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Dihydroquercetin: A Novel Potent Flavonoid Antioxidant as an Adjuvant for Effective Cancer Treatment

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Abstract

Dihydroquercetin (DHQ) was discovered in 1938, by Albert Szent-Gyorgyi, a Hungarian biochemist. Its biological and antioxidative activities have been studied for several decades. DHQ is more active than ascorbic acid, tocopherol or carotene, it is also more stable. DHQ is a polyphenolic bioflavonoid extracted from Siberian Larch wood, which belongs to a limited flavanone subclass of flavonoid compounds. It is the strongest known natural antioxidant and as such, it has the ability to bind free oxygen radicals, dramatically prevent lipid peroxidation, and inhibit inflammatory processes.

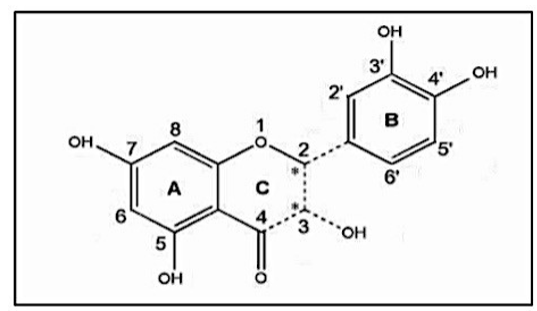


Figure 1: *DHQ chemical structure.*

Keywords:*Dihydroquercetin (DHQ); Flavonoid Antioxidant; Cancer Treatment*

Introduction

The human body is extremely complex biological system. For the long life, the system must be in balance. Any violation causes a reaction on an intracellular level. At further, provoking the disease.

Free radicals are atoms or groups of atoms, which have a single unpaired electron. A free radical substitution reaction is one involving these radicals.

Free radicals (ROS-reactive oxygen species) have unusually high reactivity associated with the characteristics of their electronic structure. They are unstable; very easy enter into chemical reactions and can derived from building a huge number of cells. That leads to oxida-

tive stress (damaged cells by oxidation). The result of this excessive activity of free radicals (ROS) is a failure in the work of the cells, and consequently, the disruption of a body.

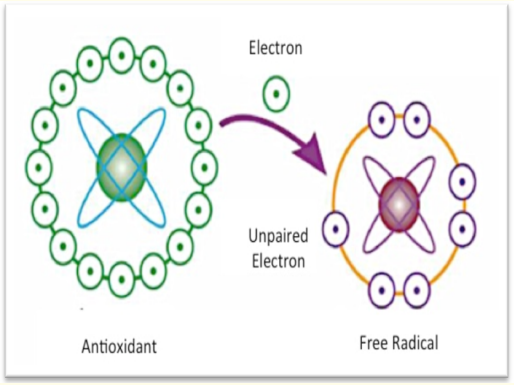


Figure 2: *Mechanism of antioxidative effect of DHQ against free radicals.*

Diet and nutrition have been shown to influence the incidence, progression and recurrence of cancer as well as patient survival [1]. As a result, dietary supplements have become very popular among cancer patients. In particular antioxidant supplements are taken by up to 87% of patients, and analyses suggest that concurrent use of supplements and chemotherapy is beneficial for the patient [2]. The primary mechanism of many chemotherapy drugs is the formation of reactive oxygen species (ROS), or free radicals. Drugs that form ROS include, alkylating agents, anthracyclines, podophylin derivatives, camptothecins, and platinum coordination complexes. Unfortunately, the free radicals generated by these treatments, while having a beneficial effect on the patient's cancer, also have serious side effects. Nephrotoxicity, ototoxicity, peripheral neuropathy, and cardiotoxicity are just some of the side effects of free radical damage induced by platinum coordination compounds such as cisplatin and anthracyclines such as doxorubicin, amongst others [3-5].

Cancer patients frequently have low antioxidant levels and exhibit marked elevation of oxidative stress before chemo- or radiotherapy when compared with healthy controls [6]. As evidenced by the elevation of lipid peroxidation products, the reduction of total radical trapping capacity of blood plasma, the reduction of antioxidants such as vitamin C, E and β-carotene, as well as the marked reduction of tissue glutathione levels, oxidative stress increases throughout the body during chemotherapy treatment [7,8]. High levels of oxidative stress have been associated with more aggressive forms of cancer and oxidative stress is further exacerbated by chemotherapy drugs during and after cancer therapy [9-12]. There has been debate as to whether the use of antioxidant supplements by patients can interfere with the mechanisms of action of therapeutic agents and therefore reduce their efficacy [13]. On the other hand, it has been argued that supplements are beneficial to patients undergoing chemotherapy because they can enhance its efficacy [8]. However, reviews of controlled clinical trials in which antioxidants were given with chemotherapy, and survival and tumor response were measured, showed that antioxidant supplementation results in the same or better tumor response and patient survival than treatment with chemotherapy drugs alone [2,14].

