คุณสมบัติ ฤทธิ์ทางเภสัชวิทยา และ ความเป็นพิษของเควอซิติน

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บทคัดย่อ

เควอซิตินเป็นสารโพลีโฟนิกฟลาโวนอยด์ธรรมชาติที่พบได้มากมายในผลไม้ หัวหอม หอมแดง สมุนไพร ชา ไวน์แดง เป็นต้น การเติมหมู่น้ำตาลให้กับโครงสร้างหลักของเควอซิตินจะได้ผลิตภัณฑ์สารอนุพันธ์ของเควอซิติน อาทิ สารไอโซเควอซิตินจากการดัดแปลงด้วยเอนไซม์ (อีเอ็มไอคิว) สารไฮโดรกซีเอทิลรูโตไซด์ (เอชอีอาร์) สารเควอซิติน-4′-โอ-เบต้า-ดี-กลูโคไซด์ ทั้งนี้เควอซิตินมีการออกฤทธิ์ที่หลากหลายอันเป็นประโยชน์ทั้งทางด้านสุขภาพและสุขภาวะที่ดี ได้แก่ ฤทธิ์ต้านการอักเสบ ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านไวรัส ฤทธิ์ต้านภูมิแพ้ และฤทธิ์ต้านมะเร็ง ปัจจุบันนี้มีการจำหน่ายผลิตภัณฑ์เควอซิตินในท้องตลาดอย่างมากมายทั้งเพื่อประโยชน์ในการรักษาโรคและเป็นอาหารเสริมเพื่อสุขภาพ อย่างไรก็ตามมีหลักฐานว่าเควอซิตินทำปฏิกิริยากับยาอื่นๆ เมื่อใช้ร่วมกัน หรือ เมื่อใช้ยาขณะที่รับประทานเควอซิตินเป็นอาหารเสริมเพื่อสุขภาพ เควอซิตินอาจรบกวนการออกฤทธิ์ของยาอื่นทั้งทางปฏิกิริยาเภสัชพลศาสตร์และเภสัชจลนศาสตร์ รวมทั้งอาจเสริมฤทธิ์หรือหักล้างฤทธิ์ของยาอื่นได้ ยิ่งไปกว่านั้นเควอซิตินอาจทำให้เกิดผลข้างเคียงเมื่อใช้ที่ขนาดสูง ทั้งนี้ปริมาณของเควอซิตินได้รับการแนะนำให้รับประทานที่ขนาด 1,000 มิลลิกรัมต่อวัน ติดต่อกันไม่เกิน 12 สัปดาห์ แม้ว่าปริมาณ เควอซิตินที่แนะนำจะมีปริมาณที่ไม่เกิดผลร้ายในสัตว์ทดลอง อย่างไรก็ตามผลข้างเคียงในระยะยาวยังไม่ทราบแน่ชัด จึงควรต้องใช้ความระมัดระวังในการใช้เควอซิตินโดยเฉพาะอย่างยิ่งเมื่อมีการรับประทานร่วมกับยาอื่น

คำสำคัญ: เควอซิติน การออกฤทธิ์ ความเป็นพิษ ปฏิกิริยาระหว่างยา

รับบทความ: *24 มิถุนายน 2565* ***แก้ไข:*** *12 สิงหาคม 2565* ***ตอบรับ:*** *17 สิหาคม 2565*

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Properties, Pharmacological Activities and Toxicities of Quercetin

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Abstract

Quercetin is a natural polyphenolic flavonoid copiously found in fruits, onions, shallots, herbs, teas, red wines, etc. By adding sugar moieties into quercetin backbone, derivatives of quercetin, like enzymatically modified isoquercitrin (EMIQ), hydroxyethylrutosides (HERs), quercetin-4′-O-β-D-glucoside, etc. are produced. Quercetin exhibits many activities beneficial for health and well-being, for instance, anti-inflammatory, antioxidant, anti-viral, anti-allergy, and anti-cancer activities. Recently, commercial quercetin products were available for both therapeutic and dietary supplement purposes. Nevertheless, quercetin was proved to interact with other medications when used together as in combination, or unintentionally from food supplement ingestion. Quercetin might interfere by pharmacodynamic and pharmacokinetic interactions, with either increase or decrease effects of other medications. Moreover, quercetin could cause adverse effects with high dose usages. Fortunately, the recommended quercetin is 1,000 mg/kg body weight orally per day, for at most 12 weeks. Even if at this dose, quercetin causes no harm in animal studies, the long-term effects were largely unknown. Careful usage of quercetin should be practiced, especially with other medication concurrently intake.

