Pelargonidin, a Dietary Anthocyanidin in the Prevention of Colorectal Cancer and Its Chemoprotective Mechanisms

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Abstract

Diseases of bowl wall mucosa stemming from sudden mutation lead to the development of colorectal cancer (CRC) cells by the transformation of normal epithelial cells into neoplastic lesions. CRC is considered to be a global burden; hence, its incidence rate is expeditiously increased up to ten-fold higher, worldwide. The epidemiological report pinpointed CRC as the utmost third common malignancy in men and second in women. Because of greater efficacy, the synthetic drugs are unsatisfactory due to higher toxic effects to the normal cells, and a chance of developing multidrug resistance by tumor cells. Therefore, dietary flavonoids with potent anticarcinogenic effects have been focused on recent investigations. Pelargonidin (PD), a bioactive molecule classified under anthocyanidin is present in red and pink pigmented berries. PD efficiently modulates intercellular antioxidant status, thereby reducing oxidative DNA damage, cellular proliferation, differentiation, apoptosis, angiogenesis, and reverse drug resistance of metastatic cells, and potentially induces cell cycle arrest, thereby interfering in colorectal carcinogenesis. PD scavenges and normalizes the intracellular reactive oxygen species (ROS), which results in gene mutation and induction of colon carcinogenesis. Therefore, the proliferation of tumor cells would be affected or blocked potentially due to disturbance in cell cycle protein by these ROS. Considering the wide pharmacological benefits of PD, this chapter deliberately reviews the cumulative research data from in vitro human colon cancer cell line studies on chemoprotective property of PD against CRC, and also summarizes the underlying mechanism in experimental models.

Keywords

Colorectal cancer (CRC) · Pelargonidin · Antioxidant · Apoptosis · Chemoprevention

6.1 Introduction

Flavonoids are low-molecular-weight large group bioactive polyphenols with a basic benzo-γ-pyrone as a backbone structure, and are widespread in a variety of plants. These bioactive compounds potentially induce apoptosis and modulate tumor cell proliferation, angiogenesis, and differentiation, thereby directly interfering at each step of carcinogenesis (Ramos 2007). A number of research studies evidenced that

secondary metabolites include flavonoids which are phenolic in nature and possess several pharmacological activities based on its unique chemical structure (Mahomoodally et al. 2005). Basically, the degree of chemical reactions such as hydroxylation, substitutions, polymerization, and conjugations determines its specific chemical properties. More interestingly, the antioxidant, metal chelating, and free radical scavenging efficiency of flavonoids were enumerated by the number of hydroxyl groups present in the flavonoids. Chelating property of flavonoids primarily prevents damage of biomolecule by stress-induced intracellular free radicals. Previous studies had evidence that flavonoids possess a chemoprotective effect against various degenerative and infectious diseases like cancer and cardiovascular diseases. Pelargonidin (PD), a bioactive molecule classified under anthocyanidin, is present in red and pink pigmented berries. This phytochemical efficiently modulates intercellular antioxidant status, thereby reducing oxidative DNA damage, cellular proliferation, differentiation, apoptosis, angiogenesis, and reverse drug resistance of metastatic cells, and potentially induces cell cycle arrest, thereby interfering in colorectal carcinogenesis. PD scavenges and normalizes the intracellular reactive oxygen species (ROS), which results in gene mutation and induction of colon carcinogenesis. Therefore, this chapter aims to provide a cumulative research data from in vitro human colon cancer cell line studies on chemoprotective property of PD against CRC, and also focuses to summarize the underlying mechanism in experimental models.

6.2 Colorectal Cancer (CRC): A General Overview

Rapid growth and progressive spreading through adjoining tissues lead to metastasis, and the formation of secondary tumors at different sites is the basic nature of the tumor cells (Tanaka 2009). Successive angiogenesis and uncontrolled proliferation of stem cells are the two important hallmarks of carcinogenesis (Neergheen et al. 2010). Colorectal cancer can be mentioned as a mucosal disease. Bowel wall mucosal lining is found to be the basal origin of colon cancer. This bowel wall anatomically consists of different layers, such as mucosa, submucosa, the serosa, and the muscularis propria. In particular, single-layered columnar epithelial goblet cells of bowel wall were termed as mucosa, which produces mucus, and identified as the primary site of colon cancer induction due to genetic mutations. The mutated cells in this region usually proliferate very rapidly and spread into the lumen. Further progression takes place at microvilli, where the crypts of Lieberkuhn were reprocessed. The strongest layer stand under mucosa is submucosa, and it is considered as an important region for carcinogenesis, and hence this layer includes vascular blood vessels and terminal and lymphatics nerve fibers by the way the tumor cells invade the circulatory system and spread throughout the body.