The majority of evidence suggests that supplementation with antioxidants reduces the toxic side effects of reactive oxygen species (ROS) generating chemotherapies. In a comprehensive review of controlled clinical trials of antioxidants being used as adjuncts to standard chemotherapy regimens of at least one drug thought to produce increased level of oxidative stress, 53% of studies showed that the antioxidant group experienced less toxicity than the control group, with 82% of those studies showing statistically significant results. No difference was seen in 43% of studies, and only 4% showing increased toxicity. Increased toxicity was statistically significant in only one study, which was not surprising taking into account that high dose vitamin A is known to have toxic effects and that N acetyl cysteine supplementation can cause diarrhea [2]. Depending on the study, decreases were seen in myelosuppression, asthenia, weight

loss, cardiotoxicity, nephrotoxicity as well as neurotoxicity. The reduction of toxic side effects from chemotherapy has clinical relevance as they often lead to dose reductions, interruptions and delays in chemotherapy treatment, and incomplete courses of treatment. Depending on the cancer, 30% to 50% of patients fail to receive the recommended doses of chemotherapy, and the earlier a patient terminates chemotherapy, the higher their mortality hazard ratio compared to those who completed treatment [15,16]. Therefore, the addition of safe and powerful antioxidants as an adjuvant can increase compliance with chemotherapy protocols and thereby improve patient care and survival [17].

Natural polyphenol antioxidants can also enhance the effects of conventional chemotherapies by mechanisms that do not involve their antioxidant capacities, but by modulating various signal transduction pathways involved in carcinogenesis. *In vivo* they have been shown to inhibit cell proliferation pathways such EGFR and ErbB2 receptor activation and ERK-MEK-Cyclin D1 as well as the expression of Ki-67. They decrease cancer cell survival by inhibiting Akt and NF-κB pathway activity, and they increase apoptosis by increasing the Bax/Bcl-2 ratio, decreasing the level of Bcl-2 and inducing PARP and caspase-3 cleavage. They have also been shown to decrease inflammation by inhibiting COX-2 expression [18].

Polyphenolic antioxidants have been shown to be synergistic with chemotherapy drugs. They have been shown to decrease cell proliferation and tumor growth, as well as to increase the apoptotic index of tumors in animal models *in vivo* and their mechanisms of action were found to be similar to those noted above [18]. Such results have led to promising human clinical trials using combinations of chemotherapy drugs and polyphenolic antioxidants. Completed trials have shown promising results in prostate, breast, and pancreatic cancers, amongst others [19-22]. Numerous other trials are currently under way which are looking at the efficacy of polyphenolic antioxidants for the treatment of what seems to be nearly every type of cancer. While the results of most of these studies have not yet been published, their increasing number speaks to the potential of these substances to enhance cancer treatment and patient care [22].

Characteristics of dihydroquercetin

DHQ, also known as taxifolin, is a unique polyphenolic flavonoid molecule isolated from the Siberian Larch tree. Like other flavonoids, DHQ has been found to have antioxidative, anti- inflammatory, pro-apoptotic, and chemo-preventative effects. However, among polyphenolic flavonoids DHQ is a unique compound with a greater potential to beneficially affect human health especially in cancer, due to several key differences with respect to other flavonoids.

Due to their water insolubility, most flavonoids require extensive glycosylation in the intestines before they can be absorbed [23]. Unlike most other bioactive flavonoids, especially quercetin, DHQ is water soluble at effective doses, which increases its direct bioavailability [24]. DHQ belongs to the flavanone subclass of flavonoids. Flavanones are directly absorbed in the intestines in their aglycone form which increases their direct bioavailability. As a consequence flavanones are the most bioavailable of all flavonoid compounds [16].

Animal studies using acute, sub-chronic and chronic administration regimens have shown that DHQ is non-toxic. Single doses up to 12,000 mg/kg were found not to be fatal in adult albino rats. At the highest dose, symptoms such as shortness of breath, languor, and unstable equilibrium disappeared one hour after onset [25]. Oral administration of 15 g/kg DHQ for 7 days to 20 adult rats resulted in no lethalities, behavioral changes or changes in organ histopathology [26]. Oral administration of 1,500 mg/kg DHQ for 6 months found no effects on behavior or physiological parameters [25]. Furthermore, no undesirable side effects were seen in human volunteers who were administered acute doses of 2g of DHQ, while DHQ metabolites was detected in blood plasma at concentrations ranging from 50 - 200 μM and DHQ metabolites were detected in urine after 12 to 18 hours [27]. These data suggest that DHQ is a well-tolerated, non-toxic and bioavailable flavonoid compound.