Keywords: Quercetin, Activity, Toxicity, Drug interaction

Received: *24 June 2022,* ***Revised:*** *12 August 2022,* ***Accepted:*** *17 August 2022*

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Introduction

Quercetin is a polyphenolic flavonoid found abundantly in edible plants and herbs. The main sources of quercetin are citrus fruits, apples (skin), onions, garlics, parsleys, sages, teas, grains, red wines, grapes, olive oils, dark cherries, and dark berries *etc*.[1,2]. Two forms of dietary quercetin are the majority glucoside form, and the less abundant aglycone form[3]. As sugar molecules attached to quercetin structure, other flavonoids were produced as derivatives of quercetin. The examples are the flavonoids rutin, quercitrin, and hesperidin from citrus fruits. Quercetin is the most active flavonoid in many medicinal plants, giving them pharmacologic activities[2].

Quercetin shows many activities such as chemopreventive, anti-oxidant, anti-proliferative, anti-inflammatory and anti-allergy effects[1]. In addition, since it is richly found in vegetation, quercetin might function in shielding the plants from detrimental UV lights[4], with its robust antioxidant properties[5]. Many dietary supplement products with quercetin as the active ingredient, in combination with other minerals and vitamins, are currently available in the market.

Granting many favorable benefits, quercetin possesses lethal potentials. UN Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Classification categorizes quercetin in H301: Toxic if swallowed [Danger Acute toxicity, oral] and H302: Harmful if swallowed [Warning Acute toxicity, oral] with Health Hazards as Acute toxicity, oral[1].

1. Characteristics

The pure substance appearance is yellow needles or powder. Quercetin converts to anhydrous form at 203-207°F (95-97.2 °C), and it melts at 316-318°C. Quercetin can be dissolved very well in ether, methanol; dissolved well in ethanol, acetone, pyridine, acetic acid; but insoluble in water. In alcoholic solutions, soluble quercetin is very bitter. Moreover, when burned to ashes, it releases pungent smoke and irritating vapors[1].

1.1 Structures

The International Union of Pure and Applied Chemistry (IUPAC) systematic name of quercetin is 2-(3,4-dihydroxy-phenyl)-3,5,7-trihydroxychromen-4-one. The molecular formula of quercetin is $C_{15}H_{10}O_7$, with a molecular weight of 302.23. The chemical structure is a pentahydroxyflavone, with the five hydroxy groups dwelling at the positions 3, 3', 4', 5 and 7 (Figure 1)[1,6]. The physical properties of quercetin are shown in Table 1 [1].

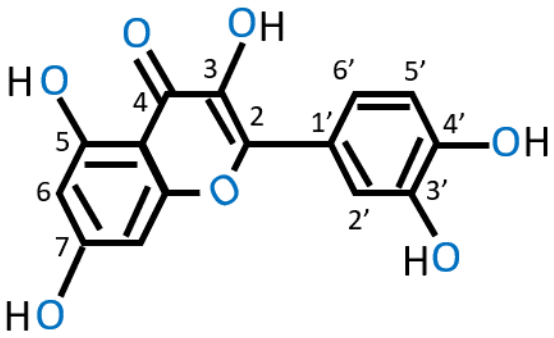

Figure 1. Quercetin Chemical Structure[1,6] The picture portrays the 2D chemical structure of quercetin with five hydroxy groups at the positions 3, 3', 4', 5 and 7.

