

Safety and Toxicology of Magnolol and Honokiol

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ABSTRACT

Magnolia officinalis and *Magnolia obovata* bark extracts have been used for thousands of years in Chinese and Japanese traditional medicines and are still widely employed as herbal preparations for their sedative, antioxidant, anti-inflammatory, antibiotic, and antispastic effects. Neolignans, particularly magnolol and honokiol, are the main substances responsible for the beneficial properties of the magnolia bark extract (MBE). The content of magnolol and honokiol in MBE depends on different factors, including the *Magnolia* plant species, the area of origin, the part of the plant employed, and the method used to prepare the extract. The biological and pharmacological activities of magnolol and honokiol have been extensively investigated. Here we review the safety and toxicological properties of magnolol and honokiol as pure substances or as components of concentrated MBE, including the potential side-effects in humans after oral intake. *In vitro* and *in vivo* genotoxicity studies indicated that concentrated MBE has no mutagenic and genotoxic potential, while a subchronic study performed according to OECD (Organisation for Economic Co-operation and Development) guidelines established a no adverse effect level for concentrated MBE >240 mg/kg b.w/d. Similar to other dietary polyphenols, magnolol and honokiol are subject to glucuronidation, and despite a relatively quick clearance, an interaction with pharmaceutical active principles or other herbal constituents cannot be excluded. However, intervention trials employing concentrated MBE for up to 1 y did not report adverse effects. In conclusion, over the recent years different food safety authorities evaluated magnolol and honokiol and considered them safe.

Introduction

MBE has been used for thousands of years in traditional Chinese, Japanese, and Korean medicine and has recently been introduced as an active ingredient in food supplements and cosmetics. Among the different magnolia species, *Magnolia officinalis* Rehder & E. H. Wilson (Magnoliaceae, commonly called Hou Po magnolia or magnolia bark) and *Magnolia obovata* Thunb are the ones most often used for these purposes. Their bark and flower extracts are used alone or combined with other herbal-derived compounds as remedies for gastrointestinal disorders, anxiety, and allergies [1, 2]. The other pharmacological activities described for MBE and its major constituents include sedative, antioxidant, anti-inflammatory, antibiotic, and antispastic effects [3]. Cosmetics contain-

ing *M. officinalis* bark extract have recently appeared on the market, although their anti-aging effects have not been scientifically proved. For its digestive and rebalancing activity on the oral microbiome, various different European national institutions have included *M. officinalis* (flos, cortex) in lists of herbal preparations suitable for inclusion in food supplements [3].

Neolignans, particularly magnolol and honokiol (► Fig. 1), are reported to be the compounds mainly responsible for MBE's beneficial properties, and their biological and pharmacological activities have been extensively investigated [2, 4–6]. The aim of this review is to have a critical look to the safety and toxicological properties of magnolol and honokiol, as pure substances or as components of concentrated MBE containing more than 90% of total neolignans, on the basis of a critical, systematic analysis of

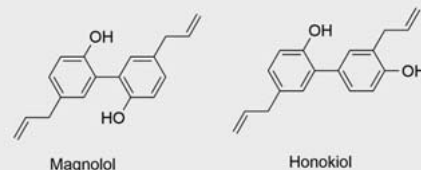
ABBREVIATIONS

CYP	cytochrome P450
CYP1A	cytochrome P450, family 1, subfamily A, polypeptide
HIM	human intestinal microsomes
HLM	human liver microsomes
MBE	magnolia bark extract
NOAEL	no adverse effect level
PICOS	patient, problem or population intervention, comparison, control or comparator, outcome with added qualitative search terms
PPAR γ	peroxisome proliferator-activated receptor gamma
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RTECS	Registry of Toxic Effects of Chemical Substances
RXR	retinoic X receptor
SULT	sulfotransferase
T _{1/2}	half-life
UDP	uridine diphosphate
UGT	glucuronosyltransferase

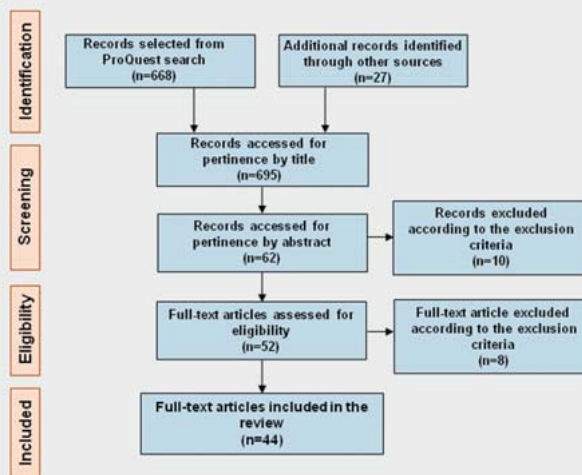
the literature. To retrieve all the pertinent toxicology evidence, we applied the PICOS conceptual framework and the applicable features of the PRISMA statement [7,8] to a structured literature search using a multidatabase platform. The search was done on May 25, 2017, with a global geographical coverage and time limits from the year 1910 to the date of the search. It involved the following databases: AdisInsight Safety, Allied & Complementary Medicine, Biosis Toxicology, Embase, Global Health, International Pharmaceutical Abstracts, Medline, RTECS. After applying pre-selected inclusion and exclusion criteria, three authors reviewed each article for inclusion or exclusion to reach consensus, and 44 original articles were deemed pertinent for inclusion in the systematic review (► Fig. 2). Additional details on the bibliographic strategy are provided as Supporting Information.