DHQ has not been found to be mutagenic or genotoxic. The bacterial Ames test has shown DHQ not to be mutagenic in *Salmonella typhimurium* [28]. Additionally, DHQ strongly inhibits benzene induced genotoxicity and lipid peroxidation, while the popular flavonoids resveratrol and catechin are only weakly inhibitory, and the ever popular quercetin is mutagenic [29]. Furthermore, in an additional study, quercetin, but not DHQ, was found to be genotoxic [30].

In summary, DHQ is a highly bioavailable flavonoid which will not interfere with chemotherapy drug metabolism. It is not toxic at any doses examined and has no mutagenic or genotoxic characteristics. In fact, it has been found to inhibit mutagenicity in some systems.

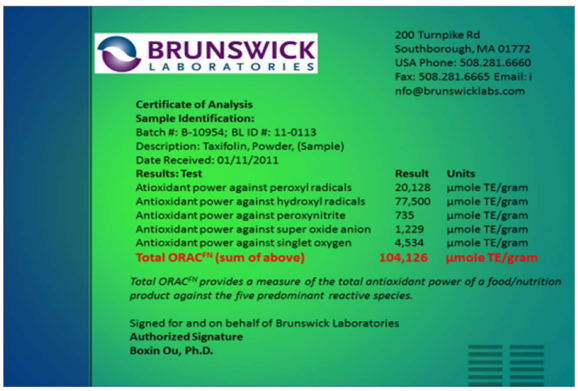
Antioxidative capacity of dihydroquercetin

DHQ has been found to be a stronger antioxidant than almost any other natural compound. Oxygen Radical Absorbance Capacity (ORAC) testing done at Brunswick Laboratories is the gold standard of antioxidant testing. Their testing has shown that 99.6% pure monomeric DHQ produced by the Russian company Taxifolia, has one of the highest single compound ORAC values ever tested, and that the antioxidative performance of DHQ greatly depends on its purity. For comparison purposes, 99.6% pure DHQ is 10 times more potent an antioxidant than quercetin, 50 times more potent than vitamin C, and 80 times more potent than vitamin E.

While all DHQ preparations are potent antioxidants in their own right and all are more potent than antioxidants than other flavonoids, it important to note that not all DHQ preparations and isolations are equally efficacious. As can be seen below, the antioxidant capabilities of DHQ exponentially increase with their purity. While it has not been confirmed, it is thought that impurities such as residual resins, terpenes, and other less potent flavonoids negatively affect the antioxidant capacity of DHQ when they are not fully removed. In less pure isolates DHQ has a tendency to polymerize, which may also negatively affect its antioxidant capacity. However, in its purest isolate, DHQ is a pure monomer, and can be considered a next generation product (see DHQ Technical Data). The advantages of this form of DHQ is high water solubility and dramatically enhanced antioxidant capacity [31].

Substance	ORAC Test
Dihydroquercetin 99.6% purity	104,000 µM TE/g
Dihydroquercetin 98% purity	64,200 µM TE/g
Dihydroquercetin 95% purity	32,744 µM TE/g
Dihydroquercetin 94% purity	21,940 µM TE/g
Dihydroquercetin 92 - 93% purity	19,925 µM TE/g
Dihydroquercetin 88 - 90% purity	15,155 µM TE/g
Luteolin	12,100 µM TE/g
Quercetin	10,900 µM TE/g
Vitamin C	2,100 µM TE/g
Vitamin E	1,300 µM TE/g

Table 1: *DHQ Technical data, Example ORAC test and antioxidant capacity.*


BRUNSWICK LABORATORIES

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Certificate of Analysis
Sample Identification:
Batch #: B-10954; BL ID #: 11-0113
Description: Taxifolin, Powder, (Sample)
Date Received: 01/11/2011

Results: Test	Result	Units
Atioxidant power against peroxyl radicals	20,128	µmole TE/gram
Antioxidant power against hydroxyl radicals	77,500	µmole TE/gram
Antioxidant power against peroxynitrite	735	µmole TE/gram
Antioxidant power against super oxide anion	1,229	µmole TE/gram
Antioxidant power against singlet oxygen	4,534	µmole TE/gram
Total ORAC^{FN} (sum of above)	104,126	µmole TE/gram

Total ORAC^{FN} provides a measure of the total antioxidant power of a food/nutrition product against the five predominant reactive species.

Signed for and on behalf of Brunswick Laboratories
Authorized Signature
Boxin Ou, Ph.D.

Figure 3: *Certificate of Analysis Brunswick Laboratories (Southborough, MA, USA).*

The ORAC test, determines the antioxidative capacity of a compound against five well known reactive oxygen species (ROS), peroxyl, hydroxyl, and peroxynitrite radicals, as well as against the superoxide anion, and singlet oxygen. Its value represents the sum of a compound's antioxidative capacities against all five ROS radicals.