Table 1. Physical Characteristics of Quercetin[1]

Properties	
Physical appearance	needles or powder
Color	yellow
Boiling Point	sublimes
Melting Point	601 to 603 °F (316 - 318 °C)
Solubility	
- very soluble	In ether, methanol
- soluble	in ethanol, acetone, pyridine, acetic acid
- insoluble	In water (60 mg/L at 16 °C)
Taste (Alcoholic solutions)	very bitter
Vapor Pressure	2.81x10-14 mm Hg at 25 °C (estimate)
Decomposition by Heat	pungent smoke and irritating vapors

2. Pharmacokinetics

The data from Egert *et al*. revealed that quercetin might possibly be better absorbed if taken at a higher dose, i.e., the 150 mg/day dose would be better taken up than at 50 or 100 mg/day doses, regardless of its bioactivity[7], while Jin *et al*. showed the absorption variabilities[8]. Nonetheless, amounts of quercetin absorption in human is highly variable and can be improved when quercetin is in glucose-bound form, available in citrus bioflavonoid and/or hydroxy-ethylrutoside (HER). Quercetin was absorbed in a small amount and degraded by gut microorganisms, thus raising the bioavailability problem[2].

In healthy adults, when quercetin was consumed, it would be converted to

isorhamnetin-3-glucuronide, quercetin-3-glucuronide, quercetin-3-sulfate, and quercetin diglucuronide[9]. After orally intake, the quercetin metabolites, quercetin-3'-O-glucuronide, would be excreted through the gastrointestinal lumen[10].

To overcome the high inconsistency in absorption, quercetin ingestion with dietary fat could help improve quercetin absorption amount through micellization via the intestine[11]. Additionally, enzymatically modified isoquercitrin (EMIQ) or phosphatidylcholine-bound quercetin showed advantages over the original quercetin by having better absorption and higher bioavailability level[11].

3. Biological activities of quercetin

3.1 Anti-inflammatory and anti-allergy effects

Quercetin demonstrated strong anti-inflammatory and anti-allergy activities by inhibiting cytokine production, mast cell and basophil degranulation, neutrophil and monocyte lysosomal secretion, leukotriene formation, and lipid peroxidation. Quercetin inhibits many enzymes in the eicosanoid metabolic pathway, like phospholipase A_2, cyclooxygenase and lipoxygenase enzymes, causing a significant leukotriene, the pro-inflammatory mediator, reduction[2,12].

Hashemi *et al*. described the study in dental pulpitis T cells. They found that quercetin decreased T-helper 17 (Th17) production by down-regulating the mitogen-activated protein kinase - toll-like receptor-4 (MAPK-TLR4) signaling pathway. The consequences were the decrease of pro-inflammatory cytokines such as interleukin-17 (IL-17) productions[13].

Likewise, in human mononuclear U937 cells activated by lipopolysaccharide, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 productions were reportedly reduced in the presence of quercetin[12]. Extracellular signal-related kinases (ERK1/2), p38 MAPK, and nuclear factor kappa B (NFκB) inhibitions were also reported in a study with *Streptococcus suis*-induced inflammation[12]. Moreover, by reducing high-valent iron, quercetin inhibits the major catalyst of lipid oxidation in the body and thus restraining lipid oxidation and reactive oxygen species (ROS)[12]. By these, quercetin shows significant anti-inflammatory effects and is greatly helpful in patients with asthma, psoriasis, atopic dermatitis, gout, ulcerative colitis, autoimmune disease and perchance cancer[2,12].

3.2 Antioxidant and antioxidative stress protective effects

In terms of potent antioxidant properties, quercetin was used as a food supplement to fight against free-radical species and oxidative stress caused by disease, drug, chemical and environmental stress. Its antioxidant activity is expressed via ROS, glutathione, and antioxidant enzyme activities[12]. Also, quercetin regulates many signal transduction pathways, for examples: MAPK, toll-like receptor 4/phosphatidyl-inositol-3-kinase (TLR4/PI3K), hemeoxy-gennase1/nuclear factor erythroid 2-related factor (HO-1/Nrf2), and 5' adenosinemono-phosphate-activated protein kinase (AMPK)[12].

