

## Role of Flavonoids in DNA Damage and Carcinogenesis Prevention

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### Abstract

High level of ionizing radiation and various other mutagenic agents can damage DNA and induce carcinogenesis which may be transferred to offspring's. Flavonoids are major antioxidant compounds obtained from plants and form a significant proportion of most diets. In this review, we have summarized the present understanding of the roles of various flavonoids as preventive approaches against DNA damage and carcinogenesis.

**Keywords:** Antioxidant; DNA damage; Flavonoid; Mutagenesis

### Introduction

In living cells, free radical species derived from oxygen (ROS) and nitrogen (NOS) are produced endogenously by mitochondria, cytochrome P450 and peroxisome, which act as signaling molecules and control metabolic pathways and the cell cycle [1]. Usually, cells have their defense mechanisms such as antioxidant enzymes catalase and superoxide dismutase and free radical scavengers like glutathione (GSH) for the removal of free radicals. However under stress conditions such as exposure to radiation or chemicals, this balance is disturbed and excessive free radicals are generated that cannot be cleared by natural defenses and can lead to DNA damage and carcinogenesis.

Many factors are known to cause DNA damage. Ionizing radiation is one of the main DNA damaging and carcinogenic agents. Radiation induced DNA damage signaling and repair pathways play important role in mutagenesis and carcinogenesis. Other major DNA damaging agents are cigarette smoke, chemical carcinogens and food borne carcinogens.

DNA damage includes various types of lesions ranging from isolated base lesions or single strand breaks (SSBs) to complex lesions like double strand breaks (DSBs) and other non-DSB such as oxidatively generated clustered DNA lesions (OCDLs) [2]. DNA damage and inefficient repair are known to cause cellular injury and initiate the process of carcinogenesis.

Various types of natural compounds are used in cancer prevention and cancer therapy [3]. Recent studies have explored the potential of flavonoids obtained from food sources in the prevention of various oxidative stress induced diseases such as cancer and cardiovascular diseases. Flavonoids regulate many cellular, biochemical and immunological events involved in cancer initiation and progress [4]. A number of studies have demonstrated ability of flavonoids to protect cellular DNA against damage [5].

Many molecular biology methods such as TUNEL assay, Flow and laser scanning cytometry, Nick translation and electron microscopy are used to detect DNA strand breaks [6]. Two different types of strategies

categorized as direct and indirect methods are used to measure radiation-induced DNA damage [7]. The most commonly used indirect methods to visualize the DNA damaging protective effects of flavonoids are micronuclei formation and comet assay, which can measure biological consequences of DNA damage. Also, calf thymus DNA and plasmid pBR322 DNA is commonly used to study protective effects of flavonoids. Many studies have also linked protective action of flavonoids with their anti-oxidant nature or ability to regulate cell signaling [8].

In this review we have focused on summarizing the current understanding of the mechanisms of action of widely used flavonoids to explore their potential use in the prevention of DNA damage and carcinogenesis.

### DNA Damage Inducing Agents and Biological Responses

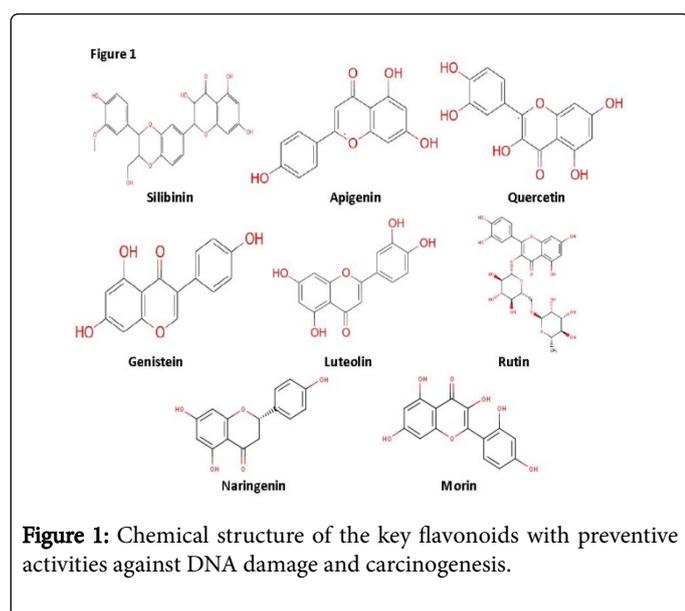
DNA can be modified and damaged by various endogenous and exogenous factors. Common endogenous factors include Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) generated during normal metabolic functions, errors during DNA replication and spontaneous or enzymatic conversions

