well as in summer season against fresh water snail *Lymnaea acuminata* (Singh and Singh, 2016b). Earlier, the toxicity of pheophorbide a was observed in different light sources against cercaria larvae of *Fasciola gigantica* and the highest toxicity of both pure and extracted pheophorbide a was maximum (8h LC_{50} 0.006 mg/10 ml and 0.12 mg/10 ml, resp.) in 650 nm spectrum band width (red light irradiance) (Singh et al., 2017). Recently the effect of photodynamic product chlorophyllin on certain biochemical parameter in *Lymnaea acuminata* causative agent of fasciolosis is done by Singh and Singh, (2018). Elhadad et al. (2018) very recently considered the outcome of Chlorophyllin on *Biomphalaria alexandrina* Snails and *Schistosoma*

mansoni larvae. Chaturvedi et al., 2019 recently reviewed on therapeutic and pharmacological aspects of photodynamic product chlorophyllin.

The present review was aimed to revise effectiveness of pheophorbide on the snails with inhibitory result on treated snails. Due to the photodynamic nature of pheophorbide, it has the potential to control fasciolosis. The mechanism of photodynamic remedy of pheophorbide cause snail mortality is not accurately known and well requires advance studies for revelation. The making of chlorophyll derived photodynamically active compound pheophorbide is reasonable and unproblematic and an advanced method to control water-borne disease in developing countries.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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