DHQ prevents oxidation by reducing the rate of chain initiation by ROS, especially the peroxyl radical, where its antioxidant capacity against the peroxyl radical is twice that of quercetin's total ORAC capacity. Furthermore, if the oxidation reaction is set in motion, DHQ is the most potent free radical chain reaction terminator (77,500 μmol TE/g against the hydroxyl radical).

DHQ, like other flavonoids, can interact with aqueous and lipophilic endogenous antioxidants, further increasing its antioxidant support by stabilizing vitamin C in the aqueous phase and by sparing vitamin E in cell membranes [32-34]. The effectiveness of antioxidant protection by flavonoids is related to their ability to interact and penetrate cell membranes causing changes in membrane structure and fluidity [35]. At the water-lipid interface of the cell membrane it provides an additional level of support. The three- dimensional structure and the number and distribution of flavonoid hydroxyl groups determine the formation of hydrogen bonds with the polar lipid head groups [35,36]. Dihydroquercetin can inhibit lipid peroxidation induced by mitochondrial free radical- producing reactions with an incredibly low and physiologically attainable IC_{50} = 10 μM [37].

DHQ suppresses lipid peroxidation

Lipid peroxidation is implicated in the pathophysiology of many disease states including cancer [38,39]. Lipid peroxidation is a result of ROS free radical attacks on polyunsaturated fatty acids (PUFA) leading to structural and/or functional damage of cell membranes [40]. Once initiated, lipid peroxidation reactions are capable of self-propagation and initiating chain reactions [41]. Lipid peroxidation results in the formation of highly reactive aldehydes which are highly diffusible and attack or form covalent bonds with distant cellular component or targets, including DNA [38]. Furthermore, lipid peroxidation products such as malondialdehyde (MDA) have been shown to be mutagenic in human cells [42].

Free radical-mediated lipid peroxidation proceeds by a chain reaction where one initiating free radical can oxidize both lipid molecules in cell membranes and low-density lipoproteins [43]. DHQ inhibits lipid peroxidation by several mechanisms. First, as a potent inhibitor of ROS, it inhibits the initiation of lipid peroxidation by scavenging the lipid peroxidation chain-initiating peroxyl, hydroxyl, and oxygen free radicals [44]. Second, as a regenerator of vitamin E, it enhances its lipid peroxidation chain-breaking properties. Third, DHQ inhibits cyclooxygenase-2 (COX-2 expression) lipoxygenase expression, two enzymes can specifically oxidize lipids [45-48]. Fourth, the propagation phase of the lipid peroxidation reaction is facilitated by divalent cations such as iron (II) and Cu(II), and all flavonoids including DHQ are divalent cation chelators, and as such DHQ inhibits lipid peroxidation via this mechanism [44,49]. It is important to note that DHQ is a more potent antioxidant when complexed with the divalent cations that it naturally chelates, and exhibits superoxide dismutase (SOD)-like properties in its complexed state [49].

Human clinical trial data support the assertion that DHQ is a potent inhibitor of lipid peroxidation without side effects. Most clinical trials examining DHQ have been performed in Russia by Plotnikov., *et al.* and have been published in one book. The trials were for a drug called Ascovertin, which is a combination of DHQ and vitamin C. Of the clinical trials performed, all 5 trials that looked at patient lipid peroxidation parameters found a significant decrease in indicators of lipid peroxidation such as MDA and liver thiobarbituric acid reactive substance (TBARS). In two trials, higher levels of endogenous antioxidants such as catalase and SOD were found in samples from patients taking Ascovertin. No trial found any undesirable side effects in the groups taking Ascovertin. Three trials were for cardiovascular diseases, one for diabetes and one for Lyme disease [50]. It is important to note that Ascovertin is now registered as a drug for use in cardiovascular applications in the Russian Federation and is only available by prescription. Lastly, Kolhir., *et al.* in a trial of 112 patients with acute pneumonia, where 50 patients received basic therapy, 32 received the basic therapy plus vitamin E and 30 patients received basic therapy plus DHQ, found that patients receiving DHQ recovered faster and had higher levels of endogenous antioxidants than the other two study groups. No patients exhibited side effects from the experimental therapies [51].

In summary, preclinical studies have shown that DHQ is capable inhibitor of lipid peroxidation *in vitro* and *in vivo*. Furthermore, multiple human trials have confirmed that oral administration of relatively low doses of DHQ inhibits lipid peroxidation in a clinical setting. Additionally, these clinical trials have shown that DHQ administration is safe and effective when applied in conjunction with other therapies. These data suggest that DHQ could be very useful in controlling side effects related to oxidative stress in cancer patients receiving chemotherapy.