A major exogenous or environmental factor is ionizing-radiation which can induce the formation of DSBs. Other exogenous factors include non-ionizing ultraviolet radiation through biochemical modifications and chemical compounds such as cisplatin, benzo[a]pyrenes (BaP), nitrogen mustards, methyl methanesulphonate (MMS), N-nitroso-N-methylurea (NMU) and N-ethyl-N-nitrosourea (ENU) [9]. Tobacco products and chemical found in food such as aflatoxins found in contaminated peanuts and heterocyclic amines in over-cooked meats are also classified as cancer-causing DNA-damaging agents [10]. Chemicals bind directly to DNA and induce damage by forming DNA adduct [11]. Cells have evolved many pathways to cope up with the harmful effects of DNA damage which are classified as 'DNA repair' or 'DNA damage tolerance' (DDT) [12]. In mammalian cells two main pathways, namely non-homologous end joining (NHEJ), which is major pathway and homologous recombination and which, provides greater repair fidelity are involved in DSBs repair [13]. In the case of single-stranded DNA lesions, DNA

damage tolerance (DDT) pathway is involved, which promotes the bypass of single-stranded DNA lesions encountered by DNA polymerases during DNA replication [14]. If the DNA damage is not repaired properly it can lead to mutations and eventually carcinogenesis.

## Flavonoids in Food Sources and Their Role in Prevention of Diseases

Flavonoids are secondary metabolites of plants that impart coloration to most flowers, fruits and seeds [15]. Flavonoids have a common structure of two aromatic rings connected to three carbon atoms. In addition, the basic structure of flavonoids allows multiple substitution patterns in benzene rings (Figure 1). Flavonoids are therefore, classified based on their chemical structure [16,17].



**Figure 1:** Chemical structure of the key flavonoids with preventive activities against DNA damage and carcinogenesis.

Few epidemiological studies have been performed to understand the health benefits of flavonoids. Flavonoids are majorly found in daily diet. Epidemiological study using smokers who ingested flavonoid rich food was performed and suggested that flavonoids protected against harmful effects of tobacco carcinogen in bladder mucosal cells [18]. To elucidate the possible roles of dietary flavonoids in cancer and the prevention of cardiovascular diseases number of cohort studies and one cross-cultural study was performed. Protective role of flavonoids in cardiovascular disease was found in few studies while reduction in lung cancer was observed only in one study [19]. Flavonoid intake was linked with lower cardiovascular disease mortality [20]. A number of studies have been performed to study protective effect of flavonoids against cancer [21]. Flavonoids showed beneficial effect in the treatment of hemorrhoidectomy with radiofrequency scalpel by reducing post-hemorrhoidectomy symptoms [22]. Many health benefits of flavonoids in food have been suggested but in general it is difficult to conclude because flavonoids are modified in different ways in mammalian biology.

Due to an improved understanding of the isolation and identification of flavonoid compounds from different food sources, it has become easier to understand health benefits of individual compounds. Flavonoids are found abundantly in vegetables and fruits and have shown many health benefits in both *in vitro* and *in vivo*

studies [23,24]. Many species of plants have evolved flavonoids as a protective mechanism against harmful effects of ultraviolet-B radiation [25]. *Zea mays* plant, which contain flavonoids anthocyanins showed protection from UV- radiation-induced DNA [26].