Magnolol and Honokiol Content in MBE

The content of magnolol and honokiol in the magnolia crude extract is a key feature of the quality of MBE and can vary by more than 10 times depending on various factors, including the *Magnolia* species (*officinalis* or *obovata*) employed for the preparation, the part of the plant used, and the method of extraction [2,9–15]. Similarly, the content of the other lignans such as 4-O-methyl-honokiol and obovatol can vary; this last may even be absent in a *M. officinalis* extract (► Table 1). The area of origin can also markedly affect the phenolic content of the extract, such as quercetin and cyaniding, reflecting differences in climatic conditions and environmental biodiversity in the different regions of China where magnolia trees are grown (► Fig. 3). Plantations of *M. officinalis* of the same age but from different Chinese regions, all at an altitude of around 1000 meters, had total magnolol plus hono-



► Fig. 1 Chemical structures of magnolol and honokiol.



► Fig. 2 Flow diagram depicting the systematic review strategy.

► Table 1 Neolignans content in crude ethanolic or methanolic MBEs from different magnolia species.

Neolignans	Neolignans content ^a (% of total weight)	
	<i>M. obovata</i>	<i>M. officinalis</i>
Magnolol	0.78–7.65	1.00–1.25
Honokiol	0.55–1.25	0.17–1.81
4-O-methyl-honokiol	0.01–0.21	0.0003–1.24
Obovatol	0.01–0.33	not detectable

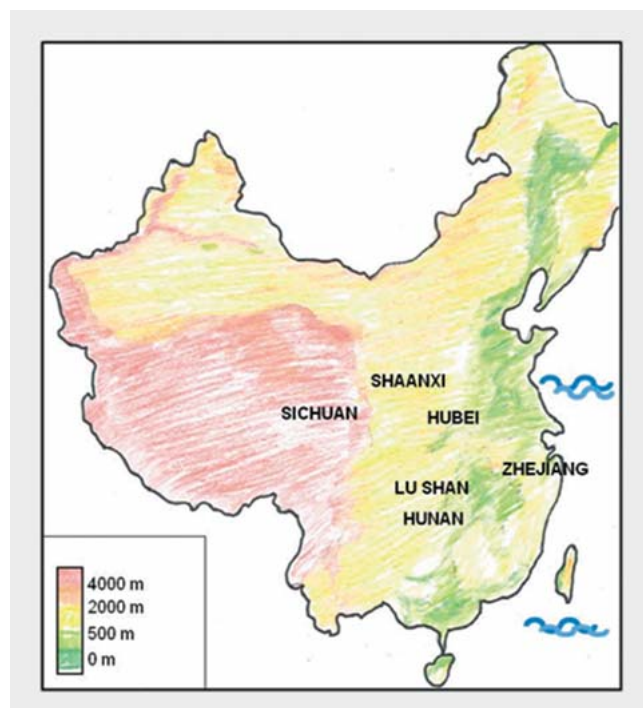
^a Data represent the minimum and maximal values, as reported by Lee et al., 2011 [2].

kiol concentrations in the bark varying by a factor of about 10 when samples from the West Hubei were compared to samples from Mount Lushan (► Fig. 3) [11]. The concentrations of magnolol and honokiol, and their ratios, in extracts from the trunks of *Magnolia officinalis* var. *biloba* trees grown in the same Zhejiang region also differ (► Table 2). The quality of the extract is also influenced by the altitude of the cultivar (► Fig. 3) and by the part of the plant collected to prepare the bark: trunk, branches, or root [2,10,11,16]. Roots seem to contain the highest concentrations

of magnolol and honokiol (87–96 mg/g of bark for each neolignan) (► **Table 2**) [10, 16]. Considering all the influencing factors, the concentrations of the two neolignans in the bark can differ even several hundred-fold, ranging for honokiol from 0.07 mg/g to 96.51 mg/g, and for magnolol from 0.05 mg/g to 91.91 mg/g [16]. Consequently, the content of total neolignans in the extracts may be different, too, as well as the ratio between the magnolol and honokiol concentrations, which can range from as little as 0.42 to as much as 12 (► **Table 2**). The age of the bark influences the content of neolignans, too. The percentages of magnolol and honokiol in the extract prepared from the trunk of a *M. officinalis* tree change with its age, peaking when the plant is 27 y old [17]. Last but not least, different methods can be employed for the extraction of neolignans from ground dried magnolia bark. These are mainly organic and/or aqueous extractions, affecting the recovery of honokiol and magnolol. Sonication, agitation, maceration, or supercritical extraction at variable temperatures for different times (from a few minutes up to 24 h) may be employed to optimize the extraction [18]. This results in widely differing concentrations of magnolol and honokiol in *M. officinalis* bark crude extracts prepared by different suppliers (► **Table 3**). From these data we can infer that similar variability is likely for the rest of the extract, whose components are often not reported but can contribute to the MBE activity. This is the case of essential oils, mainly β -eudesmol and α - and β -pinenes, suggested to exert pharmacological effects on the nervous system, and phenolic and polyphenolic compounds that alter microbial cell permeability and permit the loss of macromolecules thus exerting an antimicrobial activity [3]. For this reason, in this review we focused only on studies using pure magnolol or honokiol and extracts containing more than 90% of total neolignans.

Mutagenicity and Genotoxicity

Investigations of the potential mutagenic and genotoxic properties of magnolol and honokiol employed only MBE and not single purified compounds (► **Table 4**). No mutagenic activity was observed with the Ames test on four bacterial strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and a strain of *Escherichia coli* (WP2 *uvrA*) treated with MBE containing 94% of magnolol and 1.5% of honokiol [19]. Results were also negative from *in vitro* genotoxicity studies on Chinese hamster ovarian carcinoma CHO cells and Chinese hamster lung fibroblasts (V79 cells) treated with a magnolia extract containing 92.5% of magnolol and 1.5% of honokiol, with or without metabolic activation [20]. A more recent study investigated the genotoxic effect of an aqueous extract of *M. officinalis* employing human fetal small intestinal epithelium noncancerous cells, widely used to study the biological effects of food or medicines. The MBE was analyzed by thin-layer chromatography according to the European Pharmacopeia 8.4 monographs and indicated that the presence of magnolol and honokiol was in line with the European Pharmacopeia description and that only traces of these two neolignans were present in the decoction [21]. However, we excluded this study from the pool of pertinent studies, since it did not focus on magnolol, honokiol, or concentrated extracts containing >90% of the two neolignans. Nevertheless, we give a brief report here for the sake of complete-