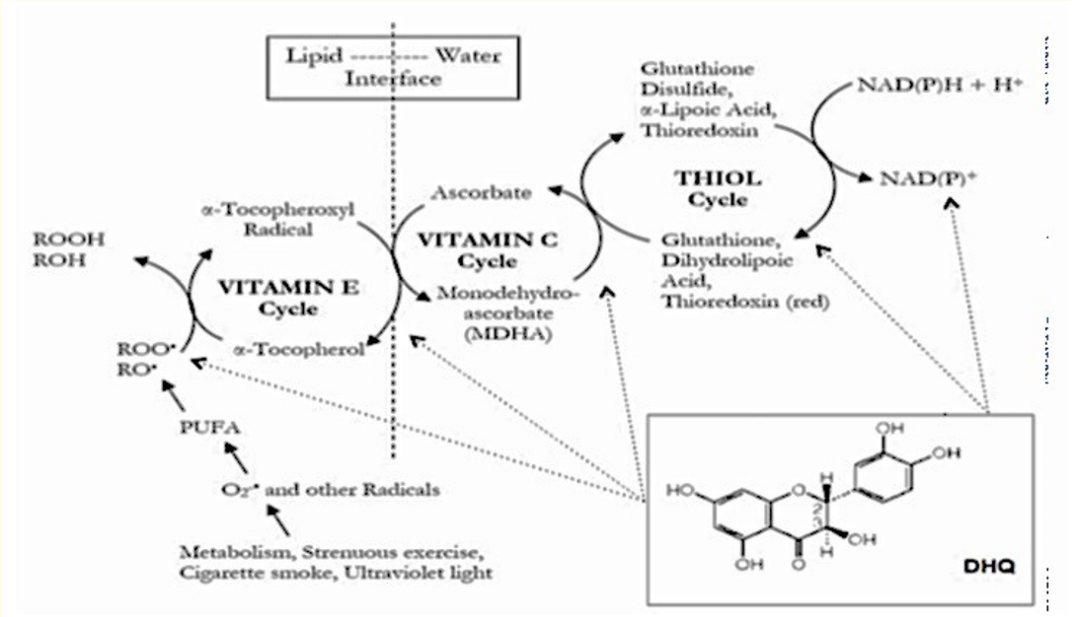

Figure 4: *Schematic depicting some of the antioxidant activities of DHQ.*

Chemo-preventative effects of DHQ

Chemotherapy drugs are genotoxic xenobiotic compounds. In general, their anticancer effects are due to their ability to induce DNA damage to the extent that it overwhelms DNA repair mechanisms in cells undergoing cell division, thus stimulating them to undergo apoptosis. However, DNA damage is not limited to rapidly dividing cancer cells. Chemotherapy treatment is genotoxic to normal healthy cells as well [52]. This is evidenced by the dramatic side effects of cancer therapies and by appearance of cancers that are refractory to chemotherapy treatment years and decades after treatment for the initial cancer [3-5,53]. Attenuation of therapy induced genotoxicity in normal tissues, as well as detoxifying the organism after treatment, should be an additional goal of cancer treatment [53].

Xenobiotic detoxification proceeds in three phases. In phase 1, the cytochrome P450 superfamily of enzymes introduces an active site into otherwise inert xenobiotics, creating a highly reactive intermediate [54]. Phase 2 enzymes exploit the newly created active site to conjugate a functional group that solubilizes the xenobiotic, enabling its excretion [55].

Phase 3 (also known as multi-drug resistant) proteins are a family of membrane transporters that remove processed toxins which become destined for excretion [55]. An imbalance between phase 1 and phase 2 systems, where there are insufficient phase 2 enzymes to transform the reactive intermediates produced by CYP450 enzymes, results in increasing oxidative stress, inflammation, as well as protein and DNA damage [56].

DHQ does not increase the expression, and is a weak inhibitor, of CYP450 enzymes thereby suggesting a low toxicological potential when co-administered with other medications [57,58]. Specifically, DHQ does not inhibit CYP1A, cytochrome b5, CYP3A4/5 isoforms at physiologically attainable concentrations, while other flavonoids such as quercetin and luteolin show considerable inhibition of this important detoxifying enzyme [59].

DHQ was also found to significantly inhibit the expression of CYP2E1, inhibition of which has been found to have anti-carcinogenic effects [60,61]. This suggests that DHQ has a low toxicological potential and will not interfere with the metabolism of co-administered chemotherapeutic drugs, and that it is a better candidate for an adjuvant to chemotherapy than other flavonoid antioxidants.