## Antioxidant and Pro-Oxidant Behavior of Flavonoids

Among various polyphenols used in human diet, flavonoids are most abundant. They are obtained from plant sources and have shown important health benefits. They show antioxidant properties and studies to understand their roles in various diseases have been undertaken. However they also show prooxidant activity [27,28] and can lead to DNA damage and mutagenic effects. Flavonoids have shown both mutagenic and anti-mutagenic properties [29]. Few plant flavonoids have been assayed for mutagenicity and their mutagenic activities were correlated with the flavonoid content [30]. Further mutagenic activities of flavonoids were correlated with their structure [31]. Ames test has been widely used to screen mutagenic potential of various flavonoids. Hydroxylation pattern and presence of metabolic activation are important determinants of the mutagenic activities of flavonoids; this was suggested by using Ames test in *Salmonella typhimurium* strains [32]. Several flavonoids such as Catechins [33], flavonoids of propolis [34] can induce oxidative DNA damage through the generation of reactive oxygen species (ROS). Dietary flavonoids such as quercetin, luteolin and genistein, reduced oxidative DNA damage through inhibition of ROS [35]. Further the protection of DNA from oxidative damage and pro-oxidative effects was shown to depend on flavonoid concentration and the incubation period [36].

## Interaction of Flavonoids with DNA

It has been proposed that flavonoids can form complexes with DNA and protect it against oxidative damage. The antioxidation potential of antioxidants are related to their binding modes to the DNA duplex [37]. Cumulative studies have suggested that flavonoids can interact with DNA; FTIR and UV-Visible difference spectroscopic methods have been used to study the interactions of flavonoids quercetin, kaempferol, and delphinidin with DNA at molecular level and found that delphinidin induces more stabilizing effect on DNA duplex than quercetin and kaempferol [38]. The interactions of flavonoids, such as quercetin, kaempferide and luteolin, with fish sperm DNA were also reported [39]. Flavonoids such as hesperitin and naringenin interacted with calf thymus DNA and stabilized it [40].

Recently it was reported that flavonoid Quercetin can interact with human telomerase sequences and stabilizes the G-quadruplex structure [41].

## DNA damage prevention by flavonoids with implications to carcinogenesis prevention

**Silibinin:** Flavonoids have shown promising results both in the inhibition of initiation and progression of cancer. DNA damage protective properties of flavonoids are well reported. We previously reported radioprotective and anti-cancer properties of flavonoid Silibinin, a major active component of Silymarin which is obtained from milk thistle [42-44]. Silibinin protected human lymphocytes against gamma-radiation induced DNA damage and micronuclei formation.

Silibinin also protected plasmid pBR322 DNA and mice leukocytes DNA against gamma-radiation-induced damage [44], Cancer

chemopreventive nature of Silibinin was also attributed to its ability to inhibit both cell growth and DNA synthesis [45]. Silibinin inhibits UVB-tumor initiation, promotion and carcinogenesis in SKH-1 hairless mouse skin [46]. Silibinin accelerated the repair of cyclobutane pyrimidine dimers (CPD) induced by UVB radiation JB6 mouse epidermal cells and SKH1 hairless mice. Silibinin protected against UVB-induced photodamage by upregulating p53-mediated GADD45a signaling (Roy S, Carcinogenesis, 2012). Further investigation showed that silibinin also induced endogenous IL-12 to protect against UVB-caused skin damages [47]. Silibinin also inhibited carcinogen azoxymethane and 1,2-dimethylhydrazine induced initiation and progression of colon cancer [48-50] and spontaneous intestinal tumorigenesis [51,52].

**Apigenin:** Apigenin is a natural flavonoid found in many plants and has shown promising results in chemotherapy [53,54]. Apigenin has also shown cancer preventive and DNA damage protective properties. For instance, Apigenin inhibited skin carcinogenesis initiated by 7,12-dimethylbenz(a)anthracene (DMBA) and promoted by 12-O-tetradecanoylphorbol-13-acetate (TPA) by inhibition of epidermal ornithine decarboxylase induction [55]. UVA/B induced skin carcinogenesis was also inhibited by Apigenin (Brit DE, 1997). Apigenin stimulated Nucleotide excision repair genes and protected against UV-B induced DNA damage in HaCaT skin keratinocytes and animal models [56]. Furthermore, it was found that that apigenin can preferentially accumulate in the nuclear matrix, binds with the nucleic acid bases and reduce oxidative DNA damage in prostate epithelial cells [57].