6.2.1 Colorectal Cancer Epidemiology

Worldwide, third most frequently recorded cancer in men and the second common cancer in women was statically reported as CRC (Ferlay et al. 2015). The incidence

rate increased up to ten-fold higher worldwide with a steep increase in notable countries, such as Australia, New Zealand, the Czech Republic, Slovakia, Kuwait, Israel, and low middle-income countries. Increased risk factors including an unbalanced diet, increased body weight, alcohol, and habitual tobacco consumption are the major reasons behind the sudden increase in the prevalence of CRC in Asian and Eastern European countries (Torre et al. 2015). Moreover, poor diagnosis at initial stage of CRC would lead to metastasis, and cordial epidemiological report had stated that it would be the universal burden which would record more new colon cancer cases approximately 2.2 million peoples and mortality may reach up to 1.1 million by the year of 2030 (Arnold et al. 2017; Ouakrim et al. 2015). Even though the mortality rates throughout the world have been declining in a huge amount in a number of countries, mortality still occurs in few countries like Romania, South America, Russia, and Brazil (Torre et al. 2015).

6.2.2 Etiology of Colorectal Cancer

The major lifestyle-related risk factors include lack of physical activity, over bodyweight, red meat and canned food intake in large amounts, habitual smoking, consumption of alcohol, and a diet lacking in vegetables and fruits. Previous family or individual history of colon polyps, and individual history of type 2 diabetes, bowel disease due to chronic inflammation, or genetic diseases such as Lynch syndrome or familial adenomatous polyposis has been proved as an important risk factor for CRC. The precise causes of colorectal cancer seem to be unknown. However, few studies state the etiology of developing colorectal cancer. The prevalence of CRC has been gradually increasing in urbanized countries, and also the rate of incidence in economically transitioning countries has been raised due to Western lifestyle culture. High fat, high calories diet, processed meats, and low fiber intake are strongly associated with the development of colorectal cancer. It has been proved that the chemical carcinogen produced while the meat was overcooked at a very high temperature, which includes frying, boiling, and grilling, is prone to cause colon cancer (Gingras and Béliveau 2011). Sedentary lifestyle and obesity play a major part in the development and progression of the number of cancer including CRC were evident by epidemiological reports. Lack of physical activity and increased body weight are the two important modifiable and inter-relatable risk factors that account third of CRC. The physical activity, which stimulates the movement of colon and the passage of waste through the colon activity, is associated with a decreased risk of CRC (Lee et al. 2007). Epidemiological evidence indicates that increased consumption of red meat such as beef, pork, and lamb are prone to cause colorectal cancer in both men and women. Stastical data had shown substantial proportion, ranging from 30% to 70%, of all colorectal cancer cases are attributable to diet with red and processed meat intakes, which is implicated as important dietary risk factors. Red meat, processed meat, and meat derived heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), nitrites, and nitrates are the known animal mutagenic compounds formed when muscle meat is cooked using high temperature. Nitrites are

harmful and are more susceptible to develop cancer at proximal colon, whereas HCAs and PHAs cause cancer in the region of the distal colon and rectum. Greater red meat and processed meat intakes were more consistently associated with colorectal carcinoma (Miller et al. 2013).

6.2.3 Pathogenesis of Colorectal Cancer

In accordance to etiology of CRC, chronic inflammation and sudden mutation resulted in the formation of polyps on distinct sites of colon and rectum, and epithelial lining is the primary characteristic of colorectal cancer development, and these polyps can be nonneoplastic or neoplastic. The microenvironment of cancer cells and epigenetic changes along with genetic mutations play a key role in multistep carcinogenesis. These epigenetic mechanisms mostly programmed in most of the cancer cells in the process of DNA methylation along with alterations in histone proteins (Bardhan and Liu 2013). The CRC development in most of the cases would be a subsequential modulation in signaling pathways as a result of respective oncogenes activation and also inactivation of tumor suppressor genes. In addition, chromosomal gains or losses are termed as chromosomal instability (CIN), and sudden insertion/deletion in the specific DNA sequence would be the reason for microsatellite instability (MSI) (Jass 2007). Most often, etiology of distal colon cancer development and progression was mainly due to CIN, which accounts for about 60–70% of total CRC cases. However, most common proximal colon cancer was observed in MSI cases. Mutations in adenomatous polyposis coli (APC) were induced by dysplastic aberrant crypt foci, where it pawed away in the transformation of polyps to cancer by activation Wnt pathway. Such mutations in APC or genes involved in Wnt signaling pathways such as KRAS or TP53 would help in the progression of dACF to tumor cells (Nosho et al. 2008). Also, epigenetic alterations in the promoter region of the respective gene including DNA methylation and histone modifications is the key factor in cancer cells epigenetic inheritance DNA methylation involves the enzymatic addition of a methyl group to the 5′-position of cytosine by DNA methyltransferases (DNMTs) to form 5-methylcytosine. The DNMTs work on specific CG dinucleotide sequences, known as CpGs. More than 70% of the cytosine (C) bases in the context of CpG dinucleotides are supposed to undergo methylation. The biallelic promoter CpG island methylation of the mismatch repair gene MLH1 is characterized to be the primary epigenetic mechanism leading to the development of sporadic colorectal cancer associated with MSI-H (Bardhan and Liu 2013). Histone deacetyl transferases (HDACs) are the most widely characterized proteins among the histone modification enzymes that play predominant roles in the development of CRC. The high HDAC expression has been shown to be associated with reduced survival in CRC patients (Ashktorab et al. 2009).