Tarasub *et al*. reported quercetin's protective effect on chromosome aberrations in male Wistar rats receiving quercetin one hour prior to 300 mg/kg nickel chloride ($NiCl_2$) administration[14]. At 24 hours after treatments, they found that the rat's spleen histology remained normal and the chromosome aberrations in bone marrow cells, usually increased in 300 mg/kg nickel chloride administration, significantly reduced by quercetin pre-treatment at > 100 mg/kg. The proposed protective mechanisms might involve the free radical scavenger, and/or the ROS reduction properties of quercetin[14]. Furthermore, despite the warning against using quercetin in kidney patients, Elbe *et al*., 2016 reported the antioxidative and therapeutic effects of quercetin on renal injury, by protecting against oxidative stress caused by ciprofloxacin in rats[15]. In addition, quercetin significantly improved liver injury in mice by upregulating the antioxidant enzyme levels, leading to restraining free radical concentration[12].

3.3 Anti-diabetes activity

Quercetin strongly inhibits aldose reductase, the enzyme responsible for the sorbitol production from blood glucose. Also, quercetin derivatives, quercitrin could delay the cataract onset by decreasing the sorbitol buildup in the animal diabetic lens[16]. In addition, quercetin enhances insulin secretion and shields the pancreatic β cells from free radicals[17]. Likewise, its inhibition of platelet aggregation could improve the diabetes patient's quality of life[18-20].

3.4 Antiviral activity

Quercetin possesses the antiviral activity against herpes virus type I, parainfluenzae 3, poliovirus type I, and respiratory syncytial virus[21]. Quercetin exhibited the ability to inhibit both viral replication and infectivity, *in vitro. In vivo* animal studies also revealed that quercetin

could inhibit viral infection[22]. Consequently, we might benefit from quercetin against viral infections.

3.5 Anticancer properties

The genotoxicity or genetic toxicity assays were used to determine quercetin's ability to damage the cell DNA, causing mutations. Quercetin did not exhibit any strong competence in a forward mutation assay, which tested quercetin's ability to induce new mutations, at least at low dose intake[23]. However, in many *in vitro* studies, quercetin showed reverse mutation abilities by correcting the existing mutations[23-26]. On the other hand, quercetin could not induce DNA repair in rat hepatocytes in one study[27].

Quercetin, one of the most effective flavonoids, displayed tumor formation inhibition via antioxidative activity, inhibition of carcinogen-activating enzymes, signal transduction modification, and through ligand-receptor interactions. It was shown to inhibit tumors and cancers, for instance: leukemia, squamous-cell carcinoma, breast cancer, ovary cancer, brain cancer, and colorectal cancer[2].

Either by epidermal growth factor receptor (EGFR) or estrogen-receptor mediated signal transduction pathways modulation, quercetin presented an antiproliferative activity[1]. Other possible anti-cancer mechanisms of quercetin were to reduce mutant p53 protein and *p21-ras* oncogene expressions, heat shock protein synthesis inhibition and G1 phase cell cycle arrest induction[1]. In addition, Chen *et al*. reported that quercetin could prevent the increase of mitochondrial DNA copy number (mtDNAcn), arised from radiation, via the decreased DNA polymerase subunit gamma (POLG) expression and the transient overexpression of mitochondrial transcription factor A (TFAM) overexpression[28].

Oršolić *et al*. demonstrated that in the model of Swiss albino mice bearing Ehrlich ascites tumor, cisplatin and quercetin combination treatment might increase tumor cell death in physiological and hyperthermic conditions[29]. Moreover, during cisplatin-quercetin combination and hyperthermia treatment, no significant differences in side effect incidences between control and experimental groups were noted. Thus, quercetin might play a role in reducing these adverse effects from the highly toxic cisplatin[29]. Furthermore, when in combination with chemotherapeutic drugs, quercetin demonstrated multitargeted effects in reversing multidrug resistance (MDR) in cancer cells, *in vitro* [30].

4. Therapeutic applications

For allergy and wound healing, topical treatment of quercetin showed anti-inflammatory effects, relieved pain and helped quickening the healing of minor aphthous ulcers. EMIQ intake could relieve the ocular symptom (ocular itching, lacrimation, and ocular congestion) during the pollen season or hay fever, but not useful for nasal symptoms[2].