**Quercetin:** Quercetin a potent antioxidant flavonoid has shown many health benefits including cancer prevention and treatment [58-60]. Quercetin prevented N-nitrosodimethylamine (NDEA)-induced lung tumorigenesis in mice [61]. Quercetin also prevented early stages of azoxymethane induced colorectal carcinogenesis [62]. Further studies showed preventive effects of Quercetin against NDEA induced hepatocellular carcinoma by inhibiting DNA specific mutations [63]. Quercetin protected Caco-2 and Hep G2 cells against H2O2-induced DNA damage [64]. Quercetin also protected against Benzo[a]pyrene-induced DNA damage and pulmonary precancerous pathologic changes in mice through inhibition of cytochrome P4501A1 activity [65]. Further study showed that quercetin can inhibit ROS formation and the formation of strand breaks in PC12 neuronal cells [66]. A recent study suggested that quercetin can interact with DNA and inhibits tumor growth by cell cycle arrest and induction of mitochondrial pathway of apoptosis [67].

**Genistein:** Genistein is a natural flavonoid which acts as phyestrogen [68]. Dietary Genistein has shown chemoprotective properties against mammary and prostate cancers by modulating specific receptors and growth factor signaling pathways such as androgen receptor, estrogen receptor- $\alpha$ , progesterone receptor, epidermal growth factor receptor, insulin-like growth factor-I, and extracellular signal-regulated kinase-1 [69]. DNA damage protective properties are also reported. Genistein inhibited DMBA initiated and TPA promoted skin carcinogenesis in mice [70]. Genistein significantly inhibited DMBA-induced bulky DNA adduct formation while TPA-induced ornithine decarboxylase activity was moderately inhibited. Later, the photoprotective effects of Genistein were found using human reconstituted skin. Genistein inhibited UVB-induced pyrimidine dimer formation and preserved cutaneous proliferation and repair mechanisms [71]. Genistein also prevented DMBA induced mammary tumors [72]. And N-methyl-N-nitrosourea induced endometrial

carcinogenesis [73]. Genistein protected calf thymus DNA against UV radiation or Fenton reaction system induced damage by inhibiting 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation [74].

Genistein also protected LAPC-4 prostate cells from hydrogen peroxide-induced DNA damage through induction of three genes with antioxidant activities, namely glutathione reductase, microsomal glutathione S-transferase 1 and metallothionein 1X, at 1-30 microM genistein [75]. In breast cancer cells, genistein depleted the G1 population of cells and increased the accumulation of cells at G2. Few cells showed chromosome abnormalities and some cells showed polyploidy, which lead to increased cell death through activation of DNA damage response. Also *Brca1* mutant cells were found to be more sensitive to genistein [76].

**Luteolin:** Anticancer properties of Luteolin are well documented [77] few studies have also shown its role in carcinogenesis initiation preventive. Luteolin protected from 2-dimethyl hydrazine (DMH) and azoxymethane-induced colon carcinogenesis [78,79]. Luteolin inhibited formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) induced by either UV or Fenton reaction in calf thymus DNA and reduced oxidative DNA damage by reducing free radical hydrogen peroxide and superoxide anion (O<sub>2</sub><sup>-</sup>) [35]. Luteolin also inhibited ROS and prevented DNA damage in PC12 neuronal cells [66].