Intracellular oxidative stress is mainly because of the imbalanced state of unstable highly reactive free radicals and the intermediate metabolites. These free radicals were commonly called reactive oxygen species (ROS), and also the inbuilt cellular mechanism to neutralize and protect cells from such ROS is termed as

antioxidants. ROS rapidly attacks the bricks of the cellular components such as all the important biomolecules that cause serious damage and defect to the whole organism (Ďuračková 2010). Intensive studies on oxidative stress mechanism during the last 20 years by a number of researchers have unmasked its key role in chronic inflammation; thereby, it has a significant role in the progression of several inflammation-related disease conditions, including cancer, cardiovascular, diabetes, pulmonary, and neurological diseases. When cells encounter a sudden attack by oxidative stress, it triggers several subsequent inflammation-related molecular pathways, such as NF-κB, HIF-1α, AP-1, PPAR-γ, p53, β-catenin/Wnt, and Nrf2 (Reuter et al. 2010). Widely occuring procarcinogens, the toxin from environmental pollution, and intracellular phagocytosis by chemo drugs generate an enormous amount of ROS as the metabolic intermediates (Klaunig et al. 2011). Oxidative stress has been found to take part in the aging process, and also it has a major impact on the prevalence of cancer in old age. More interestingly, any serious damage to the genomic DNA or mitochondrial DNA due to ROS will imitate carcinogenesis in most cases. Further, such a process may vary based on the type of tissue or triggered/suppressed transcription of certain gene or modulation in the signaling pathway or some mutational errors in replication, which would significantly result in genetic instability. It has also been studied that a notable amount of intracellular ROS was generated in tumor cells which leads to a specific microenvironment that maintains constant oxidative stress in tumor cells which favors cancer progression. Human cancer is a major activator of antioxidant defense mechanisms. With respect to its apoptosis-inducing capacity, molecules that promote ROS production may be novel therapeutics, proposed to selectively target cancer cells by elevating ROS levels beyond a tolerable threshold to induce mitochondrial outer membrane permeabilization (MOMP) and cell death (Graham et al. 2010).

Most of the common physical work and intermediates of cellular metabolism results in the inevitable production of oxidants and free radicals. At the same time, an inbuilt protective mechanism for the human body is antioxidants and that will be different for each cell type and tissues, and its mode of action might vary as a synergistic effect or even antagonistic effect based on the site of action. The cellular antioxidant defense system includes natural enzymatic antioxidant, namely catalase (CAT) which hydrolyzes H_2O_2, superoxide neutralizing enzyme superoxide dismutase (SOD), glutathione-dependent catalytic enzymes glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), and nonenzymatic antioxidants such as retinol (Vit A), ascorbic acid (Vit C), and tocopherol (Vit E) which includes vitamin A, vitamin C, vitamin E, polyphenols, pigmented carotenoids, and other natural by-products as antioxidants, paying attention great interest in recent decades. Antioxidants at very low concentrations have a higher efficacy to prevent any oxidative damage to cells when compared to antioxidants that donate an electron to stabilize free radicals and thereby inhibit its detrimental effects. SOD is an endogenous antioxidant that involves catalysis dismutation reaction, thereby producing hydrogen peroxide (H_2O_2) from most of the superoxides (O_2^-). Further, CAT catalyzes H_2O_2, the end product of SOD, and splits them into oxygen and water. On the other hand, glutathione-dependent enzyme glutathione peroxidase