The Nrf2 protein and the Keap/Nrf2/ARE pathway is a, if not the, key regulator of the phase 2 antioxidant and chemo-protective enzyme response to xenobiotics [62]. Nrf2 is a transcription factor, that when activated, translocates from the cytoplasm to the nucleus where it binds to the antioxidant response element (ARE) of ARE-responsive genes thereby increasing their expression. Most phase 2 detoxifying enzymes are regulated by an ARE-responsive element, and phytochemicals have been shown to have Nrf2 activation capabilities [63]. Low-level stimulation of Nrf2 activates the phase 2 response while inhibiting inflammation. Therefore, transient activation of Nrf2 in the absence of oxidative stress inducers can up regulate phase 2 enzymes which increases the cellular capability to more robustly respond to oxidative stress without activating intense inflammatory responses [62].

DHQ has been shown to stimulate the expression of phase II detoxifying enzymes by activation of the Keap/Nrf2/ARE signaling pathway [64]. DHQ activated the ERK, Akt, and JNK signaling pathways, directly leading to Nrf2 activation. DHQ up regulated the Nrf2- related antioxidant genes heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1) and glutamate-cysteine ligase modifier subunits. Not only that, but DHQ was found to increase the expression of Nrf2 itself. DHQ possessed considerable protective activity from oxidative DNA damage which was reduced when expression of the Nrf2 gene [65]. DHQ was shown to also increase the expression levels of the endogenous ARE- responsive antioxidant enzymes SOD, catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) at the physiologically attainable concentrations of 0.1, 1.0 and 10 μM [66]. DHQ activates the ERK, JNK, and p38 cell signaling pathways, and thereby significantly enhancing heme oxygenase-1 (HO-1) via increased Nrf2 expression in macrophages/Kupffer cells. This also results in simultaneous decreased expression of the inflammatory mediators tumor necrosis factor- α (TNF-α), and interferon-γ (INF-γ) [67]. Additionally, DHQ increases the expression of the Nrf2 protein in colon carcinoma cells. As a result, it inhibits NF-κB signaling and down regulates the levels of pro-inflammatory mediators such as TNF-α and COX-2, as well as cell cycle progression by inhibiting cyclin D1 [68].

Cell signaling effects of DHQ

In addition to its considerable and varied antioxidant properties, DHQ is a true signaling molecule that has been shown to inhibit survival and proliferation, as well as to stimulate apoptotic pathways in cancer cells.

The induction of phase 2 detoxification enzymes is not the only result of DHQ activation of ARE responsive elements. Using DNA chip technology, DHQ was found to up regulate the expression of 65 genes and down regulate the expression of 363 genes in an ARE-dependent fashion. DHQ was found to up regulate the expression of a number of tumor suppressor genes, and down regulate well-known proto-oncogenes (see table below). The data suggest that DHQ acts as a potential chemo preventative agent by regulating gene expression in a manner that is beneficial for cancer therapy [60].

Aberrant lipid metabolism is one of the key features of cancer cells. Cell proliferation requires increased lipid biosynthesis, which produces bioactive molecules, which act as signaling molecules to regulate cancer metastasis [69]. The expression of fatty acid synthase (FAS) is extremely low in normal adult tissues; however, it is significantly up regulated in solid and aggressive cancers [70]. DHQ was shown exert anticancer effects by the induction of apoptosis in prostate cancer cells by inhibiting lipogenesis by direct inhibition of fatty acid synthase (FAS), a phenomenon that could be rescued by the addition of exogenous fatty acids [71]. Subsequently, it was determined that DHQ also reduces apoB secretion in hepatoma cells by 63%. DHQ inhibits microsomal triglyceride (TG) synthesis by 37%, by inhibi-

Gene symbol	Gene name	Accession number	Fold change
Up-regulation			
XPA	Xeroderma pigmentosum, complementation group A	NM_000380	3.1±1.182
XRCC4	X-ray repair complementing defective repair in Chinese hamster cells 4	NM_022550	2.3±1.542
GDF15	Growth differentiation factor 15	NM_004864	2.5±0.263
GAS6	Growth arrest-specific 6	NM_000820	2.5±1.045
TRAF3	TNF receptor-associated factor 3	AF110908	2.4±0.405
DCC	Deleted in colorectal carcinoma	NM_005215	2.1±0.488
NQO1	NAD(P)H dehydrogenase, quinone 1	NM_000903	2.4±0.283
TXNRD1	Thioredoxin reductase 1	NM_003330	1.8±0.031
GSTM1	Glutathione *S*-transferase M1	NM_000561	1.7±0.097
Down-regulation			
CYP2E1	Cytochrome P450, family 2, subfamily E, polypeptide 1	NM_000773	0.6±0.070
JUN	V-jun sarcoma virus 17 oncogene homolog (avian)	NM_002228	0.5±0.018
CCNA1	Cyclin A1	NM_003914	0.4±0.083
CDC25A	Cell division cycle 25A	NM_001789	0.5±0.044
RASA1	RAS p21 protein activator (GTPase activating protein) 1	NM_002890	0.4±0.021
PDGFB	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	NM_002608	0.3±0.048
FGF18	Fibroblast growth factor 18	NM_003862	0.3±0.017
EGF	Epidermal growth factor (beta-urogastrone)	NM_001963	0.3±0.066
FGF3	Fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog)	NM_005247	0.3±0.057

