NTP
National Toxicology Program

Draft NTP Technical Report TR 578
Ginkgo biloba Extract

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Nomination

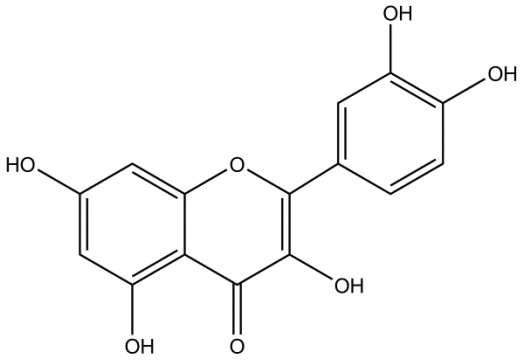
Nominated by the National Institute of Environmental Health Sciences based on:

- Widespread use as an herbal supplement
- Known mutagenicity of the *Ginkgo biloba* extract constituent quercetin
- Lack of toxicity and carcinogenicity data

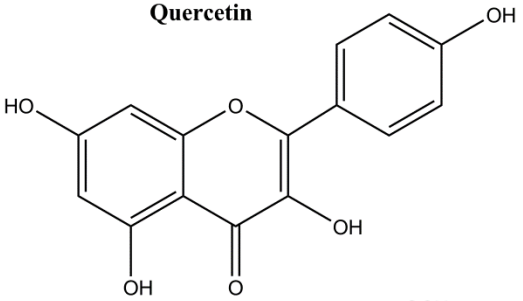
Ginkgo biloba Extract (GBE) Constituents

Class	Identified Chemical Constituents	Target Specification in EGb 761® (range in other preparations)
Terpene trilactones	Ginkgolides A, B, C, J, K, L, M (found in root only); bilobalide (sesquiterpene)	6% (0.2%-11%)
Flavonol glycosides	Major flavonoids: quercetin, kaempferol, isorhamnetin; minor flavonoids: apigenin, luteolin, myricetin	24% (24%-36%)
Biflavones	Bilobetin, ginkgetin, isoginkgetin, sciadopitysin	0% (0.05%-1.7%)
Proanthocyanidins	Dimers of procyanidin and prodelphinidin classes	7% (4%-12%)
Alkylphenols	Ginkgolic acids, cardanols	≤ 5 ppm (0.5 %-4.8% in leaves; <500 ppm – approximately 90,000 ppm)
Carboxylic acids	Non-phenolic acids (ascorbic acid, D-glucaric acid, quinic acid, shikimic acid), phenolic acids (protocatechuic, p-hydroxybenzoic, vanillic, caffeic, p-coumaric, ferulic and chlorogenic acids)	13% (N/A)
Flavanols	Catechin, epicatechin, gallocatechin, and epigallocatechin	2% (N/A)
Polyprenols	C_{85}, C_{90}, C_{95} polyprenols	0 %(1.9%-2.0% in leaves)

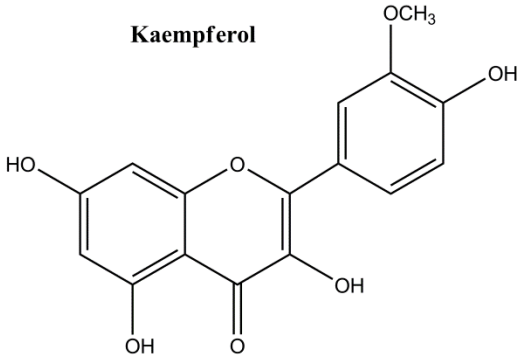
Structure of Major Constituents



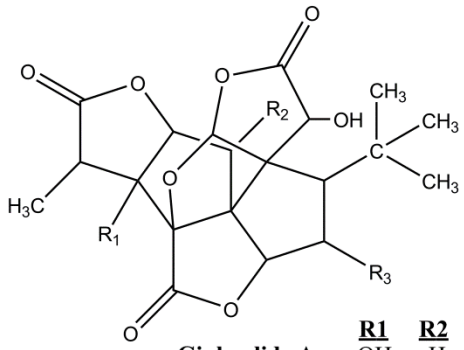
Quercetin



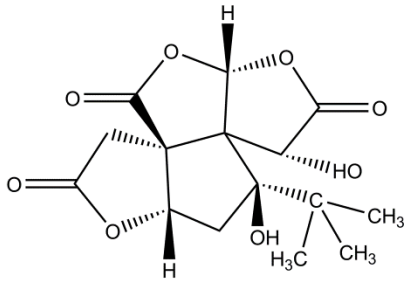
Kaempferol



Isorhamnetin



	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



Bilobalide

Exposure and Use

- *Ginkgo biloba* has been used in medicine for thousands of years
- Among the top 5 herbal supplements on the market with an estimated 7.7 million Americans taking it in 2002
- Current use of *Ginkgo biloba* is often as a leaf-based extract to promote circulation and brain function
- Typically taken in tablet or capsule form with recommended doses of 120-240 mg per day