► **Fig. 3** Map of China showing the areas with the main *Magnolia* plantations.

ness. Cells were exposed to a dose of extract in line with the estimated concentration of the plant found in the small intestine after ingestion of slimming pills containing magnolia (~ 1–2 mg/mL). Phosphorylation of the serine 139 residue of histone γ -H2AX, a biomarker of either DNA damage or DNA repair, was determined by two different methods: immunofluorescence and whole-cell ELISA. Immunofluorescence indicated that *M. officinalis* aqueous extract could cause DNA damage, since it significantly increased γ -H2AX foci, whereas the extract showed no genotoxicity according to the ELISA test. The unexpected possible DNA effect of the *M. officinalis* extract may be due to the highly sensitive test response to genetic insults as well as to the presence of some contaminants in the extract such as alkaloids, which were detected in the lyophilized extract used in the test but not in the raw material extract [21]. These results underline the need for further investigation on the magnolol and honokiol pure compounds in the MBE and contaminants of the extracts to identify the causative agent of the increase of γ -H2AX foci.

At the same time, the safety of *Magnolia* was supported by *in vivo* studies indicating the absence of any genotoxic potential. Swiss albino CD-1 mice (male and female, 7–9 wk old) were treated for 14 d with increasing oral doses (625–2500 mg/kg b.w.) of an extract containing 94% magnolol and 1.5% honokiol (► **Table 4**). To assess cytogenetic damage, erythrocytes were isolated from the femoral bone marrow and the micronuclei test was done according to OECD guidelines. There were no significant changes, at any of the doses, in the proportions of immature to total erythrocytes or increase in the number of micronucleated polychromatic erythrocytes [19]. Moreover, data obtained *in vitro*

► **Table 2** Neolignans content in bark of trunks, branches, and roots of various plant interspecies from different China regions^a.

Region	Magnolia species	Part of the plant	Honokiol (mg/g)	Magnolol (mg/g)	Magnolol/honokiol
Mianyang, Sichuan	<i>officinalis</i>	trunk	6.05	9.34	1.54
Yinshan, Hubei	<i>officinalis</i>		0.08	0.05	0.62
Hanzhong, Shaanxi	<i>officinalis</i>		2.69	4.54	1.69
Sichuan	<i>officinalis</i>		0.70	5.99	8.56
Huaihua, Hunan	<i>officinalis</i>		4.60	5.85	1.27
Sichuan	<i>officinalis</i>		29.68	22.92	0.77
Zhejiang	<i>officinalis</i>		35.28	14.83	0.42
Enshi, Hubei	<i>officinalis</i>		0.07	0.84	12.00
Xupu, Hunan	<i>officinalis</i>		2.40	25.10	10.46
Shuangpai, Hunan	<i>officinalis</i>		0.38	0.25	0.66
Jingzhou, Hubei	<i>officinalis</i> var. <i>biloba</i>		8.80	17.94	2.04
Nan, Hunan	<i>officinalis</i> var. <i>biloba</i>		3.37	9.37	2.78
Anhua, Hunan	<i>officinalis</i> var. <i>biloba</i>		13.12	18.97	1.44
Ruanlin, Hunan	<i>officinalis</i> var. <i>biloba</i>		18.37	31.94	1.74
Zhejiang	<i>officinalis</i> var. <i>biloba</i>		3.18	25.13	7.90
Xupu, Hunan	<i>officinalis</i>	branches	6.64	24.00	3.61
Yunaling, Hunan	<i>officinalis</i>	roots	96.51	90.52	0.94
Xiangxi, Hunan	<i>officinalis</i>		87.56	91.91	1.05

^aAdapted from Jiang et al., 2012 [16].

and in animal models indicated that not only the magnolia extract have no mutagenic and genotoxic effect, but it even showed anti-mutagenic activity [2, 22, 23]. In particular, magnolol and honokiol inhibited UV-induced mutations in *S. typhimurium* TA102 by scavenging of OH [24]. Ames tests performed on *S. typhimurium* TA98 and TA100 also showed that magnolol strongly inhibited mutagenicity induced by indirect mutagens 2-amino-3-methylimidazo[4,5-f]quinoline, 2-aminodipyrido[1,2-a:3',2-D]imidazole, benzo(a)pyrene, 2-aminoanthracene and 7,12-dimethylbenz[a]anthracene through the suppression of CYP1A1 and CYP1A2 activity [25]. In mice, magnolol exerted an *in vivo* antimutagenic effect against clastogenicity induced by benzo(a)pyrene, evaluated using the micronucleus test, and the DNA damage-induced by X-ray irradiation [25]. These findings indicated that magnolia extracts containing a concentration of neolignans > 90% can be classified as safe.

Metabolism

To fully understand the mechanism of action of magnolol and honokiol and their potential toxic effects, it is very important to clarify their metabolism.

Magnolol's metabolism has been widely investigated, showing that it can be extensively metabolized by tissues and intestinal bacterial enzymes to hydrogenated and hydroxyl derivatives, glucuronides, and sulfates (► **Table 5**). After oral doses of [ring-¹⁴C] magnolol, a total of 65% of the radioactivity dose was recovered in the feces and 11% in the urine after 24 h. The main fecal derivatives of oral magnolol in rats are magnolol and a series of metab-

► **Table 3** Magnolol and honokiol contents in aqueous magnolia bark crude extracts from different suppliers.

