

Ginkgo Biloba Leave Extract: Biological, Medicinal, and Toxicological Effects

Po-Chuen Chan,¹ Qingsu Xia,² and Peter P. Fu²

¹National Institute of Environmental Health Sciences, Research Triangle Park, NC

²National Center for Toxicological Research, Jefferson, AR

Ginkgo biloba leave extract is among the most widely sold herbal dietary supplements in the United States. Its purported biological effects include: scavenging free radical; lowering oxidative stress; reducing neural damages, reducing platelets aggregation; anti-inflammation; anti-tumor activities; and anti-aging. Clinically, it has been prescribed to treat CNS disorders such as Alzheimer's disease and cognitive deficits. It exerts allergy and changes in bleeding time. While its mutagenicity or carcinogenic activity has not been reported, its components, quercetin, kaempferol and rutin have been shown to be genotoxic. There are no standards or guidelines regulating the constituent components of *Ginkgo biloba* leave extract nor are exposure limits imposed. Safety evaluation of *Ginkgo biloba* leave extract is being conducted by the U.S. National Toxicology Program.

Key Words: *Ginkgo Biloba* leave extract; biological and toxicological effects; ginkgolide; quercetin

INTRODUCTION

In 1994, the U.S. Congress passed the Dietary Supplement Health and Education Act (DSHEA) that amended the U.S. Federal Food, Drug, and Cosmetic ACT (FFDCA) and created a new regulatory category, safety standard, and other rules for the FDA to regulate dietary supplements. According to DSHEA, a dietary supplement is considered unsafe only if it presents a significant or unreasonable risk of illness or injury under conditions of use recommended or suggested in labeling, or if no conditions of use are suggested or recommended in the labeling, under ordinary conditions of use. Since then, herbal products

Address correspondence to Po-Chuen Chan, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. E-mail: chanp@niehs.nih.gov

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represent the fastest growing segment of the vitamin, mineral supplements, and herbal products industry. It is estimated that there are approximately 1500 herbal plants used as herbal dietary supplements or ethnic traditional medicines. Many commonly used herbal dietary supplements in the United States are prepared by using herbal plants.

Since the manufacturers do not have to submit safety reports of the herbal dietary supplement products to the FDA, the concentrations and safety aspects of their botanical ingredients are not known. Consumers who take dietary supplements may not be safely protected; as such, it is timely and important to ensure safety on dietary supplement products. The U.S. National Toxicology Program (NTP), which performs research focused on the most critical public health issues, has been conducting a series of long-term studies on the toxicity of herbal medicines and related dietary supplements products nominated by the public and Federal agencies. The nominated herbs and active ingredients are among the most widely sold and/or most potentially toxic products, and the objective of the studies is to characterize the potential adverse health effects of the products, including reproductive toxicity, neurotoxicity, immunotoxicity, and tumorigenicity. The studies also investigate potential herb/herb and herb/drug interactions, and determine responses of the sensitive subpopulations including pregnant women, the young, the developing fetus, the elderly, etc. toward the herbal products. Echinacea, golden seal, ginseng, kava, ginkgo and aloe vera are among the most widely sold herbal dietary supplements in the United States. *Ginkgo biloba* extract is listed as the fifth or sixth most frequently used herbal dietary supplement in the United States, and the third-best-selling herbal product in health food stores in the United States in 1997. In this review, we address the pharmacological and toxicological effects of extracts of ginkgo leaves, a form of commercial herbal dietary supplement used worldwide, with focus on its toxicity.

GENERAL INFORMATION OF *GINKGO BILOBA*

Historical Background

Ginkgo biloba is a unique tree with no close living relatives. The ginkgo tree is a species that flourished 150 million years ago during the Mesozoic era, reaching its greatest development during the Jurassic and Cretaceous periods (1). Thus, it is one of the best known examples of a living fossil. Ginkgo is a gymnosperm; as such, its seeds are not protected by an ovary wall. The ginkgo tree is now cultivated extensively in Asia, Europe, North America, New Zealand, and Argentina (2). Ginkgo seed has been listed as a source of medicine since the early Chinese herbals. The leaf has been recommended for medicinal uses as early as 1509 and is still used in the form of teas. Nowadays, extracts of ginkgo leaves in the form of film-coated tablets, oral liquids or injectable solutions can be purchased in Europe and America.

Table 1: The main constituents of *Ginkgo biloba* leaves (3–5)

Class	Major chemical constituents
Terpenoids	Diterpenes: ginkgolides A, B, C, J (M is found in the root) Sesquiterpene: bilobalide Triterpenes: sterols
Flavonoids (flavone, flavonol glycosides, and aglycones)	kaempferol, quercetin, isorhamnetin, rutin, luteolin, delphidenon, myricetin
Biflavonoids	Sciadopitysin, ginkgetin, isoginkgetin, amentoflavone, bilobetin, 5'-methoxybilobetin
Organic acids	Benzoic acid derivatives (ginkgolic acid), N-containing acids
Polyprenols	di- <i>trans</i> -poly- <i>cis</i> -octadecaprenol
Others	waxes, steroids, 2-hexenal, cardanols, sugars, catechins, proanthocyanidins, phenols, aliphatic acids, rhamnose

Chemical Constituents of *Ginkgo biloba* Leaves

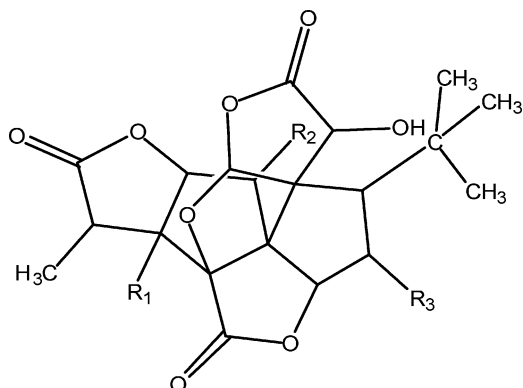
There are numerous chemical constituents contained in *Ginkgo biloba* leaves. The main constituents of are listed in Table 1.

The principal diterpene terpenoids (Table 1) are ginkgolides. The names and structures of ginkgolides in *Ginkgo biloba* leaf extract are shown in Figure 1. They have the same molecular geometrical skeleton; the structural differences are the number and geometric location of the hydroxyl functional groups (Figure 1).

The structure of bilobalide is shown in Figure 2. Both ginkgolides and bilobalide are the principal constituents of *Ginkgo biloba* that exhibit either biological and/or pharmacological activities of *Ginkgo biloba*.

The flavonoids frequently occur as glycoside derivatives. Quercetin, kaempferol, and isorhamnetin are the principal flavonoids in *Ginkgo biloba* and are structurally related. Their structures are shown in Figure 3. The Chemical Abstracts Service name of isorhamnetin is 4H-1-benzopyran-4-one,3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-(9CI). Kaempferol is a metabolite of quercetin and isorhamnetin is a metabolite of kaempferol.