(GPx) significantly neutralizes the transition metals, and with the help of GSH as cofactor, it also catalyzes H_2O_2 in the aqueous phase of the cell membrane (Valadez-Vega et al. 2013). GR catalyzes the reduction of oxidized glutathione and thereby maintains the cellular GSH level, thus altering the GSH/GSSH ratio. Further, GSH and intracellular electrophile conjugation were facilitated by glutathione-S-transferase (GST), also called Phase II detoxifying enzymes. The conjugation of GSH to electrophilic compounds is catalyzed by Phase II detoxifying enzymes called glutathione-S-transferase (GST). GSH has been considered as an important natural intracellular antioxidant and has a major role in neutralization and detoxification of ROS such as peroxides, which is produced as a result of increased LPO. It also takes a role in conjugation and excretion of toxic molecules, and thereby it maintains the normal cell structure and function (Dickinson and Forman 2002).

Human beings are constantly bombarded by ROS by means of exogenous factors, such as tobacco, smoking, chemicals, ultraviolet (UV) rays, and many other agents. It also includes drugs or medicines which are used for medical practice. The aerobic cellular metabolism generates highly reactive free radicals in terms of oxidants, which cause serious damage to different types of cells and tissues due to normal physiological conditions. The protection mechanism against infection involves NADPH enzyme activity found in neutrophils and macrophages, and also the electron transfer system of mitochondria or microsomes and peroxisomes takes a vital role in the production of ROS endogenously (Noda and Wakasugi 2001). ROS are not only prone to cause cancer but also many other human diseases (Hippeli et al. 1999). The necrotic lesion was formed by the peroxidation of the biological membrane by highly reactive oxygen with lipids (Halliwell 1994). These ROS in higher amounts alter the biomolecules, such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA). The active oxygen species also induce gene mutation, which results in the development of cancer; on the other hand, it also modulates signal transduction and either triggers/suppresses transcription factors which are also a mechanism of induction of carcinogenesis (Floyd et al. 1986). Tumor suppressor genes p53 and gene involved in cell cycle regulation are highly susceptible to mutations as a result of DNA damage in the presence of ROS. Also the product of LOP, malondialdehyde, potentially induces mutation. Many researchers have pointed out the necessity of enzymatic and nonenzymatic antioxidants to maintain the natural oxidative homeostasis of the organism especially in tumor therapy. Endogenous enzymatic antioxidants, such as SOD, CAT, GPx, GR, and GSt, play a vital role in chelating superoxides and peroxides. Therefore, tumor therapy is basically based on oxidative stress and antioxidant homeostasis (Čipak gašparović et al. 2010).

Plant products are widely used in the present world as medicines, due to the fact that they are very effective therapeutically. Moreover, they are relatively more safe when compared to any synthetic drugs (Singh and Tripathi 2018; Datir 2018; Akhtar and Swamy 2018). Natural products, mainly plant metabolites, exert superior antitumor properties by reducing the action of reactive oxygen species and protecting the critical constituents of cells, that is, macromolecules like nucleic acids and proteins from oxidative damages (Ravichandra et al. 2018). As a result, plant-derived metabolites have attracted researchers in the field of natural medicines to design effective chemodrugs for

treating cancers. Thus, plant-derived compounds are well recognized as anticancer agents, and some of them are being used in the present market for treating various cancer types (Akhtar and Swamy 2018). Drug molecules like camptothecin, vincristine, and vinblastine are some of the examples of chemodrugs derived from plant sources. However, these drugs pose a challenge of toxicity and cause side effects. Hence, a continuous search is in progress to innovate novel drug molecules against cancers. In this regard, different plant species are being explored to isolate new phytochemicals and to evaluate their bioactivities. Likewise, plant molecules are also evaluated for their antiproliferative potentials. Pelargonidin, an anthocyanidin, is among them, and the details of it are given in the following section. Also, the role of a phytochemical against different CRC cells is discussed, emphasizing on its mechanisms of actions.