Supplier	Honokiol ^a (mg/g)	Magnolol ^a (mg/g)	Magnolol/honokiol ^a
1	1.6	15.1	9.4
2	1.2	1.6	1.3
3	1.0	0.5	0.5
4	6.6	5.7	0.9
5	0.2	1.0	5.0
6	0.6	0.8	1.3

^a Each data is the mean of four values, as reported by Lee et al., 2007 [18].

olites in free forms (tetrahydromagnolol and *trans*-isomagnolol), which account for more than 90% of the administered dose; only 6% were glucuronides and sulfates, the latter being abundant in the bile [26]. The magnolol metabolites tetrahydromagnolol and *trans*-isomagnolol tended to increase after repeated doses, suggesting that their formation is associated with the induction of metabolic enzymes in the animal tissues and/or intestinal bacteria [26].

In view of the structural similarity of honokiol and magnolol, it was assumed that the two compounds are metabolized similarly [26, 27] (► **Table 5**). Honokiol, like magnolol, can be extensively metabolized in rats [28–31]. Studies *in vitro* using human liver mi-

► **Table 4** Studies investigating the mutagenic and genotoxic properties of magnolol and honokiol.

Reference	Assay	Treatment	Results
[19]	Bacterial reverse mutation assay	<i>S. typhimurium</i> (TA98, TA100, TA1535 and TA1537 strains) and <i>E. coli</i> (WP2 <i>uvrA</i> strain) were treated with 18.75–300 µg/plate MBE (94% magnolol and 1.5% honokiol) ± metabolic activation for 48–72 h at 37 °C	No mutagenic activity at all the tested doses
	Micronucleus test	Swiss albino male and female CD-1 mice were orally treated for 14 d with 625–2500 mg/kg b. w. of MBE (94% magnolol and 1.5% honokiol)	No effect on the proportion of immature to total erythrocytes nor on the number of micronucleated polychromatic erythrocytes
[20]	Chromosomal aberration assays	CHO cells treated with 0–30 µg/mL of MBE (92.5% magnolol and 1.5% honokiol) ± metabolic activation for 3–18 h	No chromosomal aberrations
		V79 cells treated with > 52–59 µg/mL of MBE (92.5% magnolol and 1.5% honokiol) ± metabolic activation for 6–24 h	No chromosomal aberrations

crosomes, and *ex vivo* in microsomes prepared from livers isolated from rats and then perfused with 10 µM honokiol [28], indicated that its metabolism resulted in the formation of two main metabolites, honokiol monoglucuronide and honokiol monosulfate, suggesting the main contributions of glucuronidation and sulfation to clearance. A recent *in vitro* study identified the metabolic profile of honokiol in different animal species and the enzymes involved in its derivatization. The neolignan was extensively metabolized by extrahepatic and hepatic pathways, producing glucuronic derivatives as main compounds and other 32 metabolites after sulfation and oxidation [32].

Human metabolism usually employs conjugation with glucuronic acid in order to increase compounds' hydrophilic properties, facilitating their excretion. This pattern of transformation, found in both magnolol and honokiol, has been described for other polyphenols as well, such as tea catechins [33] and resveratrol [34].

Absorption and Biodistribution

Different studies have examined the bioavailability of magnolol and honokiol administered by different routes (► **Table 6**). After intravenous (i.v.) injection of magnolol and honokiol (2–10 mg/kg b. w.) to Sprague-Dawley rats the two compounds had comparable pharmacokinetic profiles [35,36]. The $T_{1/2}$ (i.e., the *time needed to halve the initial circulating drug concentration*) of both compounds was not affected by the dose. As shown in ► **Table 7**, the $T_{1/2}$ of magnolol administered at the dose of 2, 5, and 10 mg/kg b. w. was respectively 54.15 min, 49.05 min, and 49.58 min and the $T_{1/2}$ honokiol was 49.22 min when administered at 5 mg/kg b. w. and 56.24 min at the dose of 10 mg/kg b. w. [35,36]. Pharmacokinetics and bioavailability studies have also been reported in rats given i.v. a higher dose of magnolol (20 mg/kg b. w.). The serum and tissue concentrations of magnolol in the free form were determined, together with magnolol glucuronides and sulfates/glucuronides, to gain a picture of the metabolism. It was observed that magnolol was immediately metabolized by the liver and that magnolol glucuronides were the main conjugates detected in the blood [37].

Only one study examined the fate of magnolol injected intraperitoneally (i.p.) [26]. Respectively 52% and 24% of the radioac-

tive ^{14}C -magnolol administered was excreted in the feces and urine within 24 h, indicating rapid elimination.

Numerous studies have investigated the oral bioavailability of magnolol. A single oral dose of radioactive ^{14}C -magnolol to rats resulted in two peaks of radioactivity in blood, the first after 15 min, and the second 8 h later, suggesting enterohepatic circulation of magnolol and its metabolites [26]. The circulating radioactivity returned to baseline within 48 h. Radioactivity distributed mostly in the gastrointestinal tract and liver, reaching 11% of the dose after 15 min, and was found also in kidney, pancreas, and lung. Similarly to what happens after i. p. injection, oral magnolol was mainly excreted in the feces (65% of the radioactivity) and the urine (11% of the radioactivity) within the first 24 h [26], suggesting that magnolol is rapidly removed from the body. Six days after the dose, more than 80% of the administered radioactivity had been excreted. Various isomers and metabolites of magnolol were also detected in urine and fecal samples. In particular, the presence of magnolol glucuronidated derivatives in bile or feces indicates that bacteria and enzymes in the animal intestines are able to metabolize magnolol.

In a similar study, rats were given nonradioactive magnolol orally at the dose of 20 mg/kg b. w. [38]. Thirty minutes later approximately 90% of magnolol had already been metabolized and transformed to the glucuronidated form, while total bioavailability of magnolol was less than 10% [38]. These authors reported that in rats given 20 mg/kg b. w. orally of magnolol the maximal circulating concentration was reached after 1 h (0.16 ± 0.023 µg/mL) and remained constant for about 6 h, with a subsequent linear decrease within 16 h [1,38]. Bioavailability was similar when magnolol was given orally at the higher dose of 50 mg/kg b. w. [37].