Test Article Selection

- Supplied by Shanghai Xing Ling Science and Technology Pharmaceutical Company, Ltd.
- Wide distribution in commerce
- Similar ratio of active ingredients to EGb 761®, which is used in clinical trials
- Key values measured in test article:
  - 31.2% flavonol glycosides
  - 15.4% terpene lactones
  - 10 ppm ginkgolic acids

Gray et al., (2005) *Journal of AOAC International* 88(6): 1613-1620
Gray et al., (2007) *Journal of AOAC International* 90(1): 43-53

Experimental Design

- Genotoxicity: in vitro and in vivo (mice)
- Toxicity/Carcinogenicity studies
  - F344/N rats and B6C3F1/N mice
  - Dosing by oral gavage with corn oil vehicle
  - Three-month studies:
    - Rats (n = 10): 0, 62.5, 125, 250, 500, 1000 mg/kg/d
    - Mice (n = 10): 0, 125, 250, 500, 1000, 2000 mg/kg/d
  - Two-year studies:
    - Rats (n = 50): 0, 100, 300, 1000 mg/kg/d
    - Special study rats (n = 10): 0, 100, 300, 1000 mg/kg/d – removed at 90 days
    - Mice (n = 50): 0, 200, 600, 2000 mg/kg/d

Genetic Toxicity

- GBE was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 and in *E. coli*, with and without S9 activation
- No increase in micronucleated erythrocytes in male mice following a 3-month exposure; results equivocal in female mice
- Significant dose-related decrease in the percentage of circulating reticulocytes in male mice

Three-Month Studies of GBE in Rats

- No significant changes in survival or body weight compared to vehicle control animals
- Absolute and relative liver weights increased in all dosed groups of males and females
- Nonneoplastic lesions
  - Liver: Hepatocyte hypertrophy in males and females, hepatocyte fatty change in males
  - Thyroid: Follicular cell hypertrophy in males and females
  - Nose: Pigmentation of olfactory epithelium in males and females

Three-Month Studies of GBE in Mice

- No significant changes in survival compared to vehicle control animals
- Significant decrease in body weight in 2000 mg/kg females
- Absolute and relative liver weights were increased in males (≥ 250 mg/kg) and females (all groups)

Three-Month Studies of GBE in Mice

- Nonneoplastic lesions
  - Liver: Hepatocyte hypertrophy in males and females, focal hepatocyte necrosis in males
  - Nose: Hyaline droplet accumulation in respiratory and olfactory epithelium, atrophy of olfactory epithelium, and pigment accumulation in macrophages in the olfactory epithelium in males and females

Dose Selection for Two-Year Studies

- The high dose in the three-month studies was based on gavageability and homogeneity of the GBE dosing solution
- GBE administration did not affect survival
- Body and organ weight changes and liver hypertrophy were not considered to be life threatening
- Dose spacing was increased to capture a range of effects

Two-Year Studies of GBE in Rats

- Survival of 1000 mg/kg males was significantly less than that of vehicle controls with increase in deaths due to mononuclear cell leukemia (MCL) late in the study
- Decrease in mean body weight of males and females in 300 and 1000 mg/kg groups after week 93 and 89, respectively

Neoplastic and Nonneoplastic Liver Lesions in Rats

	Vehicle control	100 mg/kg	300 mg/kg	1000 mg/kg
Number examined	50	50	50	50
Male				
Hepatocellular Adenoma[a]	*0*	*3*	*3*	*0*
Hepatocyte, Hypertrophy	1 (1.0)	17** (1.4)	26**(2.1)	27** (2.7)
Bile Duct, Hyperplasia	32 (1.1)	43** (1.5)	46** (2.0)	46** (2.0)
Oval Cell, Hyperplasia	0	1 (2.0)	1 (1.0)	10** (1.8)
Necrosis	1 (3.0)	4 (1.5)	6 (2.0)	7* (2.0)
Degeneration, Cystic	4 (1.0)	14** (1.1)	10 (1.0)	14** (1.1)
Female				
Hepatocyte, Hypertrophy	7 (1.4)	15* (1.7)	27** (2.2)	33** (2.5)
Bile Duct, Hyperplasia	11 (1.0)	31** (1.1)	31** (1.1)	33** (1.1)
Fatty Change, Focal	11	25**	30**	25**