6.3 Pelargonidin: An Anthocyanidin

The chemical structure of anthocyanins consists of flavylium cation, which is linked to hydroxyl (–OH) and/or methoxyl ($–OCH_3$) groups along with one or more sugar molecules. Anthocyanins without sugars are known as anthocyanidins. Six major anthocyanidins which are abundant in fruits and vegetables are pelargonidin, malvidin, delphinidin, cyanidin, peonidin, and petunidin. They differ by position and number of –OH and $–OCH_3$ group present in it. Based on the position of the functional group, either hydroxyl, methyl, sugar moiety, or several other functional substituents, about 500 and more anthocyanins have been identified and are classified under 31 anthocyanidins. Cited previous literature stated that 31 anthocyanidins account for cyanidin (30%), delphinidin (22%), and pelargonidin (18%). Also, other methylated derivatives of anthocyanidins account for 20% of all anthocyanins. Consequently, about 90% of all the anthocyanins are cyanidin, delphinidin, pelargonidin, and its derivatives (methylated). Naturally, these anthocyanidins are present in the form of glycosides with the respective sugar moiety at 3-position on the C-ring or the 5-position on the A-ring to the aglycone chromophores. Basically, pelargonidin can be chemically structured as 1-benzopyrylium, 3,5,7-trihydroxy-2-(4-hydroxyphenyl), chloride, n anthocyanidin cation that is flavylium substituted by a hydroxy group at positions 3′, 5′, 7′, and 4′ (Barnes et al. 2011).

6.3.1 Natural Occurrence of Pelargonidin

Anthocyanins are one among the subgroup of water-soluble flavonoids found widespread in the plant kingdom, and their specific compounds are responsible for floral pigmentation and other parts of plants. The color of the pigments may vary from vibrant red, purple, to blue pigments, and this is because of its conjugated structure that draws light at 500 nm (Wang et al. 2012). In particular, widespread and usually found anthocyanins are the 3-*O*-glycosides or 3,5-di-*O*-glycosides of delphinidin, cyanidin, petunidin, peonidin, malvidin, and pelargonidin. Anthocyanins are important polyphenolic components of fruits, especially berries. Bioactive component

pelargonidin is rich in berries, such as strawberry, blackberry, black currant, elderberry, sour cherry, and pomegranate. Consumption of anthocyanin and polyphenol-rich juice enhanced antioxidant status, reduced oxidative DNA damage, and stimulated immune cell functions (Ferretti et al. 2010).

6.3.2 Pharmacological Activities of Pelargonidin

Flavonoids most prevalently found in floral, fruit, and vegetable have been reported as pelargonidin and consuming them as fresh or processed food has a major impact in prevention and control of devastating chronic human diseases, such as inflammatory diseases, cancer, and cardiovascular diseases, where it directly acts as potent antioxidant, plays significant role in detoxification, induces apoptosis, fights against inflammation, etc. (Nikkhah et al. 2008). The antioxidant property of pelargonidin (anthocyanidin) was due to scavenging efficacy of ONOO− function group, and it was evidently first reported by Tsuda et al. (2003) (anthocyanidin). The mechanism behind its antioxidant activity was due to the conversion of pelargonidin to p-hydroxybenzoic and further formation of 4-hydroxy-3-nitrobenzoic acid by the catalytic conversion with ONOO− present in its structure. In vitro studies on anthocyanin had significantly downregulated the signaling pathways including NF-κB and MAPK pathways, resulting in the reduced proinflammatory cytokine expression and inhibiting inflammation (Wang et al. 1999; Pergola et al. 2006). In addition, extensive study on literature of anthocyanidin unraveled its anti-inflammatory mechanism, where significant downregulation of certain vital inflammatory markers at both transcription and translational levels in some cell line models such as downregulation of cyclooxygenase-2 (COX-2) expression in stimulated macrophage (RAW 264 cells) with lipopolysaccharide (LPS) and another case potential inhibition was observed against the transcription and expression of inducible nitric oxide (iNOS) murine macrophages (J774) activated with LPS (Hou et al. 2005; Hämäläinen et al. 2007). Isolated leucopelargonidin-3-*O*-alpha-L rhamnoside from the bark of *Ficus benghalensis*, oral administration at a dose of 100 mg/kg, showed a significant decrease in glucose level with a rise in serum insulin level of diabetic dogs induced by alloxan at a time period of 2 h. The findings state that leucopelargonidin-3-*O*-alpha-L rhamnoside stimulates insulin secretion (Augusti et al. 1994). Also, few studies have reported the estrogenic property of anthocyanidins, including pelargonidin, delphinidin, and cyanidin. These anthocyanidins were categorized as nongenotoxic, and it efficiently modulates DNA damage caused by oxidative stress and 4-nitroquinoline 1-oxide by its estrogenic activity. Therefore, the stated study has substantial evidence for the chemopreventive effects of anthocyanins against carcinogenesis on HL-60 cells (Abraham et al. 2007). A recent study on *F. benghalensis* root extract showed an antimicrobial activity when compared to the standard drug. Also, two important flavonoid compounds such as leucopelargonidin 3-*O*-alpha-L rhamnoside and 5, 3′-dimethyl ether of leucocyanidin 3-*O*-alpha-D galactosyl cellobioside were isolated from the bark of *F. benghalensis*, which inhibits the Gram-negative bacteria and also the fungal species (Aswar et al. 2008).