To clarify the steady-state pharmacokinetics of magnolol, rats were orally given 50 mg/kg b. w. of magnolol three times a day for seven doses [37]. The serum concentration of magnolol was lower and more stable than after a single dose, while the magnolol sulfates/glucuronides were much higher, with three peaks at 15, 60, and 240 min, indicating the enterohepatic circulation of magnolol conjugates [26]. These results show that repeated doses result in a temporary accumulation of magnolol sulfates/glucuronides but not free magnolol.

► **Table 5** Studies investigating the metabolism of magnolol and honokiol.

Reference	Study	Results
[52]	Magnolol (0–250 μ M) was incubated with HLM or HIM or recombinant UGT enzymes (0.01–0.1 mg/L) for 20 min at 37 °C	Magnolol inhibited the activity of multiple UGT enzymatic isoforms in microsomes
[51]	Magnolol (0.1–10 μ M) was incubated with HLM or UGT enzymes (10–2000 μ M) for 20 min at 37 °C	Magnolol, at concentrations lower than those possibly reached in human gut lumen and blood, inhibited the activity of different UGT isoforms and abolished propofol glucuronidation activity in microsomes
[53]	Honokiol (0.01–200 μ M) was incubated with HLM at 37 °C	Honokiol inhibited the activity of different CYP and UGT enzymes in a dose-dependent manner
[54]	Liver microsomes from Swiss–Häusli mice, Sprague–Dawley rats, Chinese Bama pigs or Cynomolgus macaque were incubated with propofol alone or together with 10 μ M magnolol	Magnolol decreased the propofol glucuronidation by liver microsomes from pigs or monkeys of 25–26% and from mice or rats of 70–78%
[56]	Microsomes from liver of male Wistar rats or HLM were incubated with magnolol or honokiol (10.0–56.2 μ M)	Magnolol and honokiol showed no inhibition or weak to moderate inhibition of CYP isoforms. No significant metabolic interaction occurred when magnolol and honokiol were co-administered with drugs acting as substrate for CYP isoforms
[32]	Hepatocytes from mouse, rat, dog, monkey, and human were treated with 20 μ M honokiol for 0–120 min at 37 °C	Honokiol was extensively metabolized in hepatocytes
	2 μ M honokiol was incubated with UGT and SULT isoforms	Multiple UGT isoforms were involved in the glucuronidation of honokiol. Different SULT isoforms were responsible for formation of honokiol sulfates
[55]	HLM, HIM, or microsomes from human kidney were incubated with propofol alone or together with magnolol	Magnolol potentially inhibited propofol glucuronidation in HLM and human kidney microsomes but not in HIM
[28]	HLM (50 μ g of protein) were incubated with honokiol for 20 min at 37 °C in the presence of uridine 5-diphosphoglucuronic acid and 3-phosphoadenosine-5-phosphosulfate	Glucuronidation (UGTs isoenzymes) and sulfation (SULTs isoenzymes) were the main metabolic pathways for honokiol
	Male Wistar rat livers were perfused with 10 μ M honokiol for 90 min and microsomes were isolated	P450-mediated oxidation by liver microsomes did not contribute to the metabolism of honokiol
[26]	Single or repeated doses of [14 C] magnolol were orally or i. p. administered to male Wistar rats	A single oral dose of [14 C]magnolol resulted in two peaks of radioactivity in blood (15 min and 8 h after the administration) indicating the enterohepatic circulation of magnolol and its metabolites. Radioactivity mainly distributed in the gastrointestinal tract and liver, but also in kidney, pancreas, and lung. A similar excretion dynamic was observed after a single oral and i. p. administration. Within 12–24 h more than 72% of magnolol was excreted in feces and 24% in urine. Repeated oral doses resulted in the accumulation of magnolol sulfates/glucuronides but not free magnolol
[27]	[14 C] magnolol (13 mg/kg b. w.) was administered i. v. to male Wistar rats	14 C-magnolol reached the maximal blood concentration 1 h after the administration and disappeared within 4 h. Biliary levels of radioactivity start to increase 30 min after administration and reached the maximal concentration of 1.3 μ mol/mL 4 h after. At this time-point radioactivity was detected also in the lungs, liver and kidneys. Minor proportion of magnolol was excreted through the gastrointestinal wall

No indications on the pharmacokinetic profile of magnolol in humans are available. Only one study employing an extract containing less than 90% of total neolignans was performed. Although it did not meet the inclusion criteria, it was still considered in this review. Seven healthy subjects and ten asthmatic patients were treated with 5 g/d of Saiboku-To, the *M. officinalis* bark extract used in China as herbal medicine for bronchial asthma, corresponding to 2.1 mg/d of magnolol [39]. The urinary excretion profile of magnolol was reported. In the asthmatic patients and the healthy subjects 10% of magnolol was excreted in the

urine within 9 h and about 95% of urinary magnolol was glucuronidated [39]. These data, comparable to findings from animal models [26], suggest that magnolol can be rapidly excreted in humans, too.

Little information is available so far on the pharmacokinetics and distribution of honokiol [40,41]. A single oral dose of honokiol (40 mg/kg b. w.) in rats was rapidly absorbed, reaching its peak plasma concentration within 20 min. It is also rapidly metabolized to mono-glucuronidated honokiol, and slowly eliminated ($T_{1/2}$ = 290.4 min) [40]. Honokiol rapidly distributed into organs

► **Table 6** Pharmacokinetic and pharmacodynamic studies.