Commercial extracts of the ginkgo leaves are enriched water-acetone or water-ethanol extracts of the ginkgo leaves, and are standardized on their flavonoid content or their terpene trilactone content. The standardized extracts contain 22–27% flavone glycosides, 5–7% terpene lactones (of which 2.8–3.4% consists of ginkgolides A, B, and C, 2.6–3.2% bilobalide), and less than 5 mg/kg (5 ppm) ginkgolic acids (6). Alkylphenol and alkylbenzoic acid derivatives, which have allergic, immunotoxic, and other undesirable properties, are completely removed from the extracts (7). Certain commercial products, including EGb 761 and LI 1370, do not contain biflavones. Although the proposed therapeutic

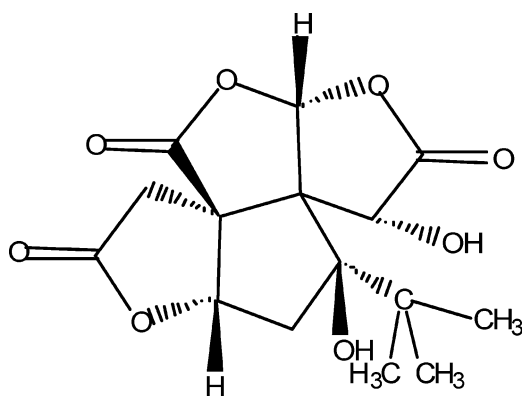


Ginkgolides

	R1	R2	R3
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH
Ginkgolide M	H	OH	OH

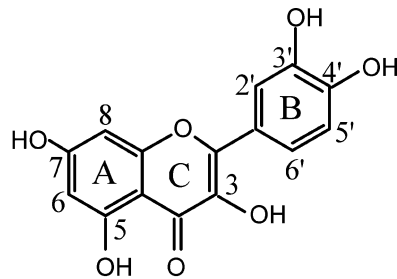
Figure 1: Structures of ginkgolides.

efficacy of the ginkgo leaf extracts is likely contributed by the terpene trilactones (ginkgolides and bilobalide) and the flavonoid glycosides, justification for the quality and quantity of the ingredients in the extracts of the ginkgo leaves has never been published (8).

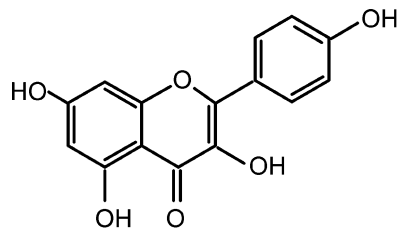


Bilobalide

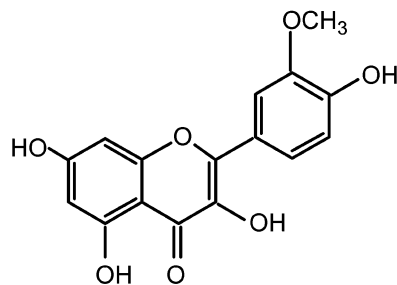
Figure 2: Structure of bilobalide.



Quercetin



Kaempferol



Isorhamnetin

Figure 3: Structures of quercetin, kaempferol, and isorhamnetin.

Commercial *Ginkgo biloba* Leaves Extracts

EGb 761 is a standardized, concentrated extract of ginkgo leaves produced by the pharmacological company Dr. Willmar Schwabe in Germany. Synthesis of this EGb 761 standardized product requires a 27-step extraction process, which starts with fifty pounds of leaves and yields one pound of extract (50:1 concentration). The final product contains 24% flavonoid glycosides (containing quercetin, kaempferol, isorhamnetin etc.), 6% terpenoids (in which 3.1% are ginkgolides A, B, C, and J and 2.9% is bilobalide), 5–10% organic acids, and other constituents (1, 9, 10). It was claimed that

EGb 761 does not contain ginkoflavone aglycones, biflavonoids, catechins (tannins), polyphenols, steroids, and proteins (6); however, analyses by independent investigators showed variation in the composition of the standardized extracts (11). EGb 761, also called Tebonin, Tanakan, Rökan, or Kaveri, is marketed in Europe as a medicine for cardiovascular disease (12). In the United States, Nature's Way has exclusive distribution rights to EGb 761 and markets this product as a dietary supplement under the trade name Ginkgold (1, 2, 13).

Many other companies in Asia, Europe, and the United States manufacture or distribute *Ginkgo biloba* extracts and dietary supplements containing extracts of the ginkgo leaves. Ginkgo leaf extract is also used in combination products to provide "special nutrients for the brain" (14). Ginkgo leaf extract and Ginkgo leaf extract products are available from bulk distributors and manufacturers in the United States and other countries.

It should be noted that the commercial extracts of Ginkgo leaves could be "full extracts," "crude extracts," or "simple extracts" that are complex mixtures consisting of active principles, inert plant constituents, and in some cases, constituents that may cause adverse side effects. The patent for the extract EGb 761 has expired. Since then, many manufacturers have standardized their extracts similar to that of EGb 761. However, it is worth noting that herbal remedies are not held to the same standards of purity and efficacy. As such, tremendous variability of the same product can occur between manufacturers and from batch to batch (8, 15).

Annual Consumption Level

Ginkgo biloba has been one of the most widely sold products in health food stores in the United States (16). Sales of *Ginkgo biloba* in the United States exceeded \$100 million in 1996 (17). Between July 19, 1996 and December 30, 1997, the Piers Imports database listed the following imports: 433,719 lb. ginkgo powders or leaves; 68,547 lb. tea; 21,922 lb. dried leaf powder; 17,339 lb. ginkgo tablets; 3,970 lb. crude natural drugs and herbs, and 476 lb. leaves extract. In the same period, approximately 31,000 lb. of ginkgo nuts were also imported (18). In Germany, more than 5 million prescriptions are written for *Ginkgo biloba* leave extract each year, with sales in 1993 amounting to \$280 million (10, 19).

GINKGO BILOBA—BIOLOGICAL EFFECTS

The purported therapeutic efficacy of *Ginkgo biloba* leave extract is likely contributed by the terpene trilactones (ginkgolides and bilobalide) and the flavonoid

glycosides. Thus, the standardized and the commercial extracts of ginkgo leave contain 22–27% flavone glycosides and 5–7% terpene lactones (6). The toxic constituent ginkgolic acids are reported to contain less than 5 mg/kg (5 ppm). Alkylphenol and alkylbenzoic acid derivatives, which have allergic, immunotoxic, and other undesirable properties, are completely removed from the extracts (7).

Ginkgo biloba leave extract may act through several mechanisms including antioxidant effects, inhibition of platelet activating factor, alterations in membrane fluidity (signal transduction), and inhibition of glucocorticoid synthesis. The purported beneficial effects of *Ginkgo biloba* leave extract might be channeled through a combination of one or more of the basic mechanisms of action. Its flavonoids components are believed to act in protecting against capillary fragility, as antioxidants, as anti-inflammatory agents, in reducing edema caused by tissue injury, and as free radical scavengers. Their biological effects include the following:

Neurological Disorders and Neural Damage

Ginkgo biloba leave extract has been reported to have effects on learning and memory processes as well as an anti-aging effect in humans. Daily oral dose of 240 mg EGb 761 was reported to be effective on presenile and senile primary degenerative dementia of the Alzheimer type and multi-infarct dementia according to DSM-III-R (20). It was reported that *Ginkgo biloba* leave extract can reduce corticosteroid production, improve cerebral blood flow, increase glucose uptake and utilization, ATP production, mitochondrial metabolism, and intra- and extra-cellular ionic gradients (21).