6.3.3 Bioavailability

Till now, very few numbers of literature are available to understand bioavailability including absorption/excretion of anthocyanins in humans, still, we found some of the contradictory conclusions. Previous studies have shown that wine from graphs rich in pelargonidin, it may or may not contain alcohol do not block the anthocyanin's absorption (Bub et al. 2001; Frank et al. 2003). Later, few other studies by Wu et al., (2006) also stated the similar pattern of specific anthocyanin pelargonidin-3-*O*-glucoside absorption form strawberries and blackberries (Wu et al. 2006). Felgines et al. (2007) stated that pelargonidin-3-*O*-glucoside present in strawberry was metabolically converted into respective pelargonidin glucuronides and pelargonidin sulfate and got excreted in the urine along with a very minute concentration of pelargonidin glucoside and pelargonidin alone a trace of pelargonidin as parent glucoside and its aglycone. More than 80% of urinary excreted pelargonidin accounts as pelargonidin glucuronides (Felgines et al. 2005). Bioactive compound like Cyanidin-3-*O*-glucoside, 3DA or 3 deoxy derivative, pelargonidin-3-*O*-glucoside, which is present in blackberry was reported as much as less than ten times its bioavailability with the corresponding 0.16% of intake and it is excreted as glucurono, sulfo and methylated metabolites which account for about 13% (Felgines et al. 2005). Pelargonidin glucuronides along with pelargonidin-3-*O*-glucoside are the two forms of anthocyanidins from strawberries found in the blood stream, and it undergoes a metabolic modification before being excreted in the urine along with trace quantities (1–3%) of pelargonidin and pelargonidin-*O*-sulfate, whereas the remaining quantity of pelargonidin was detected in plasma. In human beings on ingestion with strawberries pelargonidin-3-*O*-glucoside, it was metabolically converted to pelargonidin-*O*-glucuronide, which is present predominantly in both plasma and urine rather than the parent glucoside. The Cmax of this metabolite was found to be 274 ± 24 nmol/l after 1.1 ± 0.4 h. The major anthocyanin in strawberries, pelargonidin-*O*-glucuronide, was excreted after its metabolic process within the period of 24 h after its intake (Mullen et al. 2008).

6.4 Anticancer Mechanisms of Pelargonidin

6.4.1 Anticancer Activity Via Inducing Apoptosis

Pelargonidin, an anthocyanin found widespread in most of the berries including blackberry, raspberry, strawberry, and pomegranate, were studied in human colon adenocarcinoma cells (HT-29) (Felgines et al. 2007; Beekwilder et al. 2005). The primary anticancer effect of pelargonidin was screened with well-defined in vitro MTT assay on HT-29 cells. Followed by treatment with a determined concentration of pelargonidin (GI_{50}) against HT-29, induction of apoptosis in cancer cells had been reported. Also, the same study had stated the activation of caspase-dependent apoptotic pathway along with the fragmentation of genomic DNA. Additionally, the microscopical observation of treated cancer cells was found to be pictured with

distinct morphology, including floating cells, cellular shrinkage, and membrane blebbing, which are the hallmark features of apoptosis. A similar pattern of cell morphology in another study was reported as characteristic features of apoptotic induction in cancer cells (Sivalokanathan et al. 2006). HT-29 cell architecture had been pictured with high alterations such as round-up of cancer cells with distorted patterns (Karthi et al. 2016). AO and EtBr (AO/EtBr) fluorescent screening had further pictured the intensive morphology of apoptosis-induced HT-29 cancer cells after pelargonidin treatment in a dose-dependent manner. Basically, AO dye stains both live cells with the rigid cell membrane and permeable dead cells, whereas EtBr stains only apoptotic cells with no cell membrane integrity (Raju et al. 2004). Pelargonidin supplement had reported the notable percentage of both early and late apoptotic colon cancer cells with morphological alterations during apoptosis such as cell shrinkage along with chromatin condensation.