Reference	Treatment	Results
[26]	Single or repeated doses of [¹⁴ C] magnolol were orally or i. p. administered to male Wistar rats	Radioactivity mainly distributed in the gastrointestinal tract and liver, but also in kidney, pancreas and lung. A similar excretion dynamic was observed after a single oral and i. p. administration. Within 12–24 h more than 72 % of magnolol was excreted in feces and 24 % in urine. Repeated oral doses resulted in the accumulation of magnolol sulfates/glucuronides but not free magnolol
[38]	Magnolol was orally administered at the dose of 20 mg/kg b. w. to male Sprague-Dawley rats	Thirty minutes after the administration the concentration of glucuronidated magnolol and free magnolol were 1.79 µg/mL and 0.16 µg/mL, respectively
[1]	Magnolol was orally administered at 5–100 mg/kg b. w. to male Sprague-Dawley rats	The absorption half-life was 0.63 h, the elimination half-life 2.33 h, the time of maximum concentration 1.12 h, and the maximum concentration is 0.16 µg/mL. Oral bioavailability was 4–9 %. The locomotor activity, measured as indicative of the pharmacodynamic profile, was affected starting from 20 mg/kg b. w.
[35]	Magnolol was administered i. v. at 2–10 mg/kg b. w. to male Sprague-Dawley rats	Increasing dosages have same half-life but increasing AUC. Magnolol distributes evenly in different brain regions with concentration higher than plasma
[37]	Magnolol was administered to male Sprague-Dawley rats as a single i. v. dose 20 mg/kg b. w. or as single or multiple oral doses (50 mg/kg/b. w.)	Comparable levels of magnolol and magnolol glucuronides were found in the blood after i. v. administration whereas in orally treated rats the levels of magnolol glucuronides and sulfates were higher than that of free magnolol. The highest concentrations were found in the liver. Magnolol was found also in kidney, brain, lung, and heart
[36]	Honokiol was administered i. v. to male Sprague-Dawley rats at the dose of 5–10 mg/kg b. w.	A biphasic process consisting of a rapid distribution phase followed by a slower elimination phase was observed from the plasma concentration-time curves
[40]	Honokiol was orally administered to male Wistar rats at 40 mg/kg/b. w.	Honokiol was rapidly absorbed reaching its maximal plasma concentration within 20 min. It was rapidly metabolized to mono-glucuronidated honokiol and slowly eliminated ($T_{1/2}$ = 290.4 min). Honokiol rapidly distributed in liver, kidney, and brain. The concentrations of honokiol and its metabolites were highest in liver, followed by kidney and brain. At central level only honokiol was detected indicating that its metabolites cannot cross the blood-brain barrier
[41]	<i>M. officinalis</i> cortex extract (corresponding to 12.78 mg/kg b. w. of magnolol) was administered intragastrically to male Sprague-Dawley rats	Within the first 35 min of administration, magnolol and honokiol crossed the blood brain barrier and accumulated in different brain regions
[39]	Healthy subjects and asthmatic patients were treated with 5 g/d of Saiboku-To (corresponding to 2.1 mg/d of magnolol)	Both asthmatic patients and the healthy subjects excreted the 10 % of administered magnolol in the urine within 9 h. About the 95 % of urinary magnolol were glucuronidated

(liver, kidney, brain) and was detected in various tissues after only 5 min. The concentrations of honokiol and its metabolites were highest in the liver, followed by kidney and brain. At central level only honokiol was detectable, suggesting that its metabolites cannot cross the blood-brain barrier [40].

In a similar study, rats were treated with a series of extracts, containing aqueous *M. officinalis* cortex extract alone or with other herbs. The amounts of single or combined extracts were standardized to deliver a dose corresponding to 12.78 mg/kg b. w. of magnolol [41]. This dose was calculated as equivalent to the effective daily dose of the Zhi-Zi-Hou-Po decoction, one of the famous Chinese antidepressant formulas containing *M. officinalis* cortex, *Gardenia jasminoides* J.Ellis (Rubiaceae) and *Citrus aurantium* L. (Rutaceae) [41,42]. The extracts also contained honokiol, allowing the determination of absorption and distribution of both neolignans. Magnolol and honokiol rapidly crossed the blood-brain barrier reaching different brain regions within the first 35 min after the dose [41].

In summary, the bioavailability of magnolol and honokiol seems to be limited, and after reaching the enterohepatic circula-

► **Table 7** Half-life of magnolol and honokiol in rats after intravenous injection of different doses.

Dose (mg/kg b. w.)	Half-life (minutes ± SE)	
	Magnolol ^a	Honokiol ^b
2	54.15 ± 5.14	not determined
5	49.05 ± 5.96	49.22 ± 6.68
10	49.58 ± 6.81	56.24 ± 7.30

The data are from Tsai et al., 1996 [35] and Tsai et al., 1994 [36].
SE: standard error.

tion, they are both eliminated mostly with the feces and partly in urine. The absorbed fraction of neolignans, either in free form or possibly bound to serum albumin [43] or conjugated with glucuronic or sulfuric acid, can reach different target organs, from which they are rapidly eliminated. Similar pathways have been described for other polyphenols, such as tea catechins [33] and resveratrol [34].

► **Table 8** Toxicological studies.

Reference	Subject	Aim of the study	Treatment	Results
[80]	Human platelets	Effect of honokiol on platelets aggregation	Platelets were incubated with 0.1–1.0 μM honokiol in the presence of collagen (1 $\mu\text{g}/\text{mL}$), 0.05 U/mL of thrombin, 1 μM U46619, or 60 μM arachidonic acid	Honokiol antagonized the platelet aggregation induced by collagen but not by other compounds. It did not affect platelet permeability nor induce platelet cytolysis
	Wild-type zebrafish embryos	Evaluation of honokiol toxicity	Zebrafish embryos were treated with 1–10 μM honokiol	Honokiol was not cytotoxic for zebrafish embryos
	Male ICR mice	Evaluation of anti-clotting potential of honokiol	Honokiol was administered i. v. to mice at 0.5 mg/kg b. w.	Honokiol inhibited platelet activation and aggregation, and increased the time of thrombotic platelet plug formation
[47]	Female and male Kunming mice	Effect of methanol extracts of <i>M. officinalis</i> on kidney functionality	Methanolic extract of <i>M. officinalis</i> was intragastrically administered as a single dose at 0.25 g (equivalent to 6.25 mg of magnolol and 2 mg of honokiol) or for 1, 2, or 3 mo at the dose of 0.25 g/d	Magnolol and honokiol accumulated in serum, urine and kidneys in mice treated for 3 mo. Increased levels of serum creatinine, urea nitrogen and serum albumin as well as alterations of the kidney ultrastructure were observed
[49]	Male and female Sprague-Dawley rats	Short-term (21-d) and sub-chronic (90-d) toxicity of MBE	Rats were exposed to diet containing 60–480 mg/kg b. w./d of MBE (95.5 % magnolol plus honokiol) for 21 d or 60–240 mg/kg b. w./d of MBE for 90 d	No treatment-related macroscopic and microscopy lesions as well as any signs of toxicity were observed. NOAEL for MBE was > 240 mg/kg b. w.