For autoimmune disease treatments, quercetin has shown to significantly improve rheumatoid arthritis symptoms in mice by inhibiting neutrophil migration, neutrophil extracellular trap formation and lowering pro-inflammatory cytokine levels[12]. Similarly, by suppressing pro-inflammatory cytokines and free radicals, quercetin might prove useful in many autoimmune conditions as well as help improve the patient's quality of life[12].

For chronic disease treatments, quercetin's antioxidant and oxidative stress protective properties might benefit many patients with diseases such as thalassemia, cardiovascular disease, diabetes, and *etc*. Quercetin's ability to reduce the plasma blood sugar level is vital in the treatment of diabetes. It might serve as protective agents against many inflammatory cytokines and substances released from white blood cells, malignant cells and probably pathogens. Also, 500 mg quercetin intake could lower plasma uric acid concentrations via xanthine oxidoreductase inhibition, therefore it should help improve the gout condition[2].

For cardiac-related treatments, quercetin helped elevate high-density lipoprotein, while lowering low-density lipoprotein, total lipid, and triglyceride plasma levels in healthy and hyperlipidemia rats. Quercetin and even more efficient EMIQ showed antihypertensive effects in both hypertensive and normotensive rats. Moreover, EMIQ was successful in preventing plaque formation in arteries of animals[2].

Quercetin and its derivatives might help improve vascular-related conditions, for instance, in diabetes and retinopathy. It could inhibit many inflammatory activations of neutrophils, mast cells and basophils. Quercetin protects the vessels by inhibiting enzyme hyaluronidase, resulting in the collagen matrix stabilization and vascular integrity. Moreover, the quercetin derivative, HER, seems to increase blood flow in small blood vessels, while the plaque formation inhibition of EMIQ keeps the vessel clear. Thus, quercetin should be useful in improving the vascular condition and blood flow improvement. Its anti-platelet aggregation along with vascular stabilizing activities,

should be practical in patients with coagulopathies as well[2].

Quercetin should be helpful in viral condition treatments. In COVID-19 treatment, quercetin could reduce the symptom severity, recovery time and viral clearance time. The blood parameters showed the decreases of lactate dehydrogenase (LDH), ferritin, C-reactive protein (CRP) and D-dimer in COVID-19 patients[31]. Additional clinical trials should prove useful, with also in other viral infections. Furthermore, quercetin possesses the reverse mutagenic activities in both cell and animal studies. More human studies should prove its value in reducing toxic mutagenesis of predisposed patients.

4.1 Dietary supplements

Commercial quercetin as dietary supplements are available in combination with many supplements such as zinc**,** vitamin C, riboflavin (vitamin B2) and bromelain, etc. These products are claimed to help support the immune system, reduce inflammation, reduce oxidative stress, reduce cholesterol levels, boost respiratory health and weight control, as seen in Table 2 [32].

5. Drug interaction with other medications

Quercetin was shown to moderately interact with other medications used in treatments of many diseases. Quercetin is possibly safe for only short-term use, recommended with up to 1,000 mg daily for 12 weeks[33,34]. There is no sufficient data about the safety of long-term use or higher dose ingestion. However, pregnant, breast-feeding women and people with kidney problems should avoid quercetin intake as there is not enough data to ensure its safety. The following are examples of drug interaction effects of quercetin in medication combinations or in supplement usages whilst taking other medications[33,36].

The interferences could be either agonistic or antagonistic of medication effects with many reported mechanisms. For pharmacokinetic interactions, quercetin interacts with cyclosporine, diclofenac, Cytochrome P450 substrates (CYP2C8, CYP2C9, CYP2D6, CYP3A4) and pravastatin (pravachol), and etc. by probably decrease a medication break down rate, resulting in possible greater side effects[33,35-36].

Organic anion transporter substrates (OAT1, OAT3, polypeptide and p-glycoprotein) substrates are interfered by changing the pump operation and medication metabolic rate, which subsequently change the effects and side effects of a medication[33,35-36]. Quercetin has both pharmacodynamic and pharmacokinetic

interactions with losartan (cozaar) as it might change the medication absorption and break down rates, respectively. Eventually quercetin could alter the effects and side effects of losartan[33].