Winter (22) reported that mice treated with *Ginkgo biloba* leave extract at 100 mg/kg orally for 4–8 weeks resulted in improved memory and learning during appetitive operant conditioning. Rats dosed with *Ginkgo biloba* leave extract at 100 mg/kg per day, p.o., for 4 to 8 weeks prior to training and then for an additional 10 weeks resulted in reduced time to acquisition of tasks and enhanced retention performance (22). Cohen-Salmon et al. (23) also showed that EGb 761 (40 mg/kg ip for 1–3 weeks) enhanced learning in young (6 months old) and aging (22 months old) Swiss mice.

Although most of the literature is consistent with the view that *Ginkgo biloba* leave extract can protect against the effects of neural damage, whether the neuroprotection is from a direct action on the neurons or from an indirect effect from modulation of blood flow and antioxidant action is unclear (24). Krieglstein and coworkers (25) determined that in rodents, both bilobalide and ginkgolides A and B reduced the infarct area on the mouse brain surface when administered before occlusion. When *Ginkgo biloba* leave extract was injected into rats after global forebrain ischemia, local cerebral blood flow was significantly elevated, but neuroprotection was not observed (26).

Vasseur et al. (9) found that the protective effects of EGb 761 against alloxan-induced cytotoxicity were due to a free radical-scavenging effect of flavonoids and an effect of bilobalide on glucose metabolism. Kim et al. (27) demonstrated that flavonoids (quercetin, kaempferol, sciadopitysin, ginkgetin, isoginkgetin) stimulated the proliferative activity and increased production of collagen and extracellular fibronectin of human skin fibroblast in vitro, suggesting the protective role of flavonoids against tissue injuries. Bilobalide was found to protect against neuronal death in global brain ischemia and in glutamate-induced excitotoxicity (28, 29).

Improvement of Blood Flow

It is purported that *Ginkgo biloba* leave extract can improve blood flow by increasing red blood cell deformability and decreasing red cell aggregation, and thus, improves red blood cell fluidity and decreases whole blood viscosity (30). The vascular effects of *Ginkgo biloba* leave extract may also be mediated via the endothelium-derived relaxing factor (EDRF), presumed to be nitric oxide (NO), which relaxes the muscular cells of blood vessels. Nitric oxide has been shown to inhibit the release of prostacyclins from cultured bovine endotheliums cells (30, 31). By scavenging nitric oxide, *Ginkgo biloba* leave extract could potentiate the effects of prostacyclins. However, an excess of the free radical nitric oxide is a deleterious factor and can result in various CNS disorders. Bastianetto et al. (32, 33) determined that *Ginkgo biloba* extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity, and that the protective and rescuing abilities of EGb 761 are not only attributable to the antioxidant properties of its flavonoid constituents, but also via their ability to inhibit NO-stimulated protein kinase C (PKC) activity.

Marcocci (31) determined that besides EDRF/nitric oxide, other factors could also be involved in the beneficial vascular effects of *Ginkgo biloba* leave extract. For example, EGb 761 has been shown to stimulate the release of prostacyclins from the endothelium of rat aortic preparations.

Antagonism and Anti-inflammatory Effect

It has been purported that the administration of *Ginkgo biloba* leave extract resulted in decreases in platelet aggregation, allergic reaction, general inflammatory response, oxygen radical discharge and other proinflammatory functions of macrophages (34, 35). The effects appear to be attributed to the combined actions of ginkgolides and flavonoids. An increase in platelets aggregation factor occurs in asthma, graft rejection and in immune disorders that induce toxic shock. Ginkgolides have been shown to possess very specific and potent antagonist activity against platelets aggregation factor, which may result in increased peripheral blood flow (34, 35). Flavonoids in *Ginkgo biloba* leave

extract reportedly inhibit cyclo-oxygenase and lipoxygenase that are involved with arachidonic acid metabolism. Cyclo-oxygenase activity produces thromboxane A₂, a potent platelet aggregator and lipoxygenase is concerned with the formation of leukotrienes, the substances associated with inflammation. Houghton (26) proposed that increased blood flow and the anti-inflammatory effect of *Ginkgo biloba* leave extract may be related to inhibition of cyclo-oxygenase and lipoxygenase activity. Lee et al. (36) determined that bilobetin, ginkgetin, and isoginkgetin also suppressed the lymphocyte proliferation induced by lipopolysaccharide.

Antianxiety

Stress causes increases in circulating concentrations of epinephrine, norepinephrine and corticosterone (37). EGb 761 significantly decreased plasma concentrations of adrenocorticotrophic hormone, corticosterone, norepinephrine and epinephrine and the hypothysial portal blood concentration in rats (38). EGb 761 acts on the CNS to decrease corticotropin-releasing hormone and arginine vasopressin in the hypothalamus and ACTH in the pituitary (5, 39).

Ginkgo biloba leave extract inhibited monoamine oxidase (40). Monoamine oxidase regulates biogenic amines in the brain and exposure to *Ginkgo biloba* leave extract elevates brain concentrations of serotonin, norepinephrine, and other biogenic amines in rats.

Satyan et al. (41) reported that ginkgolic acid exhibited anxiolytic activity.

Modulation of Drug Metabolizing Enzymes and Cause of Herb-Drug Interactions

Ginkgo biloba was reported to inhibit CYP 450 enzyme activity and consequently may produce CYP-mediated herb-drug interactions (30,42–44).

Bilobalide reduced the duration and incidence of 4-O-methylpyridoxine-induced convulsions in mice. Sasaki et al. (45) determined that bilobalide potentiated hepatic 7-methoxycoumarin O-demethylase activity in mice (metabolizing 4-O-methylpyridoxine). The authors suggested that along with the anticonvulsant effects of bilobalide, the induction of drug-metabolizing enzymes by bilobalide may render a chemoprotective effect against toxic chemicals. Duche et al. (46) reported that no change in hepatic microsomal enzyme activity 4–24 hours after taking a 400 mg/day dose of *Ginkgo biloba* leave extract for 13 day in healthy male volunteers. Hepatic microsomal drug oxidase activity was measured by estimating plasma antipyrine concentration with the use of high performance liquid chromatography.

Ginkgo biloba are often taken in combination with prescription and conventional medications causing herb-drug interactions (47–51). It was purported

that Ginkgo may alter bleeding time and should not be used concomitantly with warfarin sodium (52, 53) or diazepam (54).

Antioxidant (Radical Scavenging) and its Beneficial Health Effects

The flavonol glycosides and proanthocyanidins have free radical-scavenging activity, and thus may play a protective role in the prevention of the atherosclerotic processes (55) and improving conditions resulting from oxidative stress (56). Scavenging superoxide, hydroxyl, and peroxy radicals and nitric oxide (31) may affect signal transduction (56). Attributing to its antioxidant and free-radical-scavenging properties, DeFeudis reported that *Ginkgo biloba* leave extract can affect neurosensory systems (5).

Terpenes except ginkgolide A are superoxide (O_2^-) scavengers (57) and flavonoids are scavengers of superoxide and hydroxyl radicals (58, 59). Topically applied *Ginkgo biloba* leave extract induced superoxide dismutase and catalase enzyme activities locally in the epidermis and systemically in the liver, heart, and kidney of Sprague-Dawley rats (60). EGb 761 inhibits or reduces the functional and morphological retina impairments observed after lipoperoxide release (61), and protects pancreatic cells against the toxic effects of alloxan probably by scavenging free radicals and stimulation of glucose utilization (9).