Toxicological Studies

The potential toxicity of different formulas containing magnolia extracts has been investigated in several animal studies (► **Table 8**). According to the National Research Institute of Chinese Medicine, MBE has low toxicity when administered orally. The oral dose that causes the death of 50% of the test animals (lethal dose 50%, LD_{50}) is > 50 g/kg b. w. When MBE was injected i. p., the LD_{50} was about six times lower (8.5 g/kg b. w.) [2,44,45], although data from traditional Chinese medicine describes similar low patterns of toxicity after oral and i. p. doses [46]. A methanol extract of *M. officinalis* was given as a single dose of 0.25 g or chronically to mice for 1, 2, or 3 mo, at doses of 0.25 g/d, equivalent to 6.25 mg/d magnolol and 2 mg/d honokiol [47]. There was significant accumulation of magnolol and honokiol in serum, urine, and kidneys only in mice treated for 3 mo. These effects were accompanied by increase in clinical parameters relevant to kidney function, such as serum creatinine, urea nitrogen, and serum albumin. There were also significant alterations of the kidney ultrastructure morphology in the mice treated for 3 mo—a further indication of the kidney impairment induced by chronic treatment with *M. officinalis* methanol extract.

A magnolia bark crude 50% ethanol extracts (5 or 10 g/kg b. w.) given by esophageal intubation to male rats for 14 d resulted in decreases in alanine aminotransferase and creatinine and increase in blood urea nitrogen [48]. However, these last observations were based on the effects of traditional Chinese preparations of MBE, so the precise concentrations of magnolol and honokiol are not known, thus not fitting the inclusion criteria of this review. Conversely, in a study with concentrated MBE (containing 95.5% magnolol plus honokiol), no mortality or clinical signs of toxicity, or alterations in the major organs at autopsy, were reported in animals given 625, 1250, or 2500 mg MBE/kg

b. w./d orally for 14 d [19]. The safety profile was also examined in two subchronic (21-d and 90-d) toxicity feeding studies in rats fed diets containing MBE at various concentrations [49]. This study is noteworthy because it was conducted according to the OECD Principles of Good Laboratory Practice standard procedures [50]. In the 21-d study, rats were fed 60–480 mg/kg b. w./d, and in the 90-d study they received 60–240 mg/kg b. w./d of MBE. No treatment-related macroscopic or microscopic lesions and no signs of toxicity were detected, confirming the safety of MBE for oral consumption [49]. From these results, the NOAEL for concentrated MBE was established at > 240 mg/kg b. w./d as an oral dose [49].

Interaction with Drugs or Other Substances

To investigate whether the consumption of products containing honokiol and magnolol can potentially result in dangerous clinical outcomes, increasing efforts have been made to assess their effects on human CYP and UGT enzymes. Magnolol and honokiol can be metabolized in humans and rats, undergoing extensive glucuronidation in the liver and intestines. The enzymes responsible for this metabolism are isoforms of UDP-UGT, which catalyze the transfer of glucuronic acid from UDP-glucuronic acid to magnolol/honokiol, making it a more soluble substrate, readily excreted by the kidney [51,52]. Human UGT isoforms are divided into three subfamilies (known as 1A, 2A, and 2B) based on various amino acid sequences and structures expressed in a tissue-specific manner [51]. Liver and intestine are the two most important glucuronidation sites for orally administered neolignans. It has been reported that magnolol and honokiol can potentially inhibit the activity of UGT isoforms 1A7 and 1A9 in humans and rodents [32,51]. This is not a trivial matter, since UGTs exert an important protective effect against many carcinogens and drugs with side or

even toxic effects, and their inhibition slows the clearance of drugs, thus raising their blood and tissue levels. Therefore, the inhibition of UGTs by magnolol may be a potential mechanism that enhances the toxicity of drugs or other active compounds contained in the herbal preparation [51, 53].

Different liver microsomes from mice, rats, pigs, and monkeys were employed to identify the best model for studying whether the inhibition of glucuronidation by magnolol can interact with the metabolism of other drugs. In a comparison of rat and human CYP enzymes, magnolol and honokiol were tested for inhibitory activity on five pairs of the corresponding enzymes. In all the tests, the human enzymes were less affected than rat enzymes by the magnolia neolignans; honokiol was only a moderate inhibitor of human CYP1A2, while both magnolol and honokiol were classified as weak or noninhibitors of the remaining enzymes [53].

The effects of magnolol on different animal species were compared in a study with propofol, a widely used anesthetic. In rats the inhibition of glucuronidation by magnolol resulted in prolongation of the propofol anesthesia, whereas pigs and monkeys, whose metabolism is very close to humans, were much less sensitive to the UGT inhibition [54]. A subsequent *in vitro* study confirmed that magnolol can inhibit propofol glucuronidation by the liver and kidney microsomes, but not by intestinal microsomes [55]. These findings indicate that magnolol inhibits UGT differently in humans and animals, highlighting the difficulty of adequately modeling clinical drug interactions involving UGT inhibition.