Figure 5: *DHQ as a potential chemo preventative agent, Source Lee., et al. 2007 [60].*

tion of diacylglycerol acetyltransferase (DGAT). DHQ also inhibits TG transfer to the microsomal lumen (by 26%), due to its inhibition of the microsomal triglyceride transfer protein (MTP) [72]. These data suggest that DHQ limits the availability of lipids to cancer cells by inhibiting their endogenous production as well as their exogenous availability.

DHQ was shown to enhance mitotic arrest and apoptosis of prostate cancer cells by the activation of the mitotic spindle assembly checkpoint in prostate cancer cells by andrographolide. Apoptosis was induced by the cleavage of poly(ADP-ribose) polymerase, and caspases-7 and -9, while activation of the mitotic spindle assembly checkpoint was shown to be a result of enhanced microtubule polymerization [73]. While both andrographolide and DHQ slightly decreased the viability of prostate cancer cells on their own, their synergistic effects were substantial. This suggests that DHQ can significantly enhance the efficaciousness of cancer therapy.

Lastly, in an elegant study, Oi., *et al.* demonstrated that DHQ suppresses UV-induced skin carcinogenesis and that it does so by directly inhibiting well-known cell signaling pathways that are dysregulated in numerous cancers [47]. EGFR is activated and/or overexpressed in a variety of cancers including UV-induced skin cancer [8-10]. UV radiation rapidly activates EGFR, which in turn activates a number of cell signaling cascades including ERK. JNK, p38 kinase and phosphoinositide 3-kinase (PI3K), all regulators of cell survival and division [14-17]. The study showed that DHQ can physically bind to EGFR and PI3K at their ATP-binding sites (which are required for their activation) and that DHQ has a higher affinity for EGFR than PI3K. Furthermore, the binding of DHQ to EGFR and PI3K blocked their UV-radiation induced activation, as well as the activation of ERK, JNK, p38 and their downstream target proteins and lipid mediators (COX-2 and PGE2). DHQ did not have any effect on cells that are EGFR deficient, suggesting that its protective effects are specifically due to EGFR inhibition. Furthermore, it was determined that DHQ can inhibit UV-induced transformation of normal cells into a cancerous phenotype, *in vitro*. This was also confirmed *in vivo*, whereby topical administration of DHQ, followed by UV irradiation inhibits the development of skin cancers in mice. When compared to vehicle alone, topical administration of DHQ decreases the number of tumors as well as the average tumor volume. Furthermore, topical administration of DHQ showed that EGFR activation and COX-2 expression were drastically reduced in response to UV-irradiation [47]. The results of this definitive study highlight the effectiveness of DHQ to inhibit not only UV-induced cancer, but to inhibit cell-signaling pathways that are over activated in various cancers.

Summary of anticancer effects of DHQ

As can be seen from the data presented there are a number of characteristics, which make DHQ an ideal adjuvant for cancer therapy.

1. DHQ is a natural flavonoid compound that has been shown to be safe and effective.
2. DHQ is not a drug, but a supplement, it can be easily added to existing therapy protocols.
3. DHQ has a low toxicological potential and will not interfere with the metabolism of co- administered chemotherapeutic drugs.
4. DHQ is the strongest natural antioxidant that is available for human consumption. In addition to its own antioxidant effects it increases the effects of other antioxidants such as vitamin C and vitamin E.
5. DHQ can increase compliance with chemotherapy protocols and thereby improve patient care and survival as:
    a. DHQ is a free radical scavenger, it activates phase 2 detoxifying enzymes, it inhibits lipid peroxidation and its associated damage.
    b. DHQ directly increases the body's own antioxidant defenses.
    c. DHQ does not significantly affect phase 1 enzymes, while up regulating phase 2 detoxifying enzymes. Thereby increasing clearance of highly reactive compounds that accumulate during chemotherapy, and reducing their toxicity.
    d. DHQ inhibits inflammation induced by oxidative stress.
6. DHQ can improve the outcomes of cancer therapies due to the fact that:
    a. DHQ directs tumor cells towards a normal gene expression profile by increasing the expression of tumor suppressor genes while simultaneously inhibiting the expression of proto-oncogenes.
    b. DHQ decreases viability of tumor cells by inhibiting the production of fatty acids necessary for their survival.
    c. DHQ synergizes with anticancer therapies in order to activate cell cycle activation checkpoints.
    d. DHQ directly binds to and inhibits cell-signaling pathways necessary for carcinogenesis, cancer cell proliferation and survival.
7. DHQ is beneficial to patients after the completion of therapeutic protocols as it decreases oxidative stress and lipid peroxidation induced by chemotherapy in non- target tissues. In this manner DHQ supports patient recovery from chemotherapy while suppressing potential long-term side effects.