The development and maintenance of a living organism have to be brought by the standard and controlled the homeostatic balance of both cell proliferation and cell death. Numerous physiological functions and pathological processes were based on the key role played by the apoptosis of individual cells. Modulation in the expression pattern of certain antiapoptotic proteins (Bcl-XL, Bcl-2, and A1) was notably unregulated by the specific transcription factor of NF-κB, and that was a key role in the inflammation process. Also, the unique transcription factors of this Bax family along with the contribution of NF-κB induce apoptosis. Apoptotic induction by the pelargonidin in HT-29 cells was illustrated with DNA fragmentation protocol, which has been considered as exemplary biochemical features of apoptosis. The ladder pattern after pelargonidin treatment was evident as a result of oligonucleosomal fragmentation of chromosomal DNA at the early apoptotic stage. The previous research study had stated that pelargonidin with short incubation time brings about early apoptosis and longer exposure of pelargonidin was reported to increase the volume of nuclear fragmentation which evidences the basal mechanism of anticancer effect of the pelargonidin, and also it may act through the apoptotic signaling. Bcl-2 family are known cytoplasmic proteins that regulate apoptosis, in particular sub-group of pro-apoptotic proteins Bax, significantly promotes cell death where other members of the Bcl-2 possess anti-apoptotic activity namely, Bcl-x Land Bcl-w, those includes four regions of similarity with Bcl-2. In this review, it is significant to pinpoint that pelargonidin alters the expression level of Bcl-2 family proteins in cancer cells and thereby induces apoptosis. Consciously, the expression of these particular proteins after pelargonidin treatment was deliberated with the immunoblot analysis, and the study has reported that the expression level of Bax was found to be markedly increased dose dependently in HT-29. At the same time, pelargonidin modulations in BH3 interacting-domain (BID) death were also upregulated. As per the study by Karthi et al., (2016), this treatment also downregulates the expression of antiapoptosis protein Bcl-2 and Bcl-xL Briefly, this review conveys that pelargonidin specifically promotes the translocation of mitochondrial Bax to the cytosol and triggers the signaling pathway for apoptosis in colon cancer cells.

6.4.2 Pelargonidin and Mitochondrial Pathway

The apoptotic proteins from the mitochondria due to its permeabilization make the cancer cells to induce apoptosis by three major toxic proteins: direct caspase activators, indirect caspase activators, and caspase-independent proapoptotic factors. Initially, the apoptosome was formed by conjugation of cytochrome c with apoptosis-protease activating factor pro-caspase-9, thereby caspase-9 gets activated. Subsequently, this initiator caspase-9 activates downstream caspases 3, 6, and 7, which were denoted as effectors, thereby it activates the last step of the pathway to induce apoptosis (Pradelli et al. 2010). Tumor suppressor p53 induces autophagy and/or apoptosis through its cytosolic and nuclear effects in response to DNA damage and other stresses. Oxidative stress, i.e., increased production of reactive oxygen species (ROS), can also induce apoptosis and autophagy (Fimia and Piacentini 2010). As in the case of pelargonidin treatment against colon cancer cells, Bcl-2 family members that come under proapoptotic activity significantly activate mitochondrial-mediated intrinsic apoptosis, where pelargonidin potentially induces the loss of mitochondrial membrane potential, which in turn activates caspase-9 cascade. Briefly, the cascade was initiated with the release of cytochrome C followed by activation of caspase 3 and caspase 7, where the program cell death was initiated with sequential degradation of cellular functional proteins (Karthi et al. 2016; Adams 2003). On the other hand, this review also explores the upregulation of apoptotic proteins on pelargonidin exposure such as PARP and P53. Based on the previous literature and the various study reports, the current review has summarized the activation of the intrinsic apoptotic mechanism of pelargonidin against colon cancer cells.

6.4.3 Pelargonidin Affects Cell Cycle Regulators

Tumor cell possesses unique characteristic futures by modulating the regulation of the cell cycle in cancer cells. This chapter also concentrates on elaborating on the mechanism of cell cycle arrest induced by pelargonidin in colon cancer cells. Basically, the transition between cell cycle phases is tightly regulated by cyclin-dependent kinases (Cdk). The role of Cdks is to control cell cycle progression by phosphorylation of protein substrates on serine and threonine amino acids. The protein substrates have specific roles that contribute to the cellular events that occur during the cell cycle. For example, Lamin B protein is phosphorylated by Cdk1 during mitosis, which causes nuclear envelope break down. The correct timing of cell cycle phase changes is regulated by specific cyclin-dependent protein kinase complexes. Cyclin-dependent kinases are composed of two proteins: a catalytic subunit known as Cdk and a regulatory protein subunit known as cyclin. The cyclin proteins do not have enzymatic activity; however, they bind and activate the catalytic 6 subunits. At least 25 catalytic Cdks have been described, and each binds to at least one of a large family of cyclin binding partners, creating many possibilities for Cdk cyclin protein pairings (Bruyère and Meijer 2013). The timing of the synthesis of specific cyclins is carefully regulated, which ensures that certain Cdk-cyclin