Emerit et al. (59) reported that the clastogenic factors in the plasma of persons irradiated accidentally or therapeutically were suppressed by EGb 761. Lee and associates (62) demonstrated that EGb 501 blocked serotonin stimulated proliferation of bovine pulmonary artery smooth muscle cells as well as Chinese hamster lung fibroblasts (CCL-39) by scavenging O_2^- . The authors demonstrated that the mitogenic activity of serotonin (inducing cellular hyperplasia and hypertrophy) were mediated via elevation of oxygen radicals.

The free radical-scavenging and anti-lipoperoxidative effects of *Ginkgo biloba* leave extract protect both nitric oxide and prostaglandin I_2 from degradation (31, 63). Nitric oxide activates the enzyme guanylate cyclase resulting in increased levels of cyclic guanosine monophosphate, producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood. This is how Viagra works.

The free radical scavenging activity may also attribute to longevity and anti-aging merits. Winter (64) reported that male Fischer 344 rats on EGb 761 (50 mg/kg/day in drinking water) for life had significantly higher survival rate than controls. The effect may be related to reduced oxidative stress and free radical production. It has been proposed that senescence of postmitotic cells is due to cellular and tissue damages induced by free radicals and that the mitochondrial DNA (mtDNA), in particular, are major targets of free radical attack. Oxidative lesions are thought to be important contributors to aging.

Indeed, oxidative lesions to mtDNA have been shown to accumulate with age in human and rodent tissues (65, 66).

Treatment with EGb 761 (100 mg/kg in drinking water daily for 3 months) partially prevented the morphological changes as well as the indices of oxidative damage observed in brain and liver mitochondria from old rats (67).

Antitumor Activity

Ginkgo biloba leave extract (100 mg/kg) given ip prior to irradiation of transplanted fibrosarcoma in C3H mice enhanced radiosensitivity of tumor cells. Growth of the tumors was delayed and there was no enhancement of radiation damage in normal tissue. Radiosensitivity of the tumors cells was increased probably by improving tumor blood flow and decreasing hypoxic fraction in the tumor (68). Anticarcinogenic activity of flavonoids has also been reported (69,70). Agha et al. (71) reported that EGb exhibits a beneficial chemopreventive effect against BP-induced gastric carcinogenesis in mice.

Protective Effects Against Drug, Chemical and Heavy Metal Induced Toxicity

Naidu et al. (72) reported protective effect of *Ginkgo biloba* leave extract against doxorubicin-induced cardiotoxicity in mice. EGb 761 is a protective agent in bleomycin-induced lung fibrosis (73) and ameliorates gentamicin-induced nephrotoxicity in rats (74). *Ginkgo biloba* leave extract protected against cisplatin-induced nephrotoxicity (75, 76). *Ginkgo biloba* exerts protective effects of against acetaminophen-induced toxicity in mice (77) and against mercury(II)-induced oxidative tissue damage in rats (78). Suzuki et al. (79) found that *Ginkgo biloba* leave extract and its component bilobalide on azoxymethane-induced colonic aberrant crypt foci in rats. EGb 761 also exhibited protective effects on vancomycin-induced nephrotoxicity, presumably through inhibition of free oxygen radical production (80). EGb 761 also protects endotoxin-induced oxidative renal tissue damage of rats (81).

Massieu et al. (82) found that EGb 761 exerted neuroprotective effect on staurosporine-induced neuronal death and caspase activity in cortical cultured neurons. This neuroprotective effect of EGb 761 might be mediated by its antioxidant activity, which in turn influences caspase-3 activation. Bastianetto and Quirion (83) examined the potential of the *Ginkgo biloba* extract EGb 761 on cell death produced by beta-amyloid (A β) peptides and oxidative stress, and its deleterious role in age-related neurological disorders. They found that EGb 761 protected hippocampal cells against toxic effects induced by A β peptides, and suggested that this effect was through the antioxidant properties of its flavonoid constituents. While rat hippocampal cells exposed to the nitric oxide (NO) donor sodium nitroprusside (SNP) resulted in cytotoxicity and

reactive oxygen species (ROS) accumulation, EGb 761 inhibited these events through their antioxidant activities and blocking SNP-stimulated activity of protein kinase C (PKC). EGb 761 inhibited amyloid beta aggregation in vitro and attenuates ROS in round worm *Caenorhabditis elegans* (84).

Anti-Microbiol Activity

Biloblidide has insecticidal properties and may be involved in protecting *Ginkgo biloba* against herbivorous insects or mammals (85).

GINKGO BILOBA—PHARMACOLOGICAL AND MEDICAL APPLICATIONS

Treatment with *Ginkgo biloba* can be traced to the origins of Chinese medicine 2,800 years ago. In the modern Chinese pharmacopeia, leaves and fruit are still recommended for treating heart and lung (asthma and bronchitis) problems. The nut, called Pak Ko, is recommended to expel phlegm, stop wheezing and coughing, urinary incontinence, and spermatorrhea. The raw seed is said to be anticancerous. It is said to help bladder ailments, menorrhoea, uterine fluxes, and cardiovascular ailments. The powdered leaf is inhaled for ear, nose, and throat disorders like bronchitis and chronic rhinitis. Locally applied boiled leaves are used for chilblains. The plant has also been used to treat conditions that may have poor circulation as a common symptom, such as brain function impairment and inner ear disorders such as hearing loss, vertigo and tinnitus (1, 86).

The standardized *Ginkgo biloba* leave extract EGb 761 is a popular dietary supplement taken by the general public to enhance memory (87–91). *Ginkgo biloba* is widely used for its reputed effectiveness in CNS disorders. (92–94). The pharmacological studies by Ahlemeyer and Krieglstein (87) supported the therapeutic use of *Ginkgo biloba* extract EGb 761 for Alzheimer's disease. Evidence has been purported that accumulation of beta-amyloid (Abeta)-derived peptides and free radicals result in Alzheimer's disease. EGb 761 exhibited neuroprotective effects in Alzheimer's disease through its antioxidant capability and inhibition of Abeta-induced toxicity and cell death (32, 84, 95, 96). As reported, *Ginkgo biloba* extract EGb 761 is widely prescribed in the treatment of cognitive deficits including Alzheimer's disease (97).

Ginkgo biloba leave extract can ameliorate learning and memory deficit induced by $AlCl_3$ (98). *Ginkgo biloba* leave extract has been shown to improve global and local blood flow and microcirculation, protect against hypoxia, inhibit platelet aggregation, improve blood rheology and reduce capillary permeability (5) and is, therefore, prescribed to treat cerebro-, cardio- and periphero-vascular problems. Ginkgo flavonoid and Ginkgolide exhibit marked protective effects

on cardio-cerebral vascular and central nerve systems, clinically applied mostly in treatment of cardio-cerebral vascular diseases (99, 100).

Current use of *Ginkgo biloba* for medical applications include:

1. Improving brain function: memory retention, mental clarity, Alzheimer's, dizziness, depression.
2. Strengthening the cerebrovascular and cardiovascular systems by inhibition of platelet aggregation and increasing blood flow and oxygen supply.
3. Neutralizing free radicals that deteriorate nerves, damage cellular health, and accelerate aging.
4. Stabilizing cellular energy production (higher concentrations of ATP, glucose, creatinine phosphate and lower levels of lactate).
5. Suppressing hemorrhoids, inflammation, migraine, allergies, and asthma.