Disclaimer

SD Investing Capital LLC represents the only two manufacturers of pure monomeric DHQ in the world and we are proud to present this product to the Medical Corporations and the National Centers for Cancer Care and Research, and we stand 100% behind our product. It is our goal to aid all those suffering from cancer, and therefore it is our sincere hope that DHQ reaches as many cancer patients in the world as possible.

We are sure that the Medical Corporations and the National Centers for Cancer Care and Research will recognize the benefits of the addition of DHQ to current treatment protocols, as well as for cancer patients about to undergo treatment, and for their recovery.

We encourage Medical Corporations and National Centers for Cancer Care and Research, to contact us with respect to information regarding the ordering of DHQ, as well as for information regarding suggested doses and other applications of the product.

DHQ technical data

Molecular formula: $C_{15}H_{12}O_7$.

Molecular weight: 304,25.

Physical Characteristics: DHQ is a white to yellowish powder.

Solubility: Soluble in acetone, methyl and ethyl alcohols, propylene glycol-1, 2, ethyl acetate.

Slightly soluble in water (99.6% pure DHQ water solubility is 2 g/L). Insoluble in chloroform, sulfuric air, hydrocarbons.

Melting temperature: 222 - 224°C (with decomposition).

Flash temperature: over 260°C.

Boiling temperature: over 680°C.

UV range: λ_{max} = 289 ± 2 nm, Emax =17900 ± 2050.

Technical Specification: 9197-001-99964074-09

DHQ tariff code: 2932990090, additional code 2501

CAS No: 480-18-2

EINECS: 207-543-4

Potential chronic health effects

Carcinogenic effects: Not detected and not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.

Mutagenic effects: Not detected. TERATOGENIC EFFECTS: Not detected. DEVELOPMENTAL TOXICITY: Not detected.

Standard chromatogram of 99.6% pure DHQ

Dihydroquercetin 99.6% Aromadendrin 0.40% Eriodictyol 0% Naringenin 0%.

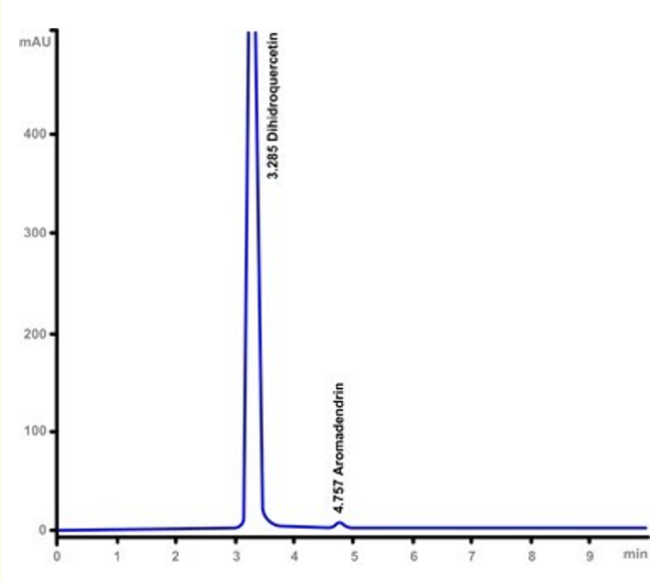

Figure 6: *Standard Chromatogram of 99.6% pure DHQ.*

Analysis conditions

Column: 4.6×250 mm, Kromasil-100-C18, 5 µm

Eluent: A - ACN/2%CH3COOH (30:70), B - 100% ACN

Gradient: from 100% A to 100% B in 13 min

Detection: UV-288 nm

Flow rate: 1 ml/min

Injection - 10 µl

Higher purity of dihydroquercetin results to significant impact on its physical and chemical properties. Dihydroquercetin differs from the other known products because of its ability to become crystallized in highly pure state.

The removal of foreign substances improves significantly its solubility as compared to the solubility of the other known products. Such significant modifications in physical and chemical properties show that dihydroquercetin manufactured using this new technology can be considered as new generation product with new technological possibilities and expanded applications.

Certification body

Federal State Scientific Research Institute of Nutrition, Russian Academy of Medical Sciences, Federal Service for Surveillance over Protection of Consumer Rights and Human Well-Being, Novel Foods and Processes (ACNFP), Council Directive (European Commission), Food Standard agency of the United Kingdom.

GMO Disclaimer

DHQ is not derived from GMO products; it is gluten, soy, and dairy free.

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