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01
[a]Historical incidence for corn oil gavage: 3/299 (1.0% ± 1.1%), range 0%-2%; all routes: 18/1249 (1.4% ± 1.9%), range 0%-6%

Neoplastic and Nonneoplastic Thyroid Lesions in Rats

	Vehicle control	100 mg/kg	300 mg/kg	1000 mg/kg
Male				
Number examined	50	50	49	45
Follicular Cell, Adenoma[a]	*2**	*1*	*3*	*5*
Follicular Cell, Hypertrophy	13 (1.0)	37** (1.2)	41**(1.3)	41** (1.8)
Follicle Hyperplasia	0	7** (1.3)	9** (2.3)	5* (2.8)
Thyroid stimulating hormone (ng/L) – week 14	6.89 ± 0.56 (n = 9)	9.10 ± 0.50** (n = 10)	9.60 ± 0.75** (n = 10)	10.90 ± 0.81** (n = 10)

*Significantly different from vehicle control by Poly-3 test (P≤0.05) or Shirley's test (TSH); ** P≤0.01; * in control indicates significant trend

[a]Historical incidence for corn oil gavage: 6/299 (2.0% ± 1.3%), range 0%-4%; all routes: 13/1239 (1.0% ± 1.7%), range 0%-6%

Neoplastic and Nonneoplastic Thyroid Lesions in Rats

	Vehicle control	100 mg/kg	300 mg/kg	1000 mg/kg
Female				
Number examined	49	50	49	49
Follicular Cell, Adenoma	*1*	*0*	*3*	*1*
Follicular Cell, Carcinoma	*0*	*0*	*1*	*1*
Follicular Cell, Adenoma or Carcinoma[a]	*1*	*0*	*4*	*2*
Follicular Cell, Hypertrophy	15 (1.0)	41** (1.0)	45** (1.1)	48** (2.0)
Follicle Hyperplasia	3 (1.3)	3 (1.0)	1 (2.0)	5 (1.6)
Thyroid stimulating hormone (ng/L) – week 14	5.56 ± 0.34 (n = 9)	5.70 ± 0.50 (n = 10)	6.40 ± 0.54 (n = 10)	7.30 ± 0.40** (n = 10)

*Significantly different from vehicle control by Poly-3 test (P≤0.05) or Shirley's test (TSH); ** P≤0.01; * in control indicates significant trend

[a]Historical incidence for corn oil gavage: 4/298 (1.4% ± 1.0%), range 0%-2%; all routes: 12/1186 (1.0% ± 1.3%), range 0%-4%

Neoplastic and Nonneoplastic Nasal Lesions in Rats

	Vehicle control	100 mg/kg	300 mg/kg	1000 mg/kg
Female				
Respiratory Epithelium, Adenoma[a]	*0*	*0*	*2*	*0*

[a]Historical incidence for corn oil gavage: 0/299; all routes: 1/1196 (0.1% ± 0.4%), range 0%-2%

Nonneoplastic lesions in male and female rats included:

- Hyperplasia of transitional and respiratory epithelium
- Atrophy, nerve atrophy, and pigmentation in the olfactory epithelium
- Chronic, active inflammation
- Hyperplasia of the goblet cells in the respiratory epithelium

Mononuclear Cell Leukemia in Male Rats

	Vehicle control	100 mg/kg	300 mg/kg	1000 mg/kg
Male				
All Organs: Mononuclear Cell Leukemia[a]	9 **	12	22**	21**

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01; ** in control indicates significant trend
[a]Historical incidence for corn oil gavage: 53/299 (17.7% ± 6.6%), range 8%-28%; all routes: 450/1249 (36.0% ± 14.4%), range 8%-58%

- Common lesion in F344/N rats
- Widely variable incidence in historical controls

Two-Year Studies of GBE in Mice

- Survival of 600 and 2000 mg/kg males was significantly less than that of vehicle controls with the increase in deaths due to liver tumors
- Decrease in mean body weight in 600 mg/kg males and 2000 mg/kg males and females