It should be noted that the therapeutic benefit of *Ginkgo biloba* depends on a combination of its constituents, each with its own pharmacological activity. Interestingly, the total extract is more active than single isolated components. This suggests that various components of *Ginkgo biloba* leave extract act synergistically on diverse processes. Thus, it is not surprising that its therapeutic benefits are polyvalent or multifactorial, i.e., it is effective in treating more than one clinical disorder. The effectiveness in treating a disorder depends on the proportions of various constituents in the extract. The *Ginkgo biloba* leave extracts marketed by different manufacturers vary qualitatively and quantitatively and their effectiveness cannot be compared (12).

Concerning *Ginkgo biloba* leave extract treatment, it is currently not known about the high dose effects, how long the effects last, the effects after stopping treatment, and treatment given preferentially for life or intermittently.

Human Exposure

The recommended dose of *Ginkgo biloba* leave extract is 120 to 160 mg daily for persons with intermittent claudication and 240 mg daily for cerebrovascular insufficiency, early stage Alzheimer's disease, resistant depression, and impotence (10). Weaker doses or cruder extracts may not be effective.

Environmental Occurrence

No information was found in the available literature on potential environmental pollution from the manufacture of *Ginkgo biloba* leave extract or dietary supplements containing *Ginkgo biloba* leave extract. However, contact with whole ginkgo plants has been associated with severe allergic reactions, including erythema and edema, similar to response to poison ivy (1).

Regulations

No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of *Ginkgo biloba* or *Ginkgo biloba* leave extract. *Ginkgo biloba* was not in the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) and biological exposure index (BEI) are made. Neither *Ginkgo biloba* nor *Ginkgo biloba* leave extract is listed in the EPA's Toxic Substances Control Act (TSCA) Inventory.

TOXICITY, GENOTOXICITY, AND TUMORIGENICITY

Ginkgo Biloba Leave Extracts

Human Data

In spite of the fact that the use of *Ginkgo biloba* leave extract has been increased due to its popularity among proponents of “smart drugs” and the purported ability of *Ginkgo biloba* leave extract to improve mental alertness and overall brain function, no epidemiological studies or case reports investigating the association of exposure to *Ginkgo biloba* leave extract or its identified active ingredients and toxicity/cancer risk were identified in the available literature. It is likely that the number of acute poisonings caused by *Ginkgo biloba* leave extract is underreported because current US law does not require manufacturers of dietary supplements to test and supply toxicity reports to the federal government (19). In Germany, the government has asked manufacturers of *Ginkgo biloba* products to include a label on their oral products that headaches, dizziness, palpitations, gastrointestinal disturbances and allergic skin reactions are possible adverse effects (1). The side effects could be reduced by lower doses of *Ginkgo biloba* leave extract. However, severe side effects have been reported to the German health authorities when *Ginkgo biloba* leave extract was used in parenteral application (11).

Kleijnen and Knipschild (21) reviewed the efficacy and safety of *Ginkgo biloba* leave extract in at least 40 clinical trials. Dosages in the 120–160 mg range daily were administered for 4 weeks to 3 months. No serious side effects were reported in any trial. A recent clinical trial by Le Bars and coworkers (101) assessed the safety and efficacy of *Ginkgo biloba* leave extract administered at 120 mg/day for 52 weeks. Adverse events were as follows in the *Ginkgo biloba* leave extract group vs. the placebo group, respectively: 30% (49/166) vs. 31% (50/161) for all adverse events, 16% (27/166) vs. 12% (19/161) for events related to the study drug, and 12 vs. 9 reported adverse events of severe intensity. Overall, the *Ginkgo biloba* leave extract group reported slightly more gastrointestinal tract complications and symptoms than the placebo group. The

findings were compromised by the high dropout rates of 50% in the *Ginkgo biloba* leave extract group and 62% in the placebo group.

It has been reported that 4-O-methylpyridoxine, a neurotoxic antivitamin B6, also known as ginkgotoxin, found in ginkgo seeds, was responsible for convulsions, loss of consciousness, and death in Japan. The chemical inhibits the formation of 4-aminobutyric acid from glutamate in the brain. A deficiency of 4-aminobutyric acid in the brain may induce seizures. *Ginkgo biloba* leave extract from ginkgo leaves also contains 4-O-methylpyridoxine (102).

Increase the Risk of Bleeding

Bent et al. (103) reported that spontaneous bleeding was associated with *Ginkgo biloba*. A number of review articles have suggested that ginkgo may increase the risk of bleeding. *Ginkgo biloba* leave extract may reduce the ability of the blood to clot. Potentially serious adverse effects associated with *Ginkgo biloba* have been reported. A 33-year-old woman had taken 120 mg of *Ginkgo biloba* leave extract daily for two years before complaining of diffuse headaches accompanied by diplopia, nausea, and vomiting (104). A MRI scan of the brain revealed bilateral subdural hematomas. The fluid was removed and identified as unclotted blood. Bleeding time, evaluated while the woman was still taking *Ginkgo biloba* leave extract, was prolonged. Thirty-five days after discontinuation of *Ginkgo biloba* leave extract, bleeding times were within the normal range. At the time the report was written, the patient continued not to take *Ginkgo biloba* and remained free of headaches and other neurologic symptoms (104). Gilbert (105) reported that a 72-year-old woman taking *Ginkgo biloba* leave extract at 50 mg thrice a day for 6–7 months developed subdural hematoma. Rosenblatt et al. (106) reported that spontaneous bleeding from the iris of the eye (hyphema) occurred in a 70-year old man one week after he began twice daily ingestion of a Ginkoba™ tablet containing 40 mg of concentrated (50:1) extract. The patient was on aspirin (325 mg) daily. In a 3-month follow-up period, the patient had stopped taking Ginkoba but continued taking aspirin and had no recurrence of bleeding (106). It was thought that the additive effects of ginkgolide B, a potent inhibitor of platelet-activating factor, in the *Ginkgo biloba* leave extract and aspirin were responsible for the bleeding.

Hauser et al. (107) reported the bleeding complications precipitated by unrecognized *Ginkgo biloba* use after liver transplantation, with potentially life-threatening toxicity. It was suggested that the history of using herbal medicines must be sought for transplant patients. A randomized, placebo-controlled, double-blind study in healthy volunteers indicated that a 7-day treatment with *Ginkgo biloba* special extract EGb 761 can influence bleeding time and coagulation (108). Dugoua et al. (109) suggested that Ginkgo must be used with caution during pregnancy.

Table 2: Acute toxicity of EGb 761 (1)

Chemical	Species	Route of Adm.	LD ₅₀ Value
EGb 761	Mice	Oral	7.73 g/kg ^a
		i.v.	1.1 g/kg
		i.p.	1.9 g/kg
	Rats	Oral	>10 g/kg
		i.v.	1.1 g/kg
		i.p.	2.1 g/kg

^aThe LD₅₀ of 7.73 g/kg corresponds to 2.3 g/kg of active ingredients, 1.9 g/kg of flavone glycosides, and 464 mg/kg of terpene lactones.

Animal Data

Acute Toxicity. The acute toxicity of EGb 761 was studied. The results are shown in Table 2.

Al-Yahya et al. (110) studied biochemical toxicity of *Ginkgo biloba* in Swiss albino mice and found that *Ginkgo biloba* caused depletion of nucleic acids, nonprotein sulfhydryl and increase of malondialdehyde, elucidating the role of free radical species in the induced changes in testis chromosomes and the reproductive function.