The levels of certain medications might be increased when taking with quercetin, therefore increase the effects and side effects of that medication, for examples; mitoxantrone, prazosin (minipress), sulfasalazine (azulfidine), quetiapine (seroquel), warfarin (coumadin), and *etc*. Overdosed warfarin (coumadin), together with quercetin's anti-platelet aggregation ability[18,20], could increase the bruising and bleeding risk in patients. In case of alpha-blocker tamsulosin, quercetin strongly increases a medication potency, therefore; it may benefit in lower tamsulosin dose[36]. However, if uninformed, the patient might be overdosed and suffer from unnecessary adverse effects. Besides, with midazolam (versed), quercetin might increase the breakdown rate, hence, lower midazolam effects[33]. For the anti-diabetes drug, quercetin's ability to lower blood sugar might cause hypoglycemia in the patients taking quercetin with the diabetes medication. This is also true in case of antihypertensive drugs[33].

Table 2. Examples of Quercetin in Dietary Supplement brands [32,37-38]

Commercially Available Dietary Supplement brands	**Claimed Benefits**	**References**
Respiratory Plus (formerly Asthma Plus)	Temporary relief of asthma symptoms including coughing and wheezing	32,37
Bio Lymph Phase (Bio Lymphomyosot)	Temporary relief of swelling due to minor injury	32,38
Doctor's Best Quercetin Bromelain	immune system and reduce inflammation	32
Natural Factors Bioactive Quercetin EMIQ	reduce oxidative stress	32
Thorne Research Quercenase	reduce cholesterol levels	32
MoxyVites Quercetin	reduce inflammation and antioxidative stress	32
Sandhu's Zinc Quercetin	Respiratory health	32

6. Quercetin toxicities

6.1 Acute toxicity and lethal dose 50 (LD50)

A study in male white mice (*Mus musculus*, strain DDY) by Lucida *et al*. reported the LD50 of solid dispersion quercetin at >16,000 mg/kg (equivalent to 1,600 mg/kg quercetin content) [39]. At this high dose, quercetin remained non-toxic which only significantly affected the feeding behavior, significant changes in the respiratory rate and rendered the mice more resistant to sound [39].

6.2 Toxicity to liver and kidneys

The liver and kidneys were damaged when a very high dose quercetin was ingested. Dibal *et al*. reported the acute toxicity of mice receiving a single oral dose of quercetin from onion (*Allium cepa*) skin (QOS) extract[3]. Photomicrograph of the liver revealed that at 100 mg/kg QOS, lymphocytes gathered in hepatic sinusoids. At 1,600 – 2,900 mg/kg QOS, the mice showed toxicity symptoms like erected hair, body weakness, and loss of appetite for only the first 3 days after QOS treatment. Photomicrograph displayed enlarged sinusoids, tissue hemorrhage, and lymphocyte aggregation. While at 2,900 mg/kg QOS, tissue hemorrhage and enlarged sinusoids were shown [3]. At 5,000 mg/kg QOS, the mice exhibited erected hair, bulged eyes, body weakness, loss of appetite and died 22 hours after the oral onion skin quercetin intake. When compare with the control receiving 10 mg/kg QOS, at the high dose QOS, the laboratory tests showed no significant changes in the serum alkaline phosphatase (ALP) and alanine transaminase (ALT) levels, while caused significant increases of aspartate aminotransferase (AST), albumin and total protein levels at 100 mg/kg QOS ($p<0.05$).

For kidney function test, at high dose quercetin, the laboratory tests showed the significant increase of serum creatinine (at 1,000 mg/kg QOS), significant decrease in the serum urea level (at 10 mg/kg QOS) when compared with the control mice ($p<0.05$). Besides, no significant change was observed in serum cholesterol, potassium, sodium, bicarbonate, and chloride levels. Photomicrograph of the kidney revealed the distorted convoluted tubules, tissue hemorrhage, and lymphocyte aggregation at 1,000 – 1,600 mg/kg QOS [3]. Likewise, a study in male Swiss albino mice (*Mus musculus*) showed that at > 1,500 mg/kg quercetin intraperitoneally administered, hepatotoxicity marker levels (AST, ALT, ALP, and LDH) were raised [40].

In addition, one study reported that the kidney of male rats, receiving 1,900 mg/kg/day quercetin, showed toxic and

neoplastic lesions, for instance, increased chronic nephropathy severity, hyperplasia, and benign renal tubular epithelial neoplasia. These kidney lesions might be caused by a nongenotoxic and genotoxic combination[41]. Moreover, the kidney photomicrograph of showed distorted convoluted tubules, enlarged Bowman's space, and tissue hemorrhage at 2,900 mg/kg QOS[3].

6.3 Parathyroid gland hyperplasia and renal papillary transitional epithelial hyperplasia

National Toxicology Program (NTP) reported an increase in the incidence and severity of parathyroid gland hyperplasia and renal papillary transitional epithelial hyperplasia in male rats, common in advanced nephropathy[42]. The relative kidney and liver weights of F344/N rat were significantly greater than those of the controls, when received 40,000 ppm quercetin. Parathyroid gland hyperplasia was found in a dose-dependent increased incidence in male rats. This indicated the renal secondary hyperparathyroidism[42].

In urinalysis, the urines from both sexes were pigmented yellow with the presence of calcium oxalate crystals only in male rats [42]. The yellow-brown pigmentation was also found in several tissues such as epithelial cells lining the glandular stomach, jejunum, ileum, and, at lesser degree in the duodenum and colon[42].

6.4 Stress and inflammatory related toxicity

In mice, the generation of oxidative stress by high dose quercetins (1,500 and 2,000 mg/kg) was shown. Such treatments induced the elevation of lipid peroxidation level, the reduction of glutathione content and greatly altered the expression of stress regulated genes[40]. This suggested the oxidative stress induction by high dose quercetin in mouse model. The altered stress regulated gene expressions were the upregulation of metabolic pathways, and the downregulated stress signaling related genes, mostly *Hsp* (heat shock protein) genes[40]. Also, genes related to endocytosis and lysosomal pathways were downregulated. In addition, a highly strong relationship between the *MAPK* expression and the stress-signaling sub-network related genes was suggested[40].

6.5 Weight control effect

Chen *et al*., 2014; Dunnick and Hailey, 1992 and NTP, 1992 reported the lower body weight gains of rats given 40,000 ppm (approximately 1,900 mg/kg/day) quercetin, compared with controls[28,41-42]. This effect might be owing to quercetin toxicity.

6.6 Tumor induction

NTP stated that the uncommon renal tubule neoplasms, adenomas, and adenocarcinoma, were seen in male rats receiving high-dose quercetin. Likewise, renal tubule focal hyperplasia and tubule renal adenomas were detected in male rats with high-dose quercetin[42].

Pamukcu *et al.* reported the increased incidence of intestinal and urinary bladder tumors in Norwegian rats after 58 weeks of 0.1% dietary quercetin treatment[43]. Although positive in cell transformation assays in mouse model[44] and Syrian golden hamster cells[45], no evidence of quercetin carcinogenic effects was confirmed even at 40,000 ppm[42] or in long-term studies in mice and rats[28,46].

Kato *et al*. revealed that the tumor formation induced by quercetin in inbred ACI male rat receiving intraperitoneally 500 mg/kg Quercetin. After 16 months allowed for tumor formation, they found that tumor incidences were not significantly different in both treatment and control groups. Furthermore, quercetin did not induce genotoxicity in DNA repair tests on rat hepatocytes[27].

6.7 Mutagenic and genotoxic effects

Earlier studies stated the mutation induced by quercetin in *Salmonella typhymulium*, and the single-stranded deoxyribonucleic acid (ssDNA) breaks in *Escherichia coli*[23-26,47]. Gene conversion without gene mutation, was also found in *Saccharomyces cerevisiae*, while sex-linked recessive lethal mutation was shown in *Drosophila melanogaster after* quercetin exposure[48,49].

The forward mutations discovered were at the thymidine kinase (*tk*) locus but not in the hypoxanthine-guanine phosphoribosyl-transferase (*HPRT*), adenine phosphoribosyl-transferase (*APRT*) or sodium–potassium adenosine triphosphatase (*Na+/K+-ATPase*) loci in experiments with mouse[44], and hamster cells[44,50-51].

For genotoxicity data, quercetin did not show any effect at low dose (200 mg/kg)[52]. However, in animal cell studies, chromosomal aberrations, ssDNA breaks, micronucleus formation and sister chromatid exchanges (SCEs) following high dose quercetin treatments were described[42,44,48,50,53-57]. Chromosomal aberrations were reported in human lymphocytes (> 8 mcg/mL quercetin) and human HE2144 fibroblasts (> 1 mcg/mL quercetin) *in vitro*[55].

The ssDNA breakage was observed at 10 mcg/mL quercetin without S9 protein, in L5178Y TK+/-mutation assay system with the selection agent trifluorothymidine (TFT) [44] and in Chinese hamster lung V79 cells[57]. In mouse bone marrow treated with quercetin,

significantly higher DNA damage was observed by alkaline single-cell gel electrophoresis (SCG) assay, compared with the negative control[58].

The flavonol quercetin had generally a strong influence for micronuclei induction in human lymphocytes *in vitro*[53]. In mouse bone marrow cells, the significant increase of damage was presented by the micronucleus test at the 2 x 1,250 mg/kg quercetin[58]. However, for *in vivo* studies, quercetin could not induce micronucleus formation in mouse bone-marrow polychromatic erythrocytes[57].

Quercetin could significantly increase SCEs in pseudodiploid Chinese hamster fibroblastic cell lines[55], and in the L5178Y TK+/-mutation assay system with the selection agent TFT[44]. Also, Quercetin induced SCE in human lymphocytes, *in vitro*[56], while the flavonol quercetin showed only a weak capability[53]. However, one study reported that quercetin had only a slight impact on either point mutation or SCE as it could only slightly increase either SCE or mutation rate in CHO-AT3-2 cells with 15 mcg/mL quercetin administered[50].

Despite many studies reporting the genotoxicity and weak mutagenic activity of high dose quercetin in cell models, no concrete evidence was sufficient to establish quercetin as a carcinogen. No substantial data of quercetin inducing genotoxicity and mutagenesis reported in any in vivo studies. For example, quercetin could not induce micronucleus formation in mouse bone-marrow polychromatic erythrocytes[57]. Besides, quercetin showed reverse mutation abilities in many studies [23-26].

Conclusion

Natural polyphenolic flavonoids are abundant in plants, fruits and herbs. Most of them are responsible for the vivid colors and therapeutic activities of the floras. Flavonoids are generally accepted as favorable for human health. Among them, quercetin is one of the most powerful substances. Quercetin is an aglycone by adding sugar molecules to its backbone, commonly at position 3-OH group. The new derivatives are formed, for example; rutin, quercitrin, kaempferol, *etc*. Many clinical applications of quercetin are proposed, such as allergy-relieved, mouth ulcer healing, antioxidative stress, anti-inflammation, anti-diabetes and anti-cancer treatments. However, quercetin exhibits many signs of acute toxicity in animal models and genotoxicity *in vitro*. Moreover, quercetin shows drug interactions when in combination with other medications. Luckily, these toxicities usually occur at high dose quercetin, over 1,900 mg/kg body weight, while the recommended dose for human is

1,000 mg/day for no longer than 12 weeks. Conversely, since quercetin absorption level is varied in each person, thus; high dose treatment might be more beneficial, but its toxicity may possibly cancel all advantages. Therefore, weighing vigilantly on helpful and hindrance properties of quercetin should be cautiously applied.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

None

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine transaminase
APRT	Adenine phosphoribosyltransferase
AST	Aspartate aminotransferase
EMIQ	Enzymatically modified isoquercitrin
HER	Hydroxyethylrutosides
MAPK	mitogen-activated protein kinase
QOS	Quercetin from onion (*Allium cepa*) skin
ROS	reactive oxygen species
SCE(s)	Sister chromatid exchange(s)
ssDNA	Single-stranded deoxyribonucleic acid
TK, tk	Thymidine kinase

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