complexes trigger different stages of the cell cycle. For example, the Cdk4/cyclin D complex functions early in the G1 phase of the cell cycle in response to growth factors, whereas the Cdk1/cyclin B complex enables cells to enter mitosis. There are several biochemical steps required to activate Cdks in addition to cyclin synthesis, thus ensuring cell cycle fidelity. The cell is blocked in the G2 phase of the cell cycle if Cdk1 remains phosphorylated on tyrosine 15, which holds the enzyme in an inactive state regardless of cyclin B1 levels. Eukaryotic cells (including human cells) have a second biochemical system that can regulate CDK activity if the cell has damaged DNA. Another protein kinase, Chk1 (Checkpoint kinase 1), can prevent the activation of Cdk1 by phosphorylating and promoting the degradation of Cdc25 phosphatases. Without Cdc25 phosphatases, cells cannot dephosphorylate tyrosine 15 of the catalytic subunit of Cdk1, thus remain blocked in interphase, usually the G2 phase. Overall, a series of protein phosphorylation and protein synthesis pathways converge to regulate cyclin-dependent protein kinases. It appears that the complexity of these steps provides cells with opportunities to ensure that their genomes are accurately copied and distributed to daughter cells while avoiding genome change (Smith et al. 2007). This part of the chapter specifically focused to reveal the cell cycle arrest and checkpoint adaptation due to induced DNA damage and interface with the cancer cells to enter mitosis. Cells with damaged DNA initiate a biochemical pathway called the "DNA damage checkpoint," which causes a delay in the cell cycle during S phase or G2 phase to allow repair. It is believed that the role of DNA damage checkpoints is to prevent damaged DNA from being transmitted to daughter cells. Cells with damaged DNA initiate a biochemical pathway called the "DNA damage checkpoint," which causes a delay in the cell cycle during S phase or G2 phase to allow repair. Thus, the review study has strongly deliberated the role of pelargonidin-induced DNA damage, and its role in cell cycle checkpoints is to prevent damaged DNA from being transmitted to daughter cells. The DNA damage checkpoint is composed of several overlapping checkpoint systems. S-phase checkpoint provides continuous monitoring of the DNA during DNA replication to ensure that blocked replication complexes or damaged DNA were repaired before replication. The successful completion of DNA replication is assessed in the G2 checkpoint. The G2 checkpoint enables cells to detect unreplicated DNA or DNA that may have been damaged by a variety of means such as genotoxic agents. The cell will delay its progression through the cell cycle at the G2 phase until errors are corrected (Palou et al. 2010). The previous study of our laboratory had strongly pinpointed that the G2/M phase arrest of colon cancer cells was significantly induced by pelargonidin treatment in a dose-dependent manner and take part in a potential role of regulating the expression of subsequential cell cycle regulatory proteins (Karthi et al. 2016). Also, pelargonidin has a minimal effect to inhibit the cells at the G1-S phase of the cell cycle in cancer cells and is mainly due to transition-related CDKs, and their expressions were highly modulated by pelargonidin with inhibiting the function of mutated P53 in colon cancer cells. Thus, the literature study on anthocyanidin, pelargonidin the drug of interest, has high efficacy and efficiency to modulate the expression of functional protein in inhibition of CRC, and thereby it possesses a significant anticancer activity which was summarized in this chapter.

6.5 Conclusions

This comprehensive review about the anticarcinogenic effect of pelargonidin concludes that most of the commercial synthetic and isolated compounds used to treat cancer not only kill cancer cells, but are also proven to be highly cytotoxic to normal cells and would cause a severe adverse effect after therapy. Whereas, pelargonidin being pinpoint and speculate that naturally occurring dietary flavonoid acts as an anticancer therapeutic agent, which inhibits colon carcinogenesis by activating the immune system and by regulating the hyperproliferation, inflammation, angiogenesis, and mitochondrial-mediated apoptosis. In particular, this chapter signifies the importance of pelargonidin in the treatment of colon cancer. Briefly, the review study states that pelargonidin can affect the basic cell functions associated with cancer development. Also, it induces significant cell cycle arrest and apoptosis in cancer cells; thereby, pelargonidin would control the formation and progression of tumors. The previous literature evidence that any alteration in the inhibition of proteins involved in the cell cycle would potentially reflect in inhibition of cell proliferation and block carcinogenesis in tumor cells. Thus, this chapter highlights the pharmacological potential of pelargonidin, and it may be very useful in the prevention of oxidative stress-induced diseases and takes part in vanquishing colon carcinogenesis. Further, a detailed study on pelargonidin would effectively promote the development of new antitumor drugs and regimens for human colorectal carcinoma.

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