Subchronic Toxicity. There was no evidence of organ damage or impairment of hepatic and renal functions when *Ginkgo biloba* leave extract was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1,600 mg/kg (1).

Chronic Toxicity. Rats exposed to EGb 761 at 4, 20, and 100 mg/kg/d for 2 years showed no histopathological changes; details of the study are not available (5, 6).

Reproductive and Developmental Toxicity. Oral administration of *Ginkgo biloba* leave extract at up to 1600 mg/kg/day to rats and 900 mg/kg/day to rabbits had no effects on reproduction and development (11). Reports of reproductive and developmental toxicity of ginkgoloid or bilobalide were not found.

Ondrizek et al. (111) reported a fertility test by mixing ginkgo with human sperm and hamster eggs. Zona-free hamster oocytes were incubated for 1 hour in ginkgo or control medium before sperm-oocyte interaction. The study showed that high concentrations (1 mg/ml) of ginkgo resulted in reduced sperm viability and oocyte penetration. Mutation of the designated sentinel BRCA1 exon 11 gene in sperms incubated with ginkgo for 7 days was not detected.

Disposition and toxicokinetics. *Ginkgo biloba* leave extract is well absorbed in humans, rats, and rabbits after oral administration. (11, 112, 113). Moreau et al. (114) showed that at least 60% of (¹⁴C)EGb 761 (360 mg/kg) was absorbed in rats after oral administration. Specific activity in blood peaked after 1.5 hours. At 3 hours, the highest amount of radioactivity was measured in the stomach and small intestine. Glandular and neuronal tissues and eyes

showed a high affinity for the labeled substance. Within 3 hours, 17% of the administered dose was expired as (^{14}C) CO_2 . After 72 hours, about 38% of the diminished dose was exhaled as carbon dioxide, 22% were excreted in urine, and 29% was present in feces. The pharmacokinetics was characteristic of a two-compartment model with an apparent first-order phase and a half-life of about 4.5 hours. The labeling was present mainly in the flavonoid glycosides. The authors also showed that ginkgolide B had a longer half-life than ginkgolide A.

Pietta et al. (115) reported that after oral administration of *Ginkgo biloba* leave extract (4 g/kg) to female Wistar rats, no intact glycosides or aglycones were detected in blood at 30 minutes or 6 hours whereas 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-(4-hydroxyphenyl)propionic acid, 3-(3-hydroxyphenyl)propionic acid were detected in blood. Metabolites identified in 24-hour urine were 3,4-dihydroxyphenylacetic acid, benzoyl-glycine (hippuric acid), 3-hydroxyphenylacetic acid, homovanillic acid, 3-(4-hydroxyphenyl)propionic acid, 3-(3-hydroxyphenyl)propionic acid, and benzoic acid. Based on AUC results obtained from pharmacokinetic studies, it was calculated that an oral dose of 240 mg in human corresponds roughly to an oral dose of 50 mg/kg in rat (5).

Genotoxicity. Mutagenicity of *Ginkgo biloba* leave extract was not detected in tests in *Salmonella typhimurium* strains TA1535, 1537, 1538, 98, and 100 with or without rat liver S9; in host-mediated-assay in mouse at 20 g/kg p.o.; in micronucleus test in mouse at 20 g/kg p.o.; and in chromosome aberration test in human lymphocytes at 100 g/ml (11).

Ginkgo biloba leave extract was nominated by the NCI for the U.S. National Toxicology Program (NTP) to conduct toxicological evaluation, mechanistic studies, and two years chronic carcinogenicity bioassay. The reasons of nomination were: (a) *Ginkgo biloba* leave extract, a defined product, and its active ingredients, the ginkgolides, especially ginkgolide B, and bilobalide, have clearly demonstrated biological activity; (b) *Ginkgo biloba* leave extract can be consumed in rather large doses for an extended period of time; and (c) some ingredients in *Ginkgo biloba* leave extract are known mutagens; in one case, a suspected high dose carcinogen, quercetin, is intentionally concentrated from the ginkgo leaf to manufacture the final product. The carcinogenicity of quercetin will be discussed in the following section.

Ginkgolides

Doses of 720 mg pure ginkgolide A, B, or C, produced no observable adverse effects and doses of 360 mg pure ginkgolide per day for one week gave also no adverse effects (11). In rabbits, plasma ginkgolide concentrations reached a peak 3 hours after a single oral dose (40 mg/kg) of Ginkoba (a 24/6 extract). After a single oral dose (40 mg/kg) of BioGinkgo, a 27/7 extract, 2 peak plasma levels of ginkgolide were observed, at 2 and 5 hours. Bioavailability of ginkgolides in

the BioGinkgo 27/7 was greater than that of the 24/6 extract (Ginkoba) 12 hours after oral administration, probably due to higher concentration of ginkgolide B (1.49%) in the former than in the latter (0.87%). Bioavailability of bilobalide (half-life 3 hours) is 70% after a dose (120 mg) of EGb 761. Ginkgolides A and B were excreted unchanged in the urine at about 70% and 50%, respectively; and bilobalide about 30% (12). Ginkgolides A and B appear to be absorbed and circulated in unmodified form in the body.

In humans after an oral dose of EGb 761 (80 mg), maximum plasma levels of ginkgolide A (15 ng/ml) and ginkgolide B (4 ng/ml) were attained at 1.4–2.0 hours. The half-lives of ginkgolide A was 3.9 hours and ginkgolide B was 7 hours. In rats, after a single oral dose of EGb 761 (30, 55, or 100 mg/kg) or 100 mg/kg/day for 5 days, the C_{max} in plasma were reached in 0.5–1 hour for ginkgolides A and B and bilobalide. The half-lives were about 1.8 hours for ginkgolide A, about 2 hours for ginkgolide B, and about 3 hours for bilobalide. About 70% of ginkgolide A, about 50% of ginkgolide B and about 30% of bilobalide were excreted unchanged in the urine (5).

Quercetin and Derivatives

Flavonols are widely present in consumed foods. Among the flavonols present in *Ginkgo biloba* leave extract, quercetin has been found cytotoxic and genotoxic.

In humans, peak plasma concentration of flavonol glycosides was attained within 2–3 hours after an oral dose of *Ginkgo biloba* leave extract (LI 1370); the half-life was 2–4 hours. The values returned to baseline levels 24 hours after intake (112). In humans, orally administered quercetin and rutin were not found in the urine or plasma in an unaltered form. However, 53% of the orally administered dose was recovered as quercetin in the feces within the first 3 days. In rats 12 hours after administration of radiolabeled quercetin orally, 80% of the radioactivity was recovered in the carcass, with the major portion (44%) found in the intestinal contents. Of the remaining radioactivity, 15% was respired, 12% was found in lung tissue, 3% was in the wall of the large intestine, less than 1% was in the blood, kidney, and gastric wall, and 4% was in the urine. It has been estimated that unaltered quercetin from ingestion of 25–50 mg daily would result in a distribution of 0.003–0.012 mol/kg body weight in a typical 70 kg man (116).

Subchronic Toxicity

No adverse effects were reported when quercetin was administered by dosed feed in male and female F344 rats at 0.1–0.2% for 64 weeks (117), in albino rats at 0.25–1% for 410 days (118), and in ACI rats at 10% for 850 days (119).

Chronic Toxicity

Quercetin has been tested for carcinogenicity in several species; this information is considered relevant to *Ginkgo biloba* leave extract and is presented below. Quercetin has also been studied extensively in initiation-promotion studies. These studies have generally been negative (120).