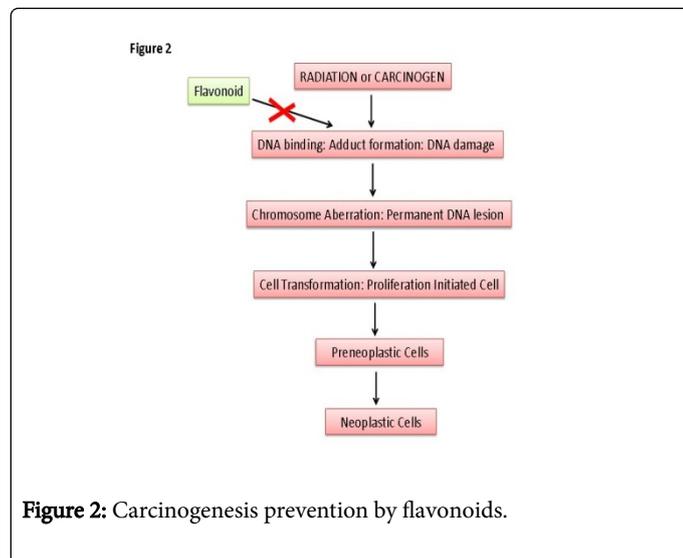
Luteolin was also reported to increase repair activity in Caco-2 cells [80]. Luteolin also inhibited UVB-induced human skin damage, erythema and the upregulation of cyclooxygenase-2 and prostaglandin E<sub>2</sub> production via MAPK pathway [81].

**Rutin:** Rutin has shown promising results in the treatment of various diseases including cancer [82,83]. Rutin also protects DNA against damage. Rutin protected against hepatocarcinogens aflatoxin B1 and N-nitrosodimethylamine induced single-strand breaks in nuclear Rutin also reduced induction of repair enzymes polymerase, DNA polymerase beta and DNA ligase [84]. Rutin also protected against hydrogen peroxide induced DNA damage in Caco-2 and HepG2 cells [64]. Flavonoids, quercetin and rutin, protected Caco-2 cells against tert-butylhydroperoxide and menadione-induced DNA single strand breaks by their metal ion chelating ability [85].

**Naringenin:** Naringenin is found in grapes and citrus fruit and has shown promising results in the treatment of obesity, metabolic disorders [86] and its anti-cancer and chemo-sensitizing properties are also reported [87-89]. For instance, Naringenin inhibited azoxymethane induced colon carcinogenesis and lowered the number of high multiplicity aberrant foci [90]. Moreover, Naringenin protected plasmid DNA from UVB-induced DNA damage [91]. Naringenin also protected against alloxan-induced DNA-damage in the peripheral lymphocytes of diabetic mice [92].

**Morin:** Morin is obtained from figs and other Moraceae have shown anti-oxidant [93] and anti-cancer properties [94]. For instance, Morin inhibited azoxymethane-induced bowl tumorigenesis [95] and 4-nitroquinoline 1-oxide (4-NQO)-induced tongue tumorigenesis and PCNA index in rats [96] and also protected from NDEA induced hepatocellular [97]. Furthermore, Morin inhibited cancer progression in breast cancer cells MDA-MB-231 [98] and protected against hydrogen peroxide induced DNA-damage by inhibiting DNA strand breaks, 8-OHdG formation and decrease of nuclear phospho histone H2AX expression [99]. Morin also protected rat hepatocytes DNA from high glucose induced DNA fragmentation, chromatin condensation and hypodiploid DNA [100] and was also observed to protect against gamma-radiation induced oxidative DNA damage by

attenuation of SEK1-JNK-AP-1 pathway [101]. These studies strongly suggest the therapeutic potential of flavonoids not only in cancer therapy but also in cancer initiation and DNA damage prevention (Figure 2).



## Concluding Remarks

Flavonoids have shown promising results in protection against various DNA damaging agents, especially since they have antioxidant properties and they can also modulate DNA damage and repair pathways. These effects of flavonoids may therefore, contribute to their anti-carcinogenic effects. In general these properties cannot be used for apoptosis induction in cancer cells. Flavonoids can be useful in the prevention of carcinogenesis by protecting DNA damage and inducing repair pathways. However their carcinogenesis preventive properties cannot be solely linked to DNA damage protection as they also regulate many other important cellular events such as cell cycle and apoptosis. Though flavonoids show excellent health benefits and anticancer properties, more studies are needed before their use in therapy. Flavonoids can be bio transformed into more harmful genotoxic agents and purified compounds may have toxicity and solubility issues. These aspects should be carefully considered before their use in therapy.

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