Neoplastic Liver Lesions in Mice

	Vehicle control	200 mg/kg	600 mg/kg	2000 mg/kg
Number examined	50	50	50	50
Male				
Hepatocellular Adenoma	*31*	*46***	*33*	*33*
Hepatocellular Carcinoma	*22***	*31**	*41***	*47***
Hepatoblastoma[a]	*3***	*28***	*36***	*38***
Female				
Hepatocellular Adenoma	*17***	*37***	*41***	*48***
Hepatocellular Carcinoma	*9***	*10*	*15*	*44***
Hepatoblastoma[b]	*1***	*1*	*8**	*11***

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01; ** in control indicates significant trend
[a]Historical incidence for corn oil gavage: 14/350 (4.0% ± 2.8%), range 2%-8%; all routes: 61/1149 (5.3% ± 7.1%), range 0%-34%
[b]Historical incidence for corn oil gavage: 1/347 (0.3% ± 0.8%), range 0%-2%; all routes: 4/1195 (0.3% ± 0.8%), range 0%-2%

Nonneoplastic Liver Lesions in Mice

	Vehicle control	200 mg/kg	600 mg/kg	2000 mg/kg
Number examined	50	50	50	50
Male				
Hypertrophy	3 (1.7)	19** (2.6)	35**(3.0)	23** (3.2)
Erythrophagocytosis	0	4* (2.0)	11** (1.2)	7** (1.3)
Hematopoietic Cell Proliferation	4 (1.0)	9 (1.1)	12* (1.2)	14** (1.0)
Inflammation	28 (1.2)	35 (1.5)	42** (1.8)	39** (1.8)
Necrosis	9 (1.9)	15 (2.1)	17* (1.9)	19* (2.3)
Female				
Hypertrophy	0	18** (2.2)	37**(2.1)	37** (2.9)
Erythrophagocytosis	0	3 (1.0)	7* (1.0)	16** (1.0)
Vacuolization Cytoplasmic	18 (1.7)	38** (2.1)	44** (2.6)	35** (2.3)
Eosinophilic Focus	26	39*	43**	45**
Mixed Cell Focus	7	27**	31**	31**

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01

Neoplastic and Nonneoplastic Thyroid Lesions in Mice

	Vehicle control	200 mg/kg	600 mg/kg	2000 mg/kg
Male				
Number examined	49	49	50	50
Follicular Cell, Adenoma[a]	*0*	*0*	*2*	*2*
Follicle, Hyperplasia	2 (1.0)	1 (1.0)	7 (1.1)	25** (1.4)
Follicular Cell Hypertrophy	2 (1.0)	0	2(1.5)	38** (1.2)
Female				
Number examined	49	48	49	48
Follicular Cell Hypertrophy	1 (3.0)	5 (1.4)	9*(1.0)	39** (1.0)

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01
[a]Historical incidence for corn oil gavage: 1/349 (0.3% ± 0.8%), range 0%-2%; all routes: 7/1143 (0.6% ± 1.0%), range 0%-2%

Nonneoplastic Nasal/Forestomach Lesions in Mice

	Vehicle control	200 mg/kg	600 mg/kg	2000 mg/kg
Number examined	50	50	50	50
Male				
Olfactory Epithelium, Hyaline Droplet Accumulation	18 (1.4)	16 (1.9)	15 (1.8)	28* (1.8)
Olfactory Epithelium, Pigmentation	0	1 (1.0)	3 (1.0)	13** (1.1)
Female				
Olfactory Epithelium, Hyaline Droplet Accumulation	5 (1.0)	3 (1.7)	12 (1.2)	17** (1.6)
Olfactory Epithelium, Pigmentation	0	1 (1.0)	6* (1.5)	13** (1.2)

*Significantly different from vehicle control by Poly-3 test (P≤0.05); ** P≤0.01

Nonneoplastic lesions in forestomach of male and female mice included:

- Inflammation
- Hyperplasia, hyperkeratosis, ulcer, and erosion of the epithelium

Conclusions

- *Some evidence of carcinogenic activity* of GBE in male F344/N rats based on increased incidences of thyroid gland follicular cell adenoma; Increased incidences of MCL and hepatocellular adenoma may have been related to GBE administration
- *Some evidence of carcinogenic activity* of GBE in female F344/N rats based on increased incidences of thyroid gland follicular cell neoplasms; Increased occurrence of respiratory epithelium adenomas in the nose may have been related to GBE administration
- *Clear evidence of carcinogenic activity* of GBE in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma; Increased incidences of thyroid gland follicular cell adenoma were also related to GBE administration
- *Clear evidence of carcinogenic activity* of GBE in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma