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Hormesis and *Ginkgo biloba* (GB): Numerous biological effects of GB are mediated via hormesis

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ABSTRACT

Ginkgo biloba (GB) extracts have been shown to commonly induce biphasic dose responses in a range of cell types and endpoints (*e.g.*, cochlea neural stem cells, cell viability, cell proliferation). The magnitude and width of the low dose stimulation of these biphasic dose responses are similar to those reported for hormetic dose responses. These hormetic dose responses occur within direct stimulatory responses as well as in preconditioning experimental protocols, displaying acquired resistance within an adaptive homeodynamic and temporal framework and repeated measurement protocols. The demonstrated GB dose responses further reflect the general occurrence of hormetic dose responses that consistently appear to be independent of the biological model, endpoint, inducing agent, and/or mechanism. These findings have important implications for consideration(s) of study designs involving dose selection, dose spacing, sample size, and statistical power. This illustrates and strengthens the need to characterize the low dose stimulatory response range and optimal dose in order to explore potential public health and clinical applications of plant-derived agents, such as GB.

1. Introduction

Ginkgo biloba (GB) is an herbal product extracted from the leaves of the ginkgo tree, which grows throughout China, Korea, Japan, Europe and the United States. The ginkgo leaf contains numerous biologically active agents, including flavonol and flavone glycosides, diterpene lactones, ginkgolides, sesquiterpenes, iron-based superoxide dismutase, p-hydroxybenzoic acid, ascorbic acid, and catechin. Bioactive ingredients are obtained using a chemical method employing an acetone-water mixture to extract and concentrate the active substances and remove toxic metabolites such as ginkgolic acids. The German government approved a standardized form of ginkgo leaf extract (*i.e.*, Egb 761). The extract contains 22 %–27 % flavonoid glycosides, 5 %–7 % terpene lactones and < 5 ppm ginkgolic acids. The flavonoid glycosides are generally recognized as a major ingredient in the extract, with commercial manufacturers often standardizing the sold product to a flavonoid glycoside content of 24 %.

Ginkgo biloba is known for its capability and use in optimizing cognitive function in humans (Rojas et al., 2016). Numerous publications support a modest efficacy, with few side effects (Hofferberth, 1994; Kanowski et al., 1996; Kleijnen and Knipschild, 1992a,b; LeBars et al., 1997; Oken et al., 1998; Cicero et al., 2018). However, research-based challenges to the cognitive efficacy effects have been published by DeKosky et al. (2008). Ginkgo leaf extract has also been approved by the German Federal Health Agency for the treatment of arterial claudication (Fetrow and Avila, 1999). In 2013 the US National Toxicology Program Technical Report, 2013 (NTP) reported on toxicology and carcinogenesis studies with GB extracts in the F344/N rats and B6C3F1/N mice. This report also provided a detailed historical review of human exposures and considerations with respect to public health risks.

The biological effects of GB have become an active area of research, suggestive of the possibility for other therapeutic applications affecting numerous cell types and biomedical endpoints. This paper provides evidence that some of the effects of GB occur in a hormetic dose-response pattern (Fig. 1). The present assessment represents the first integrative report on the hormetic effects of GB and how these actions may affect study design features, understanding of GBs dose-response features, and potential consideration and use in clinical applications. Hormetic responses induced by GB were found in a variety of biological systems and endpoints (Fig. 2), and also were shown to be contributory to

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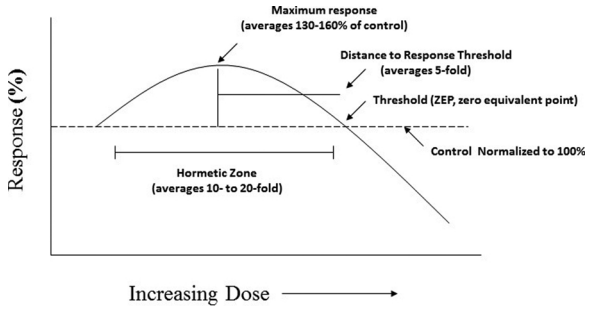

Fig. 1. Hormetic dose response: quantitative characteristics.

preconditioning effects (Fig. 3). Dose- response features in these various conditions are provided in Figs. 4A–4G. Evaluative perspectives are offered for those instances for which the most substantial findings are available (*i.e.*, hearing loss, lipolysis/cellulitis, ethanol metabolism, erythropoietic stem cell viability, and function of neuronal and glial cells).

2. Hearing loss and GBE

There is considerable interest in finding effective, reliable and efficient ways of preventing (and/or mitigating) hearing loss. Auditory hair cells located along the basilar membrane of the cochlea can become dysfunctional and/or damaged as a consequence of age-induced metabolic changes, or environmental insult (*e.g.*- high decibel sound exposure), and exposure to various chemotherapeutic/antineoplastic agents, leading to decreased auditory sensitivity. Hearing loss may also occur as a consequence of neuronal degeneration prior to or after hair cell loss. One proposed strategy to restore hair-cell or neuronally-mediated hearing loss is the replacement of damaged/dead neurons, since inner ear stem cells can form neurospheres (*in vitro*) and differentiate into hair cells and neurons (Wang and Wang, 2016).

While certainly a viable possibility, it is also important to consider protection of extant hair cells and XIII cranial (auditory) nerve cells (both prior to injury, and subsequent to therapeutic replacement with the aforementioned stem cell-based neurospheres/neurons. Thus, a role for substances that afford protective effects remains of interest and importance. It has been hypothesized that GB protects cochlea neural stem cells (NSC) from age- and environmentally-induced damage (Choi et al., 2013). This suggestion was based on studies demonstrating the ability of GB to affect numerous neurological processes such as memory retention (Hofferberth, 1994; Semlitsch et al., 1995), protection against B-amyloid neuropathology (Kampkotter et al., 2007; Wu et al., 2006), reduction of hypoxic stress effects (Kampkotter et al., 2007) and supported by research on prophylaxis against lead-induced neurotoxicity

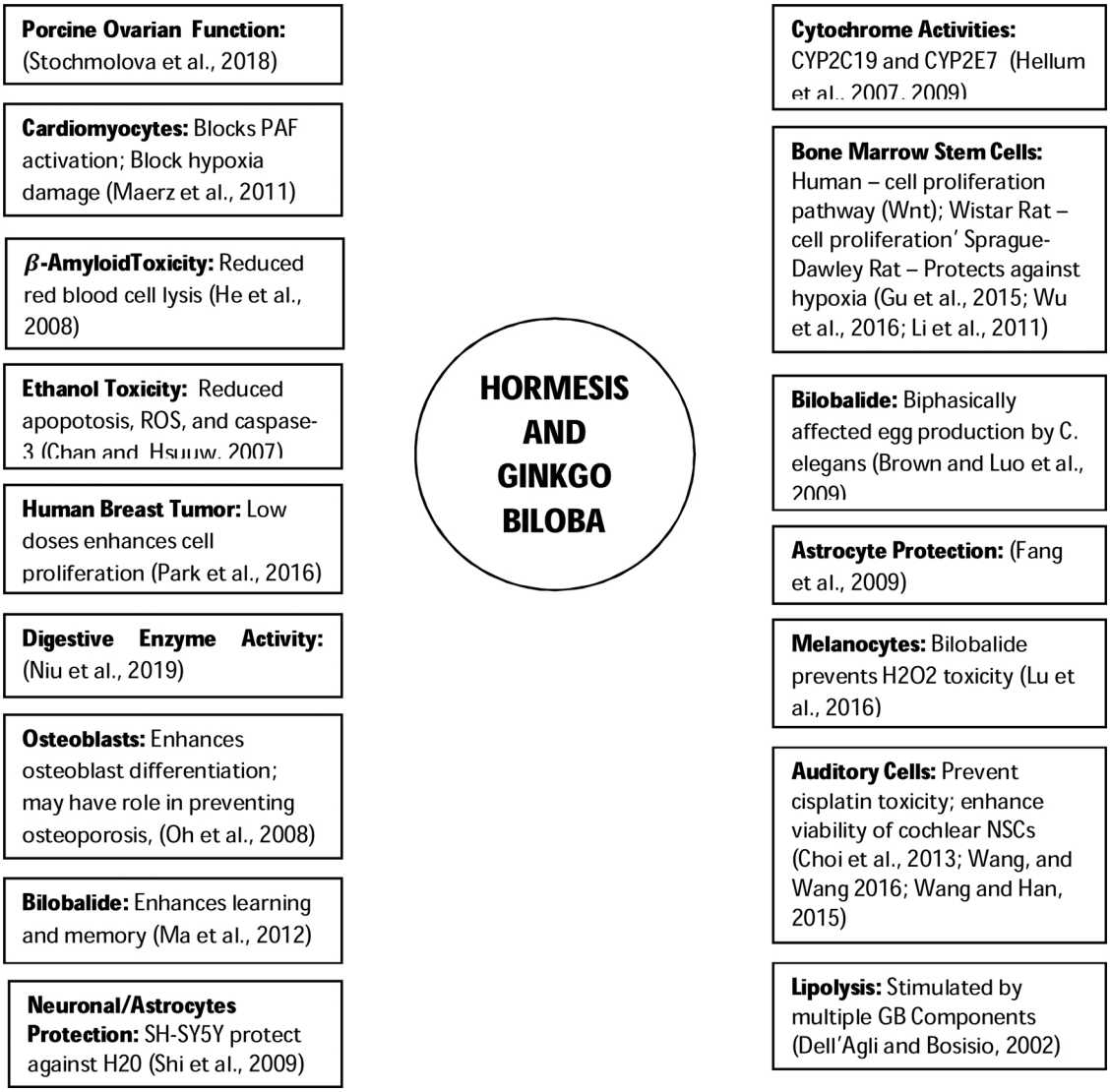

Fig. 2. The range of *Ginkgo biloba* induced hormetic effects.

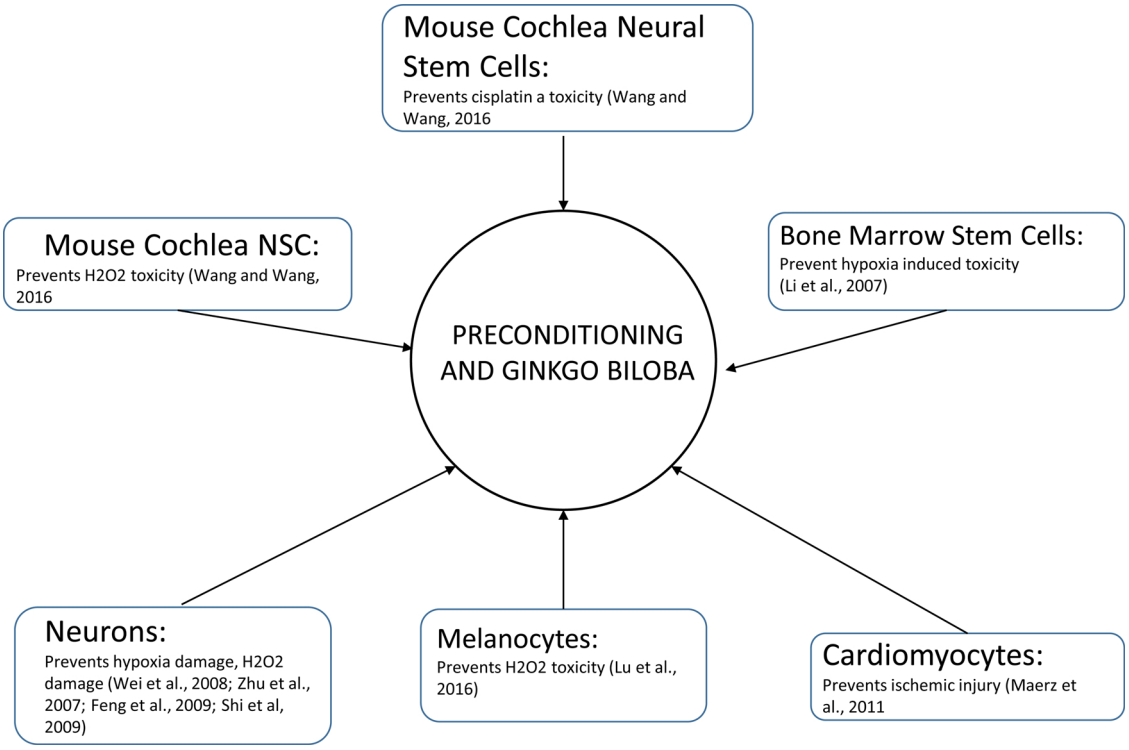

Fig. 3. Examples of *Ginkgo biloba* induced preconditioning effects.

(Yallapragada and Velaga, 2015).

Wang and Wang (2016) and Wang and Han (2015) extended the above findings with research that extracted cochlear NSCs from one day old mice, and showed that perfusion with a commercial GBE product enhanced the viability and proliferation of these cells in a hormetic-like biphasic pattern, being stimulatory in the range of 25 − 50 μg/ml, and inhibitory by 75 μg/ml (Figs. 4A1 and 2). The response at 75 μg/ml represents a rapid transition from optimized stimulation to inhibition within a less than 2-fold increase in dose. The optimized 50 μg/ml concentration was then employed to enhance neurosphere formation by about 20 %. A similar GB-induced stimulatory response in the differentiation of NSCs into neurons using various neuron-specific cytoskeletal protein markers was also reported (Wang and Han, 2015) (Fig. 4A.1).

Other studies (Wang and Han, 2015) indicated that GB enhanced neurite outgrowths [*e.g.*, number of end tips, and the average length of neurites and growth-associated protein-43 (GAP-43)] and tree-like projections (*i.e.*, dendrites) that enhance inter-neuronal contact and connectivity. These GB-induced developments were then correlated to the number of neurons generating spontaneous calcium oscillations in differentiated neural networks. A follow-up study using the same model showed that the 50 μg/ml concentration significantly prevented peroxide (H_2O_2)-induced neuronal cell damage (Wang and Wang, 2016).

Similar findings by Choi and co-workers (2013) indicated that Egb 761, biphasically enhanced the viability of HEI − OCI (*i.e.*, mouse auditory hair cells and cochlea cells) and also protected against cisplatin-induced damage to auditory hair cells. Here too, the relationship of the maximum stimulatory response and the onset of neurotoxicity represents a narrow dose range relationship (*i.e.*, approximately two-fold). However, in auditory cells the stimulatory range was shown to be at least 12-fold (5–300 μg/ml; Fig. 4A.3; Choi et al., 2013).

3. Bone marrow stem cells (BMSC)

The effects of GB on bone marrow stem cells (BMSC) were assessed in three studies using young adult Sprague-Dawley males (Li et al., 2011), one day old Wistar rats (Wu et al., 2016) and adult humans (age/sex not indicated) (Gu et al., 2015). Three different GB formulations were employed in these studies, and therefore it is not possible (or at least imprudent) to attempt making direct comparison of results and findings. These research teams approached their experiments with differing perspectives and contexts. Li et al. (2011) demonstrated that GB protected against hypoxic-induced toxicity within a preconditioning experimental framework (Figs. 4B3 and 4). Using human subjects, Gu et al. (2015) assessed the role of the Wnt-β-catenin signaling pathway in osteogenic differentiation (Fig. 4B1 and 2). The study by Wu and colleagues (2016) used Wistar rats to examine cell proliferation and osteogenic and adipogenic differentiation of BMSCs. In each case, the GB formulation induced a biphasic dose response, with both rat model studies employing an overlapping concentration wherein the lowest concentration used exceeded the highest human concentration by > 100 fold. In rat experiments (Li et al., 2011; Wu et al., 2016), GB induced stimulation was mediated, at least in part, by the P13 signaling pathway, as the hormetic stimulation was blocked by the specific pathway inhibitor, wortmannin. In human studies, GB enhanced cell proliferation in BMSCs by activating the Wnt-β-catenic pathway (Gu et al., 2015).

4. Preconditioning neurons, astrocytes, neuronal tumor cells

Preconditioning is a process whereby an initial low dose of a stressor agent upregulates adaptive mechanisms that enhance resilience against subsequent and acute stressor agents within a time-sensitive window of ~ 10–14 days. Preconditioning is a generalized phenomenon that is independent of thebiological model and level of biological organization (*i.e.*, acting at the cell, organ, and organismal levels; Calabrese, 2016a).

Preconditioning experiments that employ multiple conditioning doses typically display hormetic dose responses (Calabrese, 2016a,b). The history of preconditioning extends to t1920s research on the effects of radiation on plant growth (see Calabrese, 2016a,b for a review). It became more widespread and prominent in the mid-1980s when Murry et al. (1986) reported that an *a priori* series of brief ischemic stresses

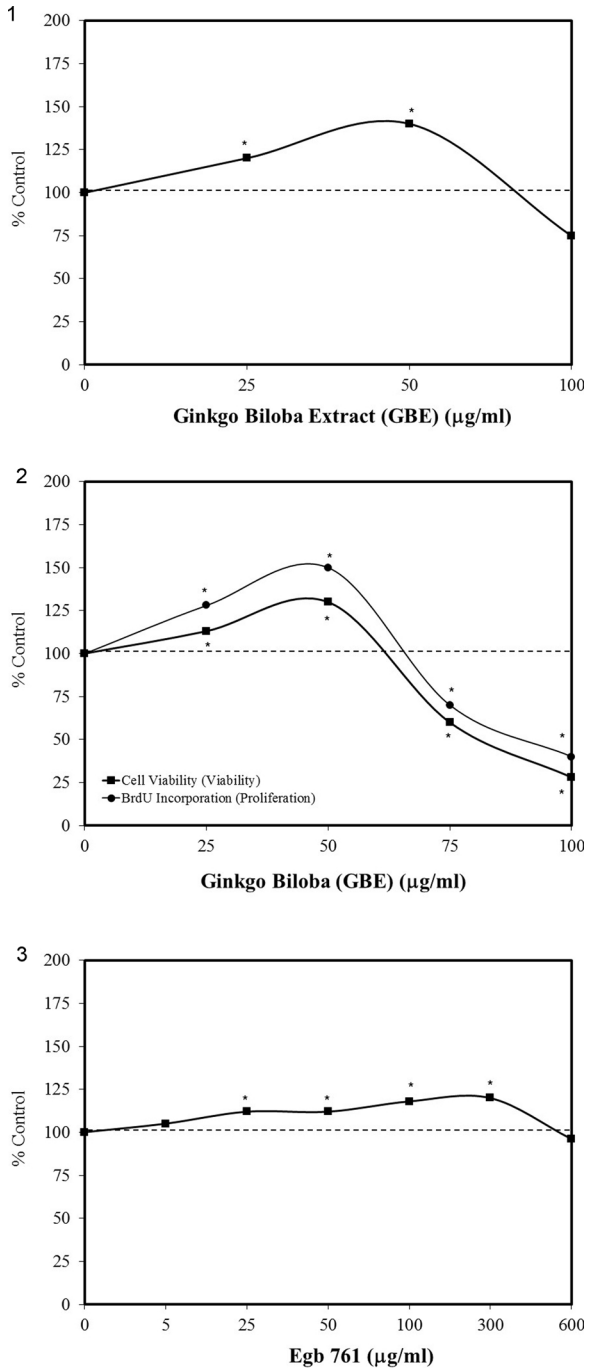

Fig. 4A. 1. Auditory Effects: Effects of Ginkgo Biloba Extract (GBE) on Cell Viability of Mouse Cochlear Neural Stem Cells (NSCs) *In Vitro*. (Wang C, Wang B. 2016. *Ginkgo biloba* extract attenuates oxidative stress and apoptosis in mouse cohlear neural stem cells. Phytotherapy research 30:774-780). *designates statistical significance. 2. Auditory Effects: Effects of Ginkgo Biloba Extract (GBE) on the Viability and Proliferation of Mouse Cochlea Neural Stem Cells *In Vitro*. (Wang and Han, 2015. *Ginkgo biloba* extract enhances differentiation and performance of neural stem cells in mouse cochlea. Cell Mol Neurobiol 35:861-869). *designates statistical significance. 3. Auditory Effects: Effects of Egb 761 (Ginkgo Biloba Extracts) on the Viability of HEI – OC1 Cells (auditory hair cells). (Choi et al., 2013. Gingko biloba extracts protect auditory hair cells from cisplatin-induced ototoxicity by inhibiting perturbation of gap junctional intercellular communication. Neuroscience 244:49-61). *designates statistical significance.

prevented damage from an acute cardiac insult a day later.

The possibility of using GB to induce preconditioning effects was first espoused by Ji et al. (2004), who were interested in finding an

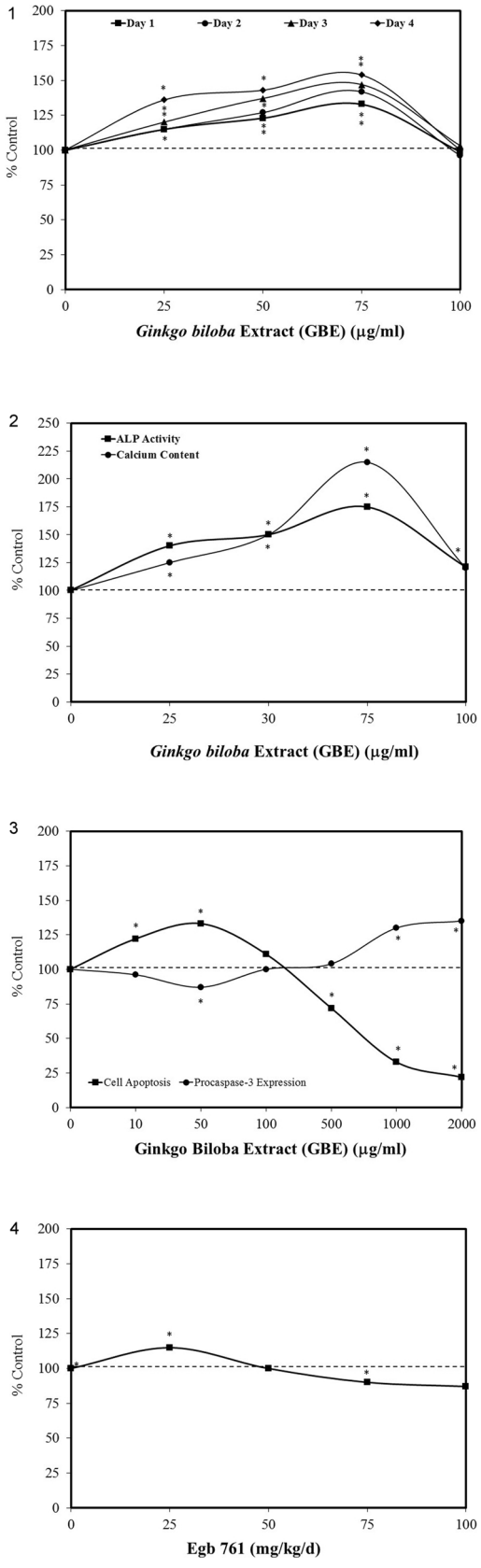

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Fig. 4B. 1. Bone Marrow: Effects of *Ginkgo biloba* Extract on the Proliferation of Human Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs). (Gu et al., 2015. *Ginkgo biloba* extract promotes osteogenic differentiation of human bone marrow mesenchymal stem cells in a pathway involving Wnt/B-catenin signaling. Pharmacological Research 97:70-78). *designates statistical significance. 2. Bone Marrow: Effects of *Ginkgo biloba* Extract on ALP Activity and Calcium Content of Human Bone Marrow Derived Mesenchymal Stem Cells (BM-MSCs). (Gu Q, Chen C, Zhang Z, Wu Z, Fan X. 2015. *Ginkgo biloba* extract promotes osteogenic differentiation of human bone marrow mesenchymal stem cells in a pathway involving Wnt/B-catenin signaling. Pharmacological Research 97:70–78. *designates statistical significance. 3. Bone Marrow: Effects of *Ginkgo Biloba* Extract (Egb 761) on Sprague-Dawley Rat Bone-Marrow Mesenchymal Stem Cell (BMSC) Survival *In Vitro*. (Li et al., 2011. Biphasic effect of EGb 761 on simulated ischemia-induced rat BMSC survival *in vitro* and *in vivo*. Life Sciences 88:853-863). *designates statistical significance. 4. Bone Marrow: Effects of Egb 761 on Apoptosis of transplanted Bone-Marrow Mesenchymal Stem Cell (BMSC) *In Vivo* (BMSC's were transplanted into rat ischemia myocardia). (Li et al., 2011. Biphasic effect of EGb 761 on simulated ischemia-induced rat BMSC survival *in vitro* and *in vivo*. Life Sciences 88:853-863). *designates statistical significance.

alternative to hypoxia as a clinical preconditioning vehicle (given medical/safety concerns if the dose and timing of hypoxic stress were not optimized). The expressed hope was that GB may offer a safer preconditioning stimulus that is practical and able to pharmacologically mimic the preconditioning effects of hypoxia (and/or other stressors).

Using cell viability and morphological changes as endpoints, this research initially demonstrated that ginkgolides induced a significant protective effect against chemically-induced hypoxic injury caused by cobalt chloride ($CoCl_2$) (Ji et al., 2004). Of note is that an earlier study showed that ginkgolides can protect against hypoxia-induced nerve damage *via* physical rather than chemical stress (Chen et al., 2001). Taken together, these prompted three subsequent investigations in which ginkgolides (A,B,C, and J), the main constituents of the non-flavone fraction of Egb 761, were employed in a preconditioning protocol and protected primary cortical neurons from 13 to 15 day old mice against hypoxic stress from potassium cyanide (Zhu et al., 2007) and ischemic stress from ODC in CG rat glioma cells (Wei et al., 2008; Fig. 4C1). Similar studies were conducted using mouse cerebral cortical astrocytes (Fang et al., 2009) which showed that ginkgolide treatment was both directly stimulating and also protective in a preconditioning framework. The mechanisms by which the ginkgolides mediated the protective responses involved activation of the MAPK and P13-Akt pathways, as well as the activation of H1F-1α, which led to the target of EPO. Ginkgolide treatment also blocked the PAF receptor, reducing inflammatory responses. The optimal concentration in each of these studies was 37.5 μM, although three different biological models were used.

The mechanism by which GB mediates hormetic-like biphasic dose responses was further explored by Shi et al. (2009) in dose-effect experiments examining Egb 761 action on H_2O_2 induced cell death in the SH-SY5Y line of human neuroblastoma cells. The experiment used a preconditioning protocol in which cells were initially treated with Egb 761 (50 − 500 μg/ml) for 24 h, after which time the Egb 761 was removed. The preconditioned cells were then challenged with H_2O_2 for 24 h and subsequently assessed for viability, apoptosis, and other parameters. It was shown that Egb 761 enhanced survival at lower concentrations (50 − 100 μg/ml) while being toxic at higher concentrations. Follow up studies revealed that at low protective concentrations, Egb 761 inactivated Akt, JNK, and caspase pathways (Fig. 4C.2). At the higher concentrations, Egb 761 activated JNK and caspase 3, while deactivating Akt, and lowering Akt levels. Other experiments suggested that Egb 761 was most likely mediating hormetic-biphasic dose responses *via* the regulation of the redox state. These findings are consistent with the general consensus that P13 kinase-Akt-PKB pathway activation enhances cell survival. In contrast, activation

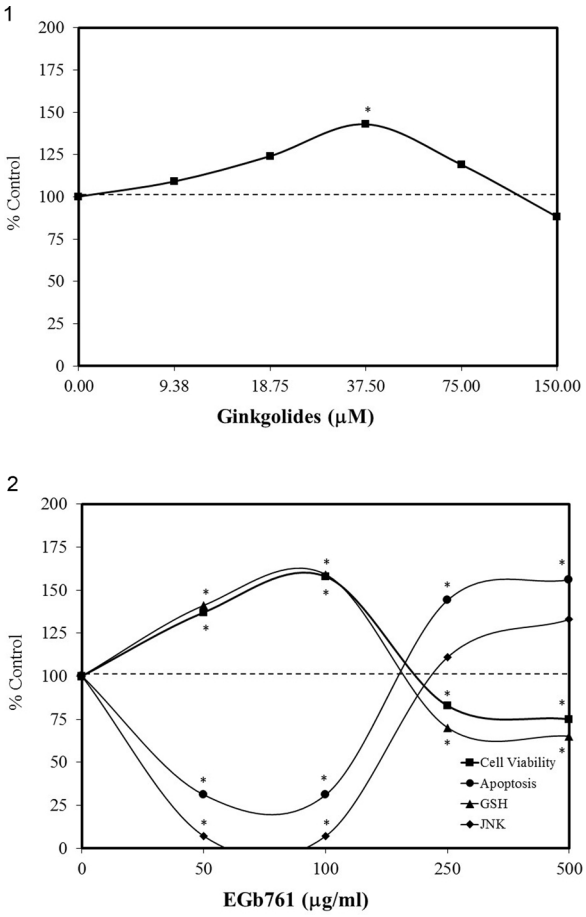

Fig. 4C. 1. Glioma Cells: Effects of Ginkgolides (Ging A-30 %; Ging B-56 %; Ging C-3 %; Ging J-0.-1.0 %) Pretreatment (24 h) on the Viability of C6 Rat Glioma Cells then Challenged with Ischemia (Oxygen-Glucose Deprivation) for 24 h. (Wei et al., 2008. Ginkgolides mimic the effects of hypoxic preconditioning to protect C6 cells against ischemic injury by up-regulation of hypoxia-inducible factor-1 alpha and erythropoietin. International Journal of Biochemistry & Cell Biology 40:651-662). *designates statistical significance. 2. Neuron-SH-SY5Y Cells: Effect of Pretreatment (24 h) EGb761 on Cell Viability of H_2O_2-Treated SH-SY5Y Cells. (Shi et al., 2009. Dosage effects of EGb 761 on hydrogen peroxide-induced cell death in SH-SY5Y cells. Chemico-Biolgoical Interactions 180:389-397). *designates statistical significance.

of the JNK pathway leads to cell death. Table 1 provides a summary of evidence supporting this pattern of activity and effect(s) (Table 2).

5. Cellulitis

Cellulitis is a regressive abiotrophic pannicular disease with multifaceted pathologic features and diverse etiology. It is exacerbated by venous stasis, as well as chronic venous insufficiency (for current overview see Cranendonk et al., 2017). The sclerotic aspect may be decreased by treatment with agents that affect microcirculation of the skin, and enhance lipolysis in adipose tissues. This commonly occurs with compounds that display anti-inflammatory, anti-oedematous and lipolytic effects. Since dimeric flavonoids from GB extracts display anti-inflammatory (Della Loggia et al., 1996), vasokinetic qualities (Bombardelli et al., 1996), and inhibit cAMP phosphodiesterase activity in adipose tissue, (Saponara and Bosisio, 1998). Dell'Agli and Bosinio (2002) proposed that GB biflavones may have some viability for treating cellutitis. In addressing this possibility, it was demonstrated that multiple GB biflavones (*i.e.*, ilobetin; sequoiaflavone; ginkgetin; isoginkgetin; amentoflavone; Fig. 4D), which vary in the position and extent of methylation of the hydroxyl groups, biphasically stimulated lipolysis.

Table 1
Hormetic mechanisms mediating *Ginkgo biloba* effects.

P-13 Kinase – Akt – PKB Pathway	
Akt/PKB Activation – Low GB Dose	**JNK Activation – High GB Dose**
Promotes cell survival	Leads to cell death
Growth factor-mediated activation of the P13-kinase/Akt pathway rescues primary neurons exposed to either high glutamate (5) or 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (Shi et al., 2009, page 389)	Lipid peroxidation products 4-hydroxynonenal and oxidized low density lipoprotein activate JNK (Shi et al., 2009, page 389)
Diminished Akt phosphorylation in neurons lacking GSH peroxidase, increases risk of cell death (Shi et al., 2009, page 389)	Inhibition of JNK prevents necrotic cell death in an *in vitro* model of excitotoxic neuron death (Shi et al., 2009, page 389)
Ginkgo Biloba/Egb 761	
Enhance phosphorylation of Akt in a human endothelial cell line and endothelial progenitor cells (Shi et al., 2009, page 390)	Down-regulates JNK-activator protein-1 signaling pathway in human peripheral blood T cells (Shi et al., 2009, page 390)
Low concentrations of ginkgolide B (5–25 uM) inhibited EtOH-induced cell death in HEPG2 via inhibition of JNK and caspase 3-but high H_2O_2 concentration (50-100 uM) enhanced EtOH-induced apoptosis via the enhancement of JNK and caspase 3 (Chan and Hsuuw, 2007, page 391)	Pre-treatment of Egb 761 inhibits EtOH-induced increase of JNK activity in gastric mucosa (Chan and Hsuuw, 2007, page 396)

Table 2
GB induced hormetic dose response concentration range.

Agent	Model	Endpoint	Hormetic Range (μg/ml)	Toxicity	Reference/Figure
GBE	BMMSC, human	Proliferation	25-75, 75 optimal	~100	Gu et al., 2015/Fig. 4B1
GBE	BMMSC, human	ALP	25-100, 75 optimal	N/A; N/A	Gu et al., 2015/Fig. 4B2
GBE	BMMSC, human	calcium content	25-100, 75 optimal	N/A; N/A	Gu et al., 2015/Fig. 4B2
GBE	BMMSC, rat	Apoptosis; procapase-3 expression	10-100, 50 optimal;	1000; 500	Li et al., 2011/Fig. 4B3
GBE	Porcine ovarian granulosa cell	Progesterone release, leptine Release	1 (no range); 1 to 10	100; 500	Stochmalova et al., 2018/ Fig. 4G.4
GBE	RBC	Lysis, AB-25-35	5 – 200	N.A	He et al., 2008
GBE	Human hepatocytes	Cytochrome CYP2D6,	2.19–21.9, 2.19 optimal;	219.0;	Hellum et al., 2009/Fig. 4G.1
GBE	Human hepatocytes	Cytochrome CYP2C19	2.19–21.0, 0.19 optimal	219.0	Hellum et al., 2009/Fig. 4G.1
GBE	Mouse cochler nerve stem cells	Cell viability	25-50, 50 optimal	100	Wang and Wang, 2016/ Fig. 4A.1
GBE	Mouse cochler nerve stem cells	Cell viability; proliferation	25-50, 50 optimal; 25-50, 50 optimal	75; 75	Wang and Han, 2015/Fig. 4A.2
Ginkgolide B	HEP G2 cells	Preventing ethanol toxicity	40-200, 100 optimal	~425	Chan and Hsuuw et al., 2007/ Fig. 4E
Ginkgolides (A,B,C,J)	Mouse cortical neuron	Cell viability, preconditioning against KCN toxicity	18.75-37.5, 7.5 optimal	N/A	Zhu et al., 2007
Ginkgolides (A,B,C,J)	Newborn ICR mouse cerebrocortid astrocytes	Cell viability	9.3–187.5, 37.5 optimal	N/A	Fang et al., 2009
Ginkgolides (A,B,C,J)	C6 rat glioma cells	Cell viability, pretreatment against OGD ischemia	18.75-75.0, 37.5 optimal	150	Wei et al., 2008

Agent	Model	Endpoint	Hormetic Range (μg/ml)	Toxicity	Reference/Figure
Egb 761	SH-SY5Y	Cell viability, preventing H2O2 toxicity	50–100, 100 optimal	250	Shi et al., 2009/Fig. 4C.2
Egb 761	BMSC rat ischemia	Apoptosis	25, 25 optimal	75	Li et al., 2011/Fig. 4B.4
Egb 761	HEI-OC1 auditory hair cells	Cell viability	25-300, 300 optimal	600	Choi et al., 2013/Fig. 4A.3
Egb 761	MCF- AROM	Cell proliferation	100-500, 250 optimal	N/A	Park et al., 2016/ Fig. 4G.3
Amentoflavone	3T3-L1 adipocytes	Lipolysis	0.55-55, 5.5 optimal	110	Dell'Agli and Bosisio, 2002/ Fig. 4D
Sequoiaflavone	3T3-L1 adipocytes	Lipolysis	0.55–555.0, 55 optimal	N/A	Dell'Agli and Bosisio, 2002/ Fig. 4D
Igoginkgetin	3T3-L1 adipocytes	Lipolysis	0.55–550, 55.2 optimal	N/A	Dell'Agli and Bosisio, 2002/ Fig. 4D
Ginkgetin	3T3-L1 adipocytes	Lipolysis	0.55–552, 55.2 optimal	N/A	Dell'Agli and Bosisio, 2002/Fig. 4D

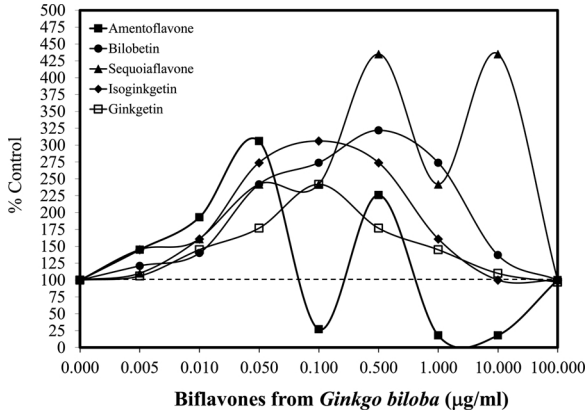

Fig. 4D. Lipolysis: Effects of Biflavones (purity ranged from 87.8 (bilobetin) to 99 (Amento flavone) of *Ginkgo biloba* L. on Lipolysis in 3T3-L1 Adipocytes. (Dell'Agli and Bosisio, 2002. Biflavones of *Ginkgo biloba* stimulate lipolysis in 3T3-L1 adipocytes. Planta Med 68:76-79).

The capacity of GB biflavones to stimulate lipolysis was related to their ability to inhibit cAMP phosphodiesterase activity. The agents that displayed a stimulatory effect on lipolysis inhibited cAMP phosphodiesterase activity and had free or partially methylated hydroxyl groups. Dell'Agli and Bosinio (2002) reported a 50–100 ratio between the concentration that enhanced lipolysis and those concentrations causing a mild cytotoxic response (20 % loss of viability), and suggested that in light of these findings, GB biflavone could be of value as a locally-applied agent to treat cellulitis and localized adiposity.

6. Ethanol

A 2007 study by Chan and Hsuuw assessed whether GB extracts might have a curative or protective effect on alcohol toxicity in human hepatoma G2 cells. Although the authors did not offer a directly supportive scientific rationale for the study, they cited multiple reports indicating that GB extract had both antioxidant and anti-angiogenetic properties. In this study, the authors assessed the effects of a 5–100 µM concentration range of ginkgolide B on apoptosis, reactive oxidative stress (ROS), and changes in JNK and caspase 3 activation (Fig. 4E). Treatment with GB displayed a hormetic concentration-response for each endpoint measured, with the maximum protection for each endpoint at 25 µM, and toxicity occurring at the next higher concentration (50 µM).

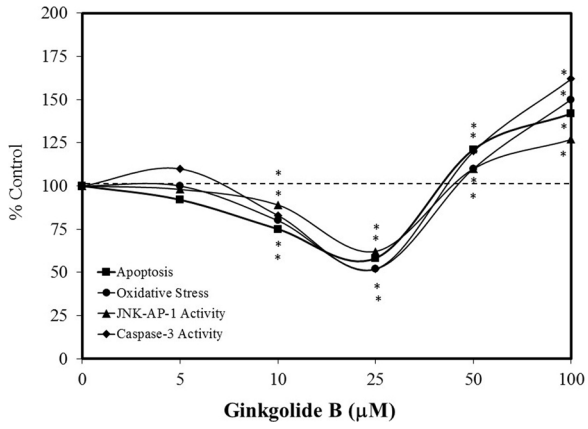

Fig. 4E. Ethanol-Liver Toxicity: Effects of Ginkgolide B (main ingredient of GBE) on Ethanol Treated Hep G2 Cells. (Chan and Hsuuw, 2007. Dosage effects of ginkgolide B on ethanol-induced cell death in human hepatoma G2 cells. Ann NY Acad Sci 1095:388-398). *designates statistical significance.

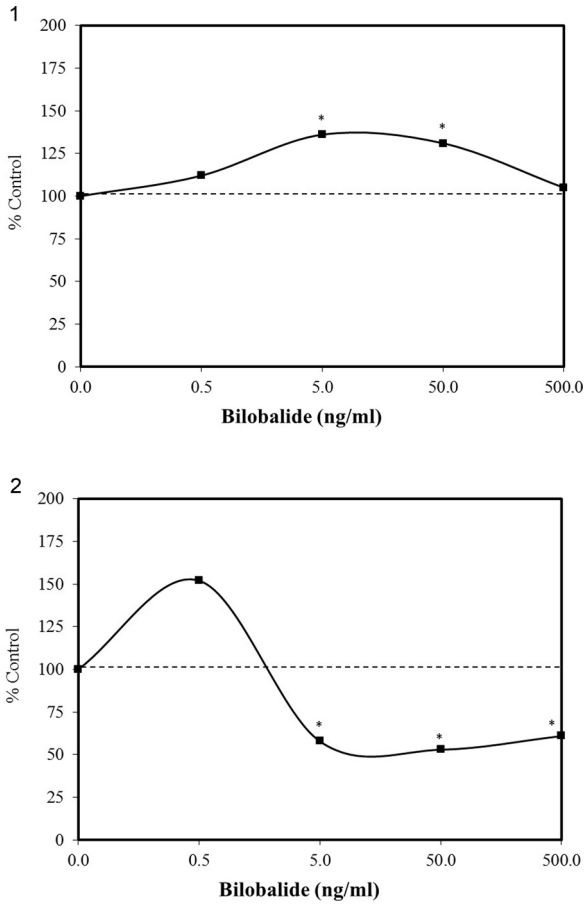

Fig. 4F. 1. Cardiomyocyte: Cardiomyocytes were Exposed to Hypoxia for Six Hours. Bilobalide Enhanced the Viability of the Cardiomyocytes for Male Sprague-Dawley Rats. (Maerz et al., 2011. Anti-ischaemic effects of bilobalide on neonatal rat cardiomyocytes and the involvement of the platelet-activating factor receptor. Biosci Rep 31:439-447). *designates statistical significance. 2. Cardiomyocyte: Cardiomyocytes were Exposed to Hypoxia for Six Hours. Bilobalide Decreased Apoptosis from 5 to 500 mg/mL While Increasing it at 0.5 mg/mL for Male Sprague-Dawley Rats. (Maerz et al., 2011. Anti-ischaemic effects of bilobalide on neonatal rat cardiomyocytes and the involvement of the platelet-activating factor receptor. Biosci Rep 31:439-447). *designates statistical significance.

7. Cardiomyocytes and platelet activation factor (PAF)

Terpenoids, including ginkgolide B, are platelet activation factor (PAF) antagonists (Braquest and Hoford, 1991). Since PAF signaling is activated during inflammation and ischemic injury, Maerz et al. (2011) hypothesized that ginkgolide B treatment diminishes inflammation damage by blocking PAF. While ginkgolide B has been a widely studied GB product, bilobalide has been shown to be the most active gingko-derived compound in hippocampal neurons (Klein et al., 1997) and endothelial cells (Janssens et al., 1995), and has been shown to mitigate apoptotic cell death (Ahlemeyer et al., 1999). Maerz et al. (2011) extended these findings to assess the cardioprotective effects of GB-induced PAF antagonism. In a series of experiments, it was initially reported that bilobalide induced a biphasic enhancement of cardiomyocyte survival, showing a similar magnitude of protection as ginkgolide but at 100-fold lower concentration (*i.e.*, 5 *vs* 500 ng/ml; Fig. 4F.1). Follow-up experiments, using hypoxic stress- induced cellular damage - measured by apoptosis - revealed that bilobalide decreased this damage at low concentrations (Maerz et al., 2011; Fig. 4F.2).

PAF is also now recognized to affect a variety of neuronal activities, acting as an endogenous neurotoxin when produced in excess, while

affording protective effects at lower concentrations (Bazan, 1998). During biologically stressful conditions (*e.g.*, ischemia, induced brain injury), PAF acts as a neuronal injury messenger *via* PAF-NMDA receptor activation, to increase glutamate release, which evokes an increase in neuronal Ca^{2+} (Mukherjee et al., 1999; Xu and Tao, 2004). Zhu et al. (2007) reported that by blocking the PAF receptor, Egb 761

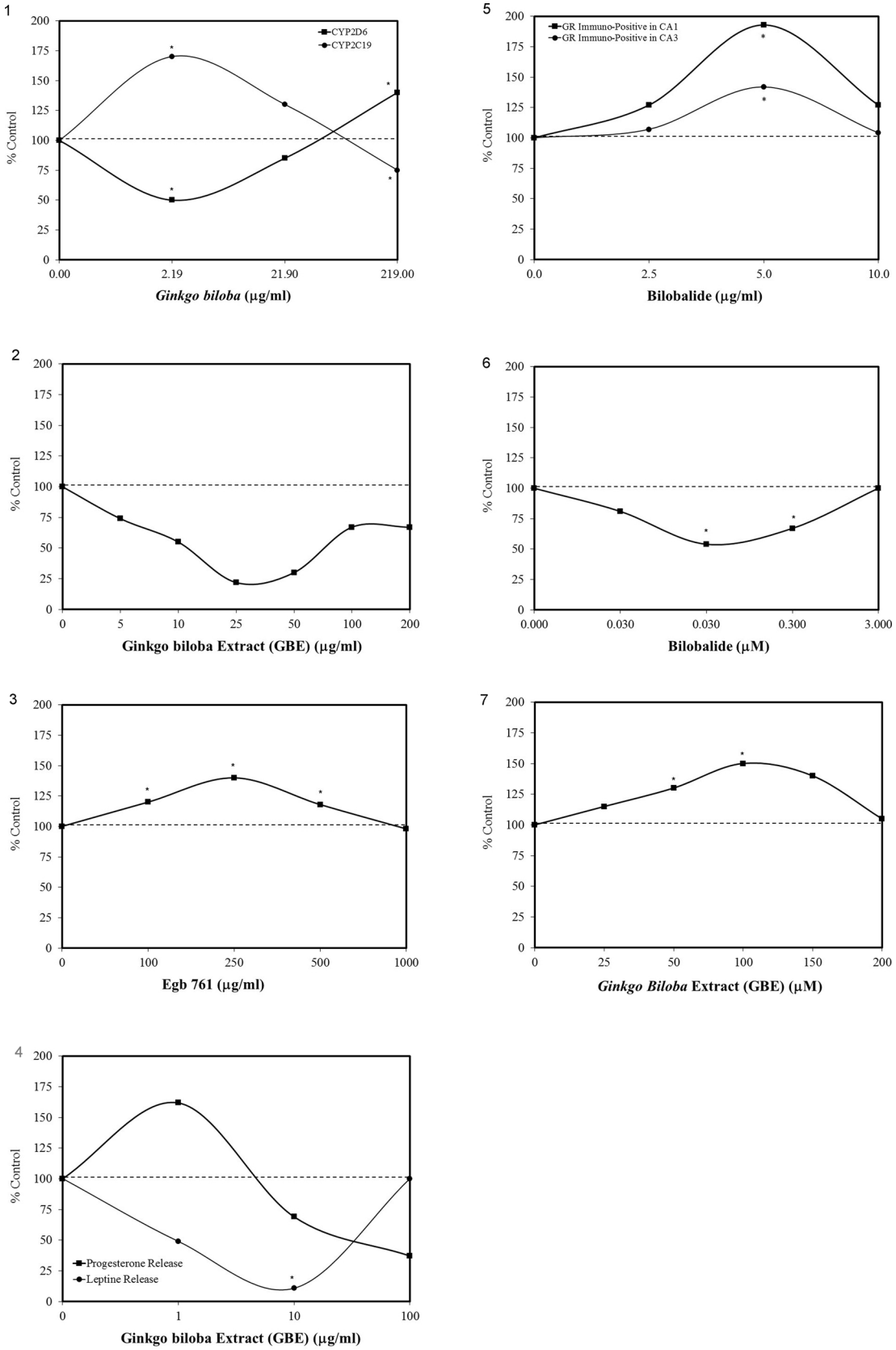

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Fig. 4G. 1. Cytochrome Effects: Effects of *Ginkgo biloba* (standard formula) on CYP2D6 and CYP2C19 Activity in Cultured Human Hepatocytes (28 year-old Chinese male). (Hellum et al., 2009. Trade herbal products and induction of CYP2C19 and CYP2E1 in cultured human hepatocytes. Basic & Clinical Pharmacology & Toxicology 105:58-63; Hellum et al., 2007. The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. Basic & Clinical Pharmacology & Toxicology 100:23–30. Cited in Fig. 1 only). *designates statistical significance. 2. Amyloid Peptide: Effects of *Ginkgo biloba* Leaf Extract (standard formula) on Amyloid Peptide (Aβ25-35) Induced RBC Lysis. (He et al., 2008. Dual effects of *Ginkgo biloba* leaf extract on human red blood cells. Basic & Clinical Pharmacology and Toxicology 104:138-144). 3. Breast Cancer Cells: Effects of *Ginkgo biloba* Extract EGb 761 on Proliferation of MCF-7 AROM Cells (aromatase overexpressing cells) for 72 h. (Park et al., 2016. *Ginkgo biloba* extract Egb 761-mediated inhibition of aromatase for the treatment of hormone-dependent breast cancer. Food and Chemical Toxicology 87:157-165). *designates statistical significance. 4. Ovarian Cell Function: Effects of *Ginkgo biloba* Extract (GBE) on Porcine Ovarian Granulosa Cell Function. (Stochmalova et al., 2018. Direct effect of polyphenol-rich plants, rooibos and ginkgo, on porcine ovarian cell functions. J Anim Physiol Anim Nutr 102:e550-e577). *designates statistical significance. 5. Behavior Related Endpoint: Effects of Bilobalide on Glucocorticoid Receptors in CA1 and CA3 in Male Kunming Mice. (Ma et al., 2012). Effects of bilobalide on anxiety, spatial learning, memory and levels of hippocampal glucocorticoid receptors in male Kunming mice. Phytomedicine 20:89-96). *designates statistical significance. 6. Behavior Related Endpoint: Effects of Bilobalide on Egg-Laying by *C. elegans* (Wild type). (Brown and Luo, 2009. Bilobalide modulates serotonin-controlled behaviors in the nematode *Caenorhabditis elegans*. BMC Neuroscience 10:62, 10 pages, doi:10.1186/1471-2202-10-62). *designates statistical significance. 7. Bone Growth: Effects of Ginkgo Biloba Extract (GBE) on Alkaline Phosphatase (ALP), a Marker for Bone Mineralization, on Osteoblast Saos-2 Cells. (Oh et al., 2008. Effects of *Ginkgo biloba* on *in vitro* osteoblast cells and ovariectomized rat osteoclast cells. Arch Pharm Res 31(2):216-224). *designates statistical significance.

and ginkgolide B could facilitate the reported hormetic properties of PAF in neurons, thereby reducing both the release of endogenous excitatory amino acids and the influx of Ca^{2+}.

8. Discussion

This paper represents the first integrated assessment of the evidence of the hormetic effects of *Gingko biloba* leaf extracts, a complex mixture of flavone glycosides and terpene lactones and other agents, as reported in the biomedical literature. This assessment reveals that GB-induced hormetic effects have been commonly demonstrated (as predominantly chemoprotective effects) for a range of biological endpoints. This is particularly notable in various types of nerve cells, including cochlea nerve stem cells (Wang and Wang, 2016). Of interest is that GB has also been shown to protect against hypoxia-induced cardiomyocyte toxicity *via* its capacity to block PAF activation (Maerz et al., 2011), and this suggests the possibility and viability of similar protective mechanisms and effects in neurons (Zhu et al., 2007). In these chemoprotective experimental frameworks, GB typically was evaluated using preconditioning protocols. When this type of experimental study included sufficient conditioning doses, the dose-response of GB and GB-derived compounds typically displayed hormetic dose responses, as is consistent in a variety of other models and systems of chemical and physical preconditioning and protection (Calabrese et al., 2007; Calabrese, 2016a, b), adding further support to the hypothesis that hormesis is a highly generalize dose response phenomenon that appears independent of biological model, endpoint, inducing agent, potency of the inducing agents, level of biological organization (cell, organ, and organism) and mechanism (Calabrese, 2008, 2013).

The studies reviewed in this paper typically employed 4–5 doses, with 50 % of the reported dose- responses having five doses. In addition, the median maximum stimulation reported was 155 %, typical of hormetic dose responses, with this response being independent of the model, endpoint, inducing agent and/or mechanism (Calabrese et al., 2019; Calabrese and Mattson, 2017; Calabrese and Blain, 2011). The width of the hormetic stimulation range displayed a median 10-fold dose range, with 95 % of observations being less than $\leq$ 20-fold and none > 100-fold dose range. This stimulatory range is consistent with findings in the hormesis literature (Calabrese and Blain, 2005, 2009, 2011). While the range of endpoints for GB-induced hormetic dose responses is relatively narrow (at least at present), the consistency of the low-modest dose/concentration stimulation range raises questions and concerns about estimating the optimal dose range, as well as possible exposures that may exceed the optimal range and its potential clinical implications.

GB and several of its active components were compared for their hormetic activity range(s) for differing endpoints in multiple biological systems. In general, it was determined that the hormetic concentrations in these *in vitro* systems were in the 10–100 μg/ml range, with the majority between 25–75 μg/ml. In observed effects on lipolysis, the hormetic concentrations had a broader range, approximately 1000-fold, from 0.5–550 μg/ml. However, the optimal response was similar to the other agents (*i.e.*, approximately 50 μg/ml). That such a wide range of biological models, endpoints and experimental protocols (*e.g.*, direct stimulation, preconditioning) displayed an overlapping hormetic concentration-response raises important questions concerning the possibility of employing hormetic approaches in strategies to affect other biological systems – both experimentally (to assess and determine mechanisms and results) and clinically (for possible therapeutic benefit).

In sum, we opine that the present findings are sufficiently extensive to suggest that the capacity of GB to induce hormetic dose responses that could be exploitable within preconditioning protocols. This position is supported by illustrations of similar hormetic effects induced by other plant-derived agents such as resveratrol (Calabrese et al., 2010), curcumin (Calabrese et al., 2019), green tea and its chief catechin component, EGCG (Kwon et al., 2015), and other polyphenolic compounds.

In this way, these findings further contribute and support a growing body of literature demonstrating hormetic mechanisms subserving the biologically active effects of several functional foods (Calabrese et al., 2018; Pilipenko et al., 2019; Amara et al., 2019). The hormetic effects have also been seen when investigating the real-life risk simulation and the effect of long term low dose exposure to combined stimuli (Docea et al., 2018, 2019; Tsatsakis et al., 2019a,b). The occurrence of hormetic dose responses indicates that dosing considerations can be critical, and that the optimal dose range is essential to define, especially when seeking to avoid potentially undesirable responses produced at and by higher doses. It is hoped that such findings may be of use to researchers' developing improved study designs, protocols (viz.- with respect to selection of doses), sample size, and statistical power.

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References

Ahlemeyer, B., Mowes, A., Kriegistein, J., 1999. Inhibition of serum deprivation- and staurosporine-induced neuronal apoptosis by *Ginkgo biloba* extract and some of its constituents. Eur. J. Pharmacol. 367, 423–430.

Amara, I., Timoumi, R., Annabi, E., Di Rosa, G., Scuto, M., Najjar, M.F., Calabrese, V., Abid-Essefi, S., 2019. Di(2-ethylhexyl)phthalate targets the thioredoxin system and the oxidative branch of the pentose phosphate pathway in liver of Balb/c mice. Environ. Toxicol. https://doi.org/10.1002/tox.22844.

Bazan, N.G., 1998. The neuromessenger platelet-activating factor in plasticity and neurodegeneration. Nitric oxide in brain development, plasticity and disease. Book Series. Prog. Brain Res. 118, 281–291.

Bombardelli, E., Cristoni, A., Curri, S.B., 1996. Activity of phospholipid-complex of *Ginkgo biloba* dimeric flavonoids on the skin microcirculation. Fitoterapia 67, 265–273.

Braquest, P., Hoford, D., 1991. Ethnopharmacology and the development of natural PAf antagonists as therapeutic agents. J. Ethnopharmacol. 32, 135–139.

Brown, M.K., Luo, Y., 2009. Bilobalide modulates serotonin-controlled behaviors in the nematode *Caenorhabditis elegans*. BMC Neurosci. 10, 62. https://doi.org/10.1186/1471-2202-10-62.

Calabrese, E.J., 2008. Hormesis: why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27 (7), 1451–1474.

Calabrese, E.J., 2013. Hormetic mechanisms. Crit. Rev. Toxicol. 43 (7), 580–606.

Calabrese, E.J., 2016a. Preconditioning is hormesis. Part I: documentation, dose-response features and mechanistic foundations. Pharm. Res. 110, 242–264.

Calabrese, E.J., 2016b. Preconditioning is hormesis. Part II: how the conditioning dose mediates protection: dose optimization within temporal and mechanistic frameworks. Pharm. Res. 110, 265–275.

Calabrese, E.J., Blain, R., 2005. The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. Toxicol. Appl. Pharmacol. 202 (3), 289–301.

Calabrese, E.J., Blain, R.B., 2009. Hormesis and plant biology. Environ. Poll. 157 (1), 42–48.

Calabrese, E.J., Blain, R.B., 2011. The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. Regul. Toxicol. Pharmacol. 61 (1), 73–81.

Calabrese, E.J., Mattson, M.P., 2017. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech. Dis Dis. 3:UNSP13.

Calabrese, E.J., Mattson, M.P., Calabrese, V., 2010. Resveratrol commonly displays hormesis: occurrence and biomedical significance. Hum. Exper. Toxicol. 29 (12), 980–1015.

Calabrese, E.J., Dhawan, G., Kapoor, R., Mattson, M.P., Rattan, S.I., 2019. Curcumin and hormesis with particular emphasis on neural cells. Food Chem. Toxicol. 129, 399–404.

Calabrese, E.J., Bachmann, K.A., Bailer, A.J., Bolger, P.M., Borak, J., Cai, L., Cedergreen, N., Cherian, M.G., Chiueh, C.C., Clarkson, T.W., Cook, R.R., Diamond, D.M., Doolittle, D.J., Dorato, M.A., Duke, S.O., Feinendegen, L., Gardner, D.E., hart, R.W., Hastings, K.L., Hayes, A.W., Hoffmann, G.R., Ives, J.A., jaworowski, Z., Johnson, T.E., Jonas, W.B., Kaminski, N.E., Keller, J.G., Klaunig, J.E., Knudsen, T.B., Kozumbo, W.J., Lettieri, T., Liu, S.-Z., Maisseu, A., Maynard, K.I., Masoro, E.J., McClellan, R.O., Mehendale, H.M., Mothersill, C., Newlin, D.B., Rattan, S.I.S., Riviere, J.E., Rodricks, J., Sapolsky, R.M., Scott, B.R., Seymour, C., Sinclair, D.A., Smith-Sonneborn, J., Snow, E.T., Spear, L., Stevenson, D.E., Thomas, Y., Tubiana, M., Williams, G.M., Mattson, M.P., 2007. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. Toxicol. Appl. Pharmacol. 222, 122–128.

Calabrese, V., Santoro, A., Trovato Salinaro, A., Modafferi, S., Scuto, M., Albouchi, F., Monti, D., Giordano, J., Zappia, M., Franceschi, C., Calabrese, E.J., 2018. Hormetic approaches to the treatment of Parkinson's disease: perspectives and possibilities. J. Neurosci. Res. 96 (10), 1641–1662.

Chan, W.-H., Hsuuw, Y.-D., 2007. Dosage effects of ginkgolide B on ethanol-induced cell death in human hepatoma G2 cells. Ann. NY. Acad. Sci. 1095, 388–398.

Chen, H.S., Wu, X.M., Zhu, L., Jin, S.Y., 2001. Protective effect of ginkgolid on hypoxic injury of cultured mouse cortical neurons. Acta. Acad. Med. Nantes. 21, 226–228.

Choi, S.J., Kim, S.W., Lee, J.B., Lim, H.J., Kim, Y.J., Tain, C., So, H.S., Park, R., Choung, Y.-H., 2013. Gingko biloba extracts protect auditory hair cells from cisplatin-induced ototoxicity by inhibiting perturbation of gap junctional intercellular communication. Neurosci. 244, 49–61.

Cicero, A.F.G., Fogacci, F., Banach, M., 2018. Botanicals and phytochemicals active on cognitive decline: the clinical evidence. Pharmacol. Res. 130, 204–212.

Cranendonk, D.R., Lavrijsen, A.P.M., Prins, M., Wiersinga, W.J., 2017. Cellulitis: current insights into pathophysiology and clinical management. Nerth. J. Med. 75, 366–378.

Dell'Agli, M., Bosisio, E., 2002. Biflavones of *Ginkgo biloba* stimulate lipolysis in 3T3-L1 adipocytes. Planta Med. 68, 76–79.

Della Loggia, R., Sosa, S., Rubaro, A., Morazzoni, P., Bombardelli, E., Griffini, A., 1996. Anti-inflammatory activity of some *Ginkgo biloba* constituents and of their phospholipid-complexes. Fitoterapia 67, 257–264.

Docea, A.O., Gofita, E., Goumenou, M., Calina, D., Rogoveanu, O., Varut, M., Olaru, C., Kerasioti, E., Fountoucidou, P., Taitzoglou, I., Zlatian, O., Rakitskii, V.N., Hernandez, A.F., Kouretas, D., Tsatsakis, A., 2018. Six months exposure to a real life mixture of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats. Food Chem. Toxicol. 115, 470–481.

Docea, A.O., Goumenou, M., Calina, D., Arsene, A.L., Dragoi, C.M., Gofita, E., Pisoschi, C.G., Zlatian, O., Stivaktakis, P.D., Nikolouzakis, T.K., Kalogeraki, A., Izotov, B.N., Galateanu, B., Hudita, A., Calabrese, E.J., Tsatsakis, A., 2019. Adverse and hormetic effects in rats exposed for 12 months to low dose mixture of 13 chemicals: RLRS part III. Toxicol. Lett. 310, 70–91.

Fang, D., Ming, Q.Z., Li, Z., Mei, W.X., Ya, K., 2009. Hypothermia attenuates protective effects of ginkgolides on astrocytes from ischemia/reperfusion injury. Neurochem. Intern. 55, 181–186.

Fetrow, C., Avila, J., 1999. Professional's handbook of complementary and alternative medicines. Springhouse Publishing, Springhouse PA.

Gu, Q., Chen, C., Zhang, Z., Wu, Z., Fan, X., Zhang, Z., Di, W., Shi, L., 2015. *Ginkgo biloba* extract promotes osteogenic differentiation of human bone marrow mesenchymal stem cells in a pathway involving Wnt/β-catenin signaling. Pharm. Res. 97, 70–78.

He, J., Lin, J., Li, J., Zhang, J.-H., Sun, X.-M., Zeng, C.-M., 2008. Dual effects of *Ginkgo biloba* leaf extract on human red blood cells. Basic Clin. Pharm. Toxic. 104, 138–144.

Hellum, B.H., Hu, Z., Nilsen, O.G., 2007. The induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. Basic Clin. Pharm. Toxic. 100, 23–30.

Hellum, B.H., Hu, Z., Nilsen, O.G., 2009. Trade herbal products and induction of CYP2C19 and CYP2E1 in cultured human hepatocytes. Basic Clin. Pharm. Toxic. 105, 58–63.

Hofferberth, B., 1994. The efficacy of Egb 761 in patients with senile dementia of the Alzheimer type, a double-blind, placebocontrolled study on different levels of investigations. Hum. Psychopharm. Clin. Exp. 9, 215–222.

Janssens, D., Michiels, C., Delaive, E., Eliaers, F., Drieu, K., Remacle, J., 1995. Protection of hypoxia-induced ATP decrease in endothelial cells by *Ginkgo biloba* extract and bilobalide. Biochem. Pharmacol. 50, 991–999.

Ji, Q.H., Gu, J.L., Gu, Y.J., Zhu, L., 2004. Effect of ginkgolides, acanthopannx senticosus saponins, ginsenosides on the injury of PC12 cells induced by cobalt chloride. Acta. Acad. Med. Nantes. 24, 33–36.

Kanowski, S., Hermann, W.M., Stephan, K., Wierich, W., Horr, R., 1996. Proof of efficacy of the *Ginkgo biloba* special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. Pharmacopsychiatry 29 (2), 47–56.

Kampkotter, A., Pielarski, T., Rohrig, R., Timpel, C., Chovolou, Y., Watjen, W., Kahl, R., 2007. The *Ginkgo biloba* extract Egb 761 reduces stress sensitivity, ROS accumulation and expression of catalase and glutathione S-transferase 4 in *Caenorhabditis elegans*. Pharmacol. Res. 55 (2), 139–147.

Kleijnen, J., Knipschild, P., 1992a. *Ginkgo-biloba* for cerebral insufficiency. Brit. J. Clin. Pharm. 34 (4), 352–358.

Kleijnen, J., Knipschild, P., 1992b. *Ginkgo-biloba*. Lancet 340 (8828), 1136–1139.

Klein, J., Chatterjee, S.S., Loffelholz, K., 1997. Phospholipid breakdown and choline release under hypoxic conditions: inhibition by bilobalide, a constituent of *Ginkgo biloba*. Brain Res. 755, 347–350.

Kwon, S.J., Lambert, J.D., Yang, C.S., Hong, J., 2015. Role of reactive oxygen species for the green tea catechin, (-)-epigallocatechin-3-gallate in growth modulation of intestinal cells. Food Sci. Biotech. 24 (4), 541–1548.

LeBars, P.L., Katz, M.M., Berman, N., Itil, T.M., Freedman, A.M., Schatzberg, A.F., 1997. A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. JAMA 278 (16), 1327–1332.

Li, S., Tang, D., Xue, Z., Zhang, Z., Sun, X., Liu, Y., Dong, H., Yin, X., Zhang, Z., 2011. Biphasic effect on Egb 761 on simulated ischemia-induced rat BMSC survival *in vitro* and *in vivo*. Life Sci. 88, 853–863.

Ma, L., Wang, S., Tai, F., Yuan, G., Wu, R., Liu, X., Wei, B., Yang, X., 2012. Effects of bilobalide on anxiety, spatial learning, memory and levels of hippocampal glucocorticoid receptors in male Kunming mice. Phytomedicine 20, 89–96.

Maerz, S., Liu, C.-H., Guo, W., Zhu, Y.-Z., 2011. Anti-ischaemic effects of bilobalide on neonatal rat cardiomyocytes and the involvement of the platelet-activating factor receptor. Biosci. Rep. 31, 439–447.

Mukherjee, P.K., DeCoster, M.A., Campbell, F.Z., Davis, R.J., Bazan, N.G., 1999. Glutamate receptor signaling interplay modulates stress-sensitive mitogen-activated protein kinases and neuronal cell death. J. Biol. Chem. 274, 6493–6498.

Murry, C.E., Jennings, R.B., Reimer, K.A., 1986. Preconditioning with ischemia – a delay of lethal cell injury in ischemic myocardium? Circulation 74 (5), 1124–1136.

National Toxicology Program Technical Report, 2013. Toxicology and Carcinogenesis. Studies of *Ginkgo biloba* Extract (CAS No. 90045-36-6) in F344/N Rats and B6C3F1/N Mice (gavage Studies) March 2013, NTP TR 578, NIH Publication No. 13-5920.

Oh, S.M., Kim, H.R., Chung, K.Y., 2008. Effects of ginkgo biloba on in vitro osteoblast cells and ovariectomized rat osteoclast cells. Arch. Pharm. Res. 31 (2), 216–224.

Oken, B.S., Storzbach, D.M., Kaye, J.A., 1998. The efficacy of *Ginkgo biloba* on cognitive function in Alzheimer disease. Arch Neurology 55 (11), 1409–1415.

Park, Y.J., Ahn, H.Y., Kim, H.R., Chung, K.H., Oh, S.M., 2016. Ginkgo biloba extract EGb 761-mediated inhibition of aromatase for the treatment of hormone-dependent breast cancer. Food Chem. Toxicol. 87, 157–165.

Pilipenko, V., Narbute, K., Amara, I., Trovato, A., Scuto, M., Pupure, J., Jansone, B., Poikans, J., Bisenieks, E., Clusa, V., Calabrese, V., 2019. GABA-containing compound gammapyrone protects against brain impairments in Alzheimer's disease model male rats and prevents mitochondrial dysfunction in cell culture. J. Neurosci. Res. 97 (6), 708–726.

Rojas, C., Rojas-Castaneda, J., Rojas, P., 2016. Antioxidant properties of a Ginkgo biloba leaf extract (EGb 761) in animal models of Alzheimer's and Parkinson's diseases. Curr. Top. Nutraceut. Res. 14 (1), 1–16.

Saponara, R., Bosisio, E., 1998. Inhibition of cAMP-phosphodiesterase by biflavones of *Ginkgo biloba* in rat adipose tissue. J. Nat. Prod. 61, 1386–1387.

Semlitsch, H.V., Anderer, P., Saletu, B., Binder, G.A., Decker, K.A., 1995. Cognitive psychophysiology in nootropic drug research: effects of *Ginkgo biloba* on event-related potentials (P300) in age-associated memory impairment. Pharmacopsychiatry 28 (4), 134–142.

Shi, C., Zhao, L., Zhu, B., Li, Q., Yew, D.T., Yao, Z., Xu, J., 2009. Dosage effects of EGb 761 on hydrogen peroxide-induced cell death in SH-SY5Y cells. Chem.-Biol. Inter. 180, 389–397.

Stochmalova, A., Kadasi, A., Alexa, R., Bauer, M., Harrath, A.H., Sirotkin, A.V., 2018. Direct effect of pholyphenol-rich plants, rooibos and ginkgo, on porcine ovarian cell functions. J. Anim. Physiol. Anim. Nutr. (Berl) 102, e550–e557.

Tsatsakis, A., Tyshko, N.V., Docea, A.O., Shestakova, S.I., Sidorova, Y.S., Petrov, N.A., Zlatian, O., Mach, M., Hartung, T., Tutelyan, V.A., 2019a. The effect of chronic

vitamin deficiency and long term very low dose exposure to 6 pesticides mixture on neurological outcomes - A real-life risk simulation approach. Toxicol. Lett. 315, 96–106.

Tsatsakis, A.M., Docea, A.O., Calina, D., Buga, A.M., Zlatian, O., Gutnikov, S., Kostoff, R.N., Aschner, M., 2019b. Hormetic Neurobehavioral effects of low dose toxic chemical mixtures in real-life risk simulation (RLRS) in rats. Food Chem. Toxicol. 125, 141–149.

Wang, C., Han, Z., 2015. *Ginkgo biloba* extract enhances differentiation and performance of neural stem cells in mouse cochlea. Cell. Mol. Neurobiol. 35, 861–869.

Wang, C., Wang, B., 2016. *Ginkgo biloba* extract attenuates oxidative stress and apoptosis in mouse cohlear neural stem cells. Phytother. Res. 30, 774–780.

Wei, H., Ming, Q.Z., Li, Z., Qian, C., Fang, D., Ho, Y.W., Ya, K., 2008. Ginkgolides mimic the effects of hypoxic preconditioning to protect C6 cells against ischemic injury by up-regulation of hypoxia-inducible factor-1 alpha and erythropoietin. Intern. J Biochem. Cell Biol. 40, 651–662.

Wu, Y., Wu, Z., Butko, P., Christen, Y., Lambert, M.P., Klein, W.L., Link, C.D., Luo, Y., 2006. Amyloid-beta-induced pathological behaviors are suppressed by *Ginkgo biloba* extract EGb 761 and ginkgolides in transgenic *Caenorhabditis elegans*. J. Neurosci. 26 (50), 3102–13113.

Wu, Z., Zhang, J., Gu, X., Zhang, X., Shi, S., Liu, C., 2016. Effects of the extract of *Ginkgo biloba* on the differentiation of bone marrow mesenchymal stem cell *in vitro*. Am. J. Transl. Res. 8 (7), 3032–3040.

Xu, Y., Tao, Y.X., 2004. Involvement of the NMDA receptor/nitric oxide signal pathway in platelet activating factor-induced neurotoxicity. Neuroreport 15 (2), 236–238.

Yallapragada, P.R., Velaga, M.K., 2015. Effect of *Ginkgo biloba* extract on lead-induced oxidative stress in different regions of rat brain. J. Environ. Pathol. Toxicol. Oncol. 34, 161–173.

Zhu, L., Xu, Y.J., Du, F., Qian, Z.M., 2007. Ginkgolides protect primary cortical neurons from potassium cyanide-induced hypoxic injury. Exp. Brain Res. 179, 665–671.