Bhattacharya and Firozi (121) determined that quercetin was very active in modulating microsomal activation of aflatoxin B1 and DNA adduct formation. The basal cytotoxicity and metabolism-mediated cytotoxicity of kaempferol, quercetin, and rutin in McCoy cells were studied (122). Kaempferol and quercetin were found cytotoxic and provoked a dose-dependent decrease in cell viability, without the S9 system. The hepatic S9 microsomal fraction metabolized these compounds to the metabolites with lower cytotoxicity. Antognoni et al. (123) demonstrated that quercetin strongly inhibited pollen tube growth in vitro, and also produced irreversible malformations in growth, while its glycoside, rutin promoted growth.

Cytotoxicity

Kim et al. (124) studied the antioxidant activity and cytotoxicity of myricetin, quercetin, kaempferol, and galangin on human umbilical vein endothelial cells and their potential antiangiogenic and cell adhesion effects. The relative antioxidant capacity of these flavonols in cell culture medium (cell-free system) and their intracellular antioxidant activity were myricetin = quercetin > kaempferol = galangin. These results indicate the greater the number of hydroxyl groups on the B-ring (Figure 3) the less toxic the flavonol. The LD50 was determined as: myricetin > quercetin > kaempferol > galangin. Kim et al. (124) proposed that the numbers of hydroxyl groups on the B-ring of these flavonols may play an important role in their antioxidant activity, toxicity, angiogenesis, and immune-endothelial cell adhesion, leading to developing cancer and atherosclerosis.

Soares et al. (122) determined that kaempferol and quercetin were cytotoxic in vitro without requiring the S9 system.

Genotoxicity

The capacity of rutin and quercetin to cause DNA damage was studied using alkaline single-cell gel electrophoresis (SCG) and the micronucleus test in the mouse bone marrow (125). Rutin did not cause DNA damage in the micronucleus test but caused DNA damage only at high dose; quercetin caused significant DNA damage in both SCG assay and micronucleus test. Yamashita and Kawanishi (126) reported that quercetin induced DNA cleavage with subsequent DNA ladder formation, characteristics of apoptosis, in HL-60 cells. In the H₂O₂-resistant clone of HL-60 cells, quercetin induced DNA cleavage and DNA ladder formation in a level less than that in HL-60 cells. In addition, quercetin

increased the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), an indicator of oxidative DNA damage, in HL-60 cells but not in the H₂O₂-resistant clone of HL-60 cells. Thus, the results obtained by Yamashita and Kawanishi (126) suggest that quercetin induces H₂O₂-mediated DNA damage, leading to apoptosis, mutations, and possibly carcinogenic effects.

Binding to DNA and Protein

Kessler et al. (127) studied the anti- and pro-oxidant activity of rutin and quercetin derivatives and determined that alkylation of the hydroxyl in position 7 enhanced the scavenging. In a Fenton reaction system, some quercetin derivatives with free catechol moiety or free hydroxyl in position 3 (or both) were pro-oxidant. Thus, to avoid pro-oxidant behavior, the hydroxyl group in position 3 should be blocked to prevent its auto-oxidation.

When quercetin acts as an antioxidant, quercetin itself is oxidized, forming quercetin *o*-quinone/quinone methide. Quercetin *o*-quinone/quinone methide is toxic, capable of interacting with proteins and forming adduct with glutathione (GSH) (128,129). This GSH-quercetin adduct is not stable, can reversibly dissociates back into GSH and quercetin *o*-quinone/quinone methide with a half life of 2 min. Evidence has been shown that GSH-quercetin adduct acts as a transport and storage form of quercetin *o*-quinone/quinone methide (128,129). Thus, the toxicity of quercetin *o*-quinone/quinone methide can be elicited by the formation of GSH-quercetin adduct that exerts quercetin *o*-quinone/quinone methide-induced toxicity. Because quercetin *o*-quinone/quinone methide reacted much faster with glutathione or protein thiols than with DT-diaphorase with isolated compounds, in human liver cytosol or blood plasma, DT-diaphorase does not play a role in the protection against quercetin *o*-quinone/quinone methide (130). Choi et al. (131) investigated the effects of long-term administration of quercetin on glutathione and the enzymes involved in its metabolism in male Sprague-Dawley rat liver in vivo. This leads to decrease in glutathione concentration and glutathione reductase activity, suggesting that quercetin acts as a pro-oxidant agent by decreasing glutathione concentration and glutathione reductase activity.

Walle et al. (132) reported that quercetin can covalently bind to cellular DNA and protein in human intestinal and hepatic cells. Woude et al. (133) studied the covalent binding of quercetin to DNA and, glutathione, protein, resulting in the formation of quercetin-*p*-quinone methide derived DNA, glutathione, and protein adducts. These adducts are chemically unstable, reversing back to quercetin-*p*-quinone methide. The study by Woude et al. (133) was conducted in four different cell lines: the cell lines HL-60 and B16-F10 with elevated levels of peroxidase and tyrosinase, respectively; and HepG2 and Caco-2 cells, which did not contain any detectable activity of tyrosinase or peroxidase-type oxidative enzymes. The results suggested that tyrosinase and/or peroxidase-type

oxidative enzyme activities do not play a major role in the intracellular formation of prooxidant metabolites of quercetin. As such, the authors suggested that oxidative metabolites of quercetin, formed in an enzymatic system containing these types of oxidative enzyme activities, may not be capable of efficiently binding to DNA or protein (133).

Mutagenicity

Extensive information suggests that quercetin and kaempferol are frameshift mutagens. Quercetin has consistently shown mutagenic activity in *S. typhmuri* strains TA97, TA98, TA100, and TA102 without metabolic activation. Responses with metabolic activation have also tended to be positive, but somewhat dependent on the activation system used (phenobarbital or Aroclor 1254; S9 or S100) and other conditions of the experiment (117, 120, 134, 135).

Brown and Griffiths (136) determined that rats could metabolize quercetin and other 3-hydroxyl flavonoids to the 3'-O-methyl ether, which is less mutagenic in the Ames test than quercetin. This ability of the rat to form 3'-O-methyl esters may be important in protecting the body against the putative carcinogenic action of quercetin.

Quercetin is mutagenic in *Drosophila melanogaster* (137), clastogenic in Chinese hamster lung cells and human lymphocytes with or without S9, negative in *in vivo* micronucleus assay (138), mutagenic in Chinese hamster lung cells (139), positive in chromosomal aberration test in CHO cells, negative and positive for SCE (139–141), and positive in inducing DNA strand breakage in HepG2 and HeLa cells, human lymphocytes (142), and L5178Y cells (143), but negative in colon tumor cell line (142).

Quercetin was administered to Sprague-Dawley rats by intraperitoneal injection or gastric intubation at a single dose of 500–2,000 mg/kg body weight. Moderate mutagenic activity was found in the urine and fecal extracts, but not in plasma samples from the treated animals as assessed in *S. typhimurium* strain TA98. The mutagenic activity in the urine accounted for about 0.5% of the administered dose. Higher mutagenic activity was shown in fecal extracts (143).

Kaempferol is mutagenic in TA98, TA100, TA102, and TA1537 with metabolic activation and induces lipid peroxidation, but is negative without activation (144). Kaempferol was positive in the mouse lymphoma L5178Y assay, inducing DNA single strand breaks with or without S9 activation (145). It also induced mutations in *Drosophila melanogaster* (137) and chromosome aberrations and micronuclei in V79 cells with S9 (146). It induced chromosomal aberrations in CHO cells with or without S9 but was negative for SCE (141). In a special study of oxidative damage, kaempferol produced DNA degradation concurrent with lipid peroxidation in rat liver nuclei (147). Kaempferol induced lipid peroxidation mediating the mutagenic activity suggested possible dual role in mutagenesis and carcinogenesis.

No information on the mutagenicity of isorhamnetin was found; rhamnetin was mutagenic in *S. typhimurium* (144) and the mouse lymphoma L5178Y TK+/- assays; rhamnetin also induced single strand breaks in DNA (145). Rutin was also reported to be mutagenic (125).

Polyphenolic flavonoids (quercetin and kaempferol) are mutagenic only under aerobic conditions. Polyphenols (quercetin and kaempferol) are known to produce active oxygen species by autoxidation in aqueous medium. The mutagenicity of polyphenolic flavonoids may be related to the active oxygen species produced by their autoxidation.

Tumorigenicity

Quercetin has been tested for carcinogenicity in several species. The results are summarized in Table 3.

Interpretation of the tumorigenicity bioassays is controversial. The NCI/NTP study is a good example. F344 rats were exposed to quercetin by dosed feed at 0, 1,000, 10,000, or 40,000 ppm (148). At 2 years, male rats fed

Table 3: Quercetin tumorigenicity bioassays

Species	Experimental conditions	Results	Reference
F344 rats, males/females	1.25 or 5% in diet for 104 weeks	Negative	(120)
F344/N rats, males/females	concentrations of 1,000, 10,000 and 40,000 ppm (estimated dose of 40-1,900 mg/kg/day in diet for 104 weeks)	renal tubule adenomas in high dose (40,000 ppm) males	(134,148)
F344/DuCrj rats	1.25 or 5% in the diet for 104 weeks	Negative	(149)
Norwegian strain rats males/females	0.1% in diet for 58 weeks	tumors of small intestine & bladder in both sexes	(150)
F344 rats	0-2% in diet	preneoplastic liver foci, hepatomas, and bile duct tumors	(135)
Syrian golden hamsters, males/females	10% in diet for 735 days, 4% in diet for 709 days, or 1% in diet for 351 days	Negative	(151)
Strain A mouse (lung adenoma assay)	5% in diet	Negative	(152)
ddY mice, males	2% in diet	Negative	(153)

40,000 ppm had increased incidences of renal tubular hyperplasia and adenoma or adenocarcinoma compared with controls; no increased incidence of lesions were observed in other dose groups of males and females. Hirono (154) criticized this study as follows.

“Most chemicals which show carcinogenic activity only after long-term administration at a high dose (>30,000 ppm) are non-mutagenic carcinogens. However, quercetin is positive in the Ames test. It seems unlikely that a mutagenic carcinogen would significantly induce tumors only after long term, high-dose administration and that its effect would only be detectable by step sectioning, and then only in males.”

It has been concluded by a number of scientists that quercetin is safe for human use, although quercetin is mutagenic to *Salmonella* (155). For example, quercetin has been tested in phase I clinical trials in cancer patients.

A preliminary epidemiological study by Ross et al. (156) demonstrated that maternal consumption of foods including bioflavonoids containing topoisomerase II inhibitors led to an approximately 10-fold higher risk of infant acute myelogenous leukemia. Recently, Strick et al. (157) provided evidence that similar to chemotherapeutic agents etoposide and doxorubicin, quercetin (as well as eight other dietary bioflavonoids) induces cleavage in the *MLL* gene by topoisomerase II (topo II) inhibition and may contribute to infant leukemia. They found that quercetin causes site-specific DNA cleavage in the *MLL* breakpoint cluster region (BCR) *in vivo*, indicating that quercetin is a possible infant leukemia inducing agent. The experiments on *MLL* BCR DNA cleavage were conducted in primary progenitor hematopoietic cells from healthy newborns and adults as well as in cell lines. It colocalized with the *MLL* BCR cleavage site induced by chemotherapeutic agents, such as etoposide and doxorubicin. Similar to these chemotherapeutic agents, topo II is the target of quercetin (and other bioflavonoids tested).

Ginkgolic Acids

Crude ginkgo extracts contain a group of alkylphenols (e.g., ginkgolic acids, ginkgol, bilobol). These constituents exhibit potential contact allergenic and toxic properties. Thus, for drug safety, a maximal concentration (< or = 5 ppm) of ginkgolic acids is requested by the Monograph of the Commission E of the former German Federal Health Agency (Bundesgesundheitsamt, BGA) (110). During the multi-step isolation of the standardized Ginkgo extract EGb 761, alkylphenols are largely eliminated as water insoluble compounds (decanter sludge) from the primary acetone extract.

Ginkgolic acids and ginkgols provoke strong allergic reactions (11). Ginkgol, bilobol, and other 3-alkylphenols are derived from ginkgolic acid and are found in the leaves, buds and nut shells of *Ginkgo biloba* (7). These compounds

reportedly inhibit dehydrogenases, cyclooxygenase, lipoxygenase, glucosidase, aldose reductase, and tyrosinase in mammalian systems. They also have antimicrobial effects and may contribute to the resistance of *Ginkgo biloba* to damaging environmental influences (7). Satyan and colleagues (41) reported that ginkgolic acid has antioxidant, radical-captivative, anti-inflammatory and antiallergic properties and is not allergic as long as the carboxylic acid group is intact, either in free or conjugated form.

Others

There are many identified and unidentified constituents in *Ginkgo*. 2-Hexenal is a plant chemical constituent for protecting plants against harmful substances, and is an identified constituent of BGE. 2-Hexenal, an alpha,beta-unsaturated aldehyde, forms exocyclic 1,N2-propanodeoxyguanosine adducts like other mutagenic and carcinogenic alpha,beta-unsaturated carbonyl compounds. Since humans have a permanent intake of 2-hexenal via intake of fruit and vegetables, 2-hexenal is considered to play a role in human carcinogenicity (158–162). As such, the presence of 2-hexenal in BGE may impose risk in cancer induction.

PERSPECTIVES

Although it is perceived that “natural green” products are safe, clinical and scientific data from epidemiological and rodent experimental studies have provided evidence that use of herbal plants is not without risk. Consequently, for human health protection, safety of herbal dietary supplements and their raw herbal plants has to be assumed. In general, information is limited on the genotoxicity and tumorigenicity of natural remedies, functional foods, and dietary supplements.

Ginkgo biloba leave extract is a complex mixture derived from a natural product. As one of the top five most selling dietary supplements in the United States, *Ginkgo biloba* leave extract is being consumed in rather large doses for an extended period of time by a large portion of people in the United States. While its toxicity is not known, some ingredients in *Ginkgo biloba* leave extract are known mutagens; and in one case, a suspected carcinogen at high dose, quercetin, is intentionally concentrated from the ginkgo leaf to manufacture the final product. *Ginkgo biloba* leave extract is currently undergoing NTP chronic toxicology and two years carcinogenicity studies, which well-illustrate the prudent actions by the U.S. federal agencies on the safety assurance of widely consumed herbal dietary supplements in the United States. Since the use of herbal dietary supplements grows rapidly in many countries, it is anticipated that safety considerations of herbal dietary supplements will increase in importance worldwide.

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