Although the UGT expression profile is different in rats and mice from that in humans, nearly all whole-animal magnolol pharmacological studies have been conducted in these rodents. Pharmacokinetic studies in rats indicated that after oral administration, magnolol in the liver can reach the concentration of about 10 μ M [37]. This concentration almost completely abolished the glucuronidation of propofol by human enzymes *in vitro*, while only limited inhibition was observed in rats [51, 52]. These results suggest that, when no significant inhibition of UGT is seen in laboratory animals, the potential inhibition and the corresponding toxic effects in humans may be underestimated.

The ability of magnolol and honokiol to interact with drugs has been investigated by assessing their effects on CYP enzymes. *In vitro* studies using human liver microsomes indicated that honokiol and magnolol inhibited the activity of different CYP1A2, CYP2C8, CYP2C9, and CYP2C19 enzymes involved in drug metabolism and hydroxylation. These two neolignans, similarly to other herb-derived compounds, may have the potential to interact pharmacologically with other co-administered drugs metabolized by CYP enzymes [53, 56].

It has also been reported that *in vitro* MBE, as well as magnolol and honokiol, can activate cannabinoid 1 and 2 receptors, which mediate analgesia, stimulate appetite, and have anti-inflammatory action [57]. These effects were also seen with magnolol's two main metabolites, isomagnolol and tetrahydromagnolol, pointing to a new potential mechanism of action for MBE and suggesting a potential synergistic effect with other molecules acting on cannabinoid receptors [57].

Magnolol has been also suggested to exert a benzodiazepine-like sleep-promoting effect by acting on the GABA_A receptor,

known to be modulated by traditional benzodiazepines as well as nonbenzodiazepine hypnotic drugs which are the first line pharmacological agents used to treat insomnia [58]. These findings suggest that the ability of magnolol to increase the effect of drugs interacting with GABA_A receptors should not be excluded.

Honokiol, for its ability to activate RXR, is considered a natural retinoid agonist [59, 60]. It has been recently reported to act also as agonist of PPAR γ , a ligand-dependent transcriptional factor member of the nuclear hormone receptor subfamily. Similar to other agonist of PPAR γ , which are used as antidiabetic drugs, honokiol stimulated glucose uptake *in vitro* in 3T3-L1 adipocytes [61, 62] efficiently stimulating differentiation *via* multiple pathways, including RXR activation. The oral administration of honokiol at 200 mg/kg b.w. for 8 wk lowered fasting blood glucose in type 2 diabetic mice by enhancing phosphorylation of insulin receptor β -subunit, which is critical to trigger insulin signaling pathway. In addition, it activated the downstream insulin signaling factors including the serine/threonine kinase Akt and extracellular signal-regulated kinases 1/2 [63]. The ability of honokiol to interact with insulin metabolism should be taken into consideration when this neolignan is administered together with other PPAR γ agonists used as antidiabetic drugs, such as pioglitazone, rosiglitazone, ciglitazone, and troglitazone. Honokiol enhanced *in vitro* the effects of rosiglitazone on the activation of PPAR γ target genes such as adiponectin, glucose transporter type 4, and adipocyte protein 2 in 3T3-L1 adipocytes, suggesting that it can synergistically stimulate the differentiation and function of adipocytes in combination with endogenous PPAR γ agonists produced in the early phase of preadipocyte differentiation or clinically administered PPAR γ agonists.

Numerous studies reported that PPAR γ agonists, due to their ability to induce cell growth inhibition, cell cycle arrest, or apoptosis of tumor cells, act also as antitumoral agents [64]. Recently, it has been reported that honokiol too exerted an antiproliferative effect on hepatoma cells through the regulation of G0/G1 phase-related proteins expression and that a more effective inhibition was induced when honokiol was administered with rosiglitazone [58].

Honokiol, alone or combined with chemiotherapeutic agents, can also contribute to reduce the chemioresistance and cancer progression of mammary carcinoma cells by suppressing the expression of multidrug resistance proteins and the transmembrane protein Mucin 1 [65].

It has been recently reported that, like some drugs, magnolol and honokiol interact *in vitro* with aristolochic acids, suggesting they may interact with other compounds derived from plants as well [66]. However, it is important to note that these data do not necessarily translate into drug interactions in clinical situations, and additional *in vivo* studies are required to draw firm conclusions on the metabolic drug interactions of magnolol and honokiol. In real-life situations, the same enzymes used to transfer glucuronic acid to drugs and to neolignans also interact with natural polyphenols taken through the diet (e.g., wine, berries, tea). Thus, it remains to be established which of the components of a whole diet are in fact responsible for the putative modulation of drug metabolism.

► **Table 9** Studies involving humans.

Reference	Subject	Investigation	Treatment	Results
[68]	16 healthy premenopausal women (20–50 years of age), overweight (BMI 25–34.9)	Effect of a dietary supplement containing <i>M. officinalis</i> extract and <i>Phellodendron amurense</i> (Relora) on anxiety, stress, and sleep	Relora (corresponding to 11.25 mg/d honokiol and 0.75 mg/d berberine) or placebo was administered for 6 wk	Two volunteers in the study group reported heartburn, trembling hands, perilabial numbness, sexual dysfunction, thyroid dysfunction, fatigue, and headaches. No serious adverse events related to treatment were recorded and no significant alterations in biochemical markers were found
[74]	40 healthy adults at high risk for caries	Effect of MBE on caries and gingivitis	Participants chewed five pieces/d of sugar-free chewing gum containing MBE (equivalent to 11.9 mg of magnolol and honokiol) for 30 d	No side effects were reported
[69]	42 healthy premenopausal women (20–50 years of age), overweight (BMI 25–34.9)	Effect of a dietary supplement containing <i>M. officinalis</i> extract and <i>Phellodendron amurense</i> (Relora) on weight control	Relora (corresponding to 11.25 mg/d honokiol and 0.75 mg/d berberine) or placebo was administered for 6 wk	Side effects “possibly related” to Relora treatment (heartburn, shaking hands, perilabial numbness, sexual dysfunction, and thyroid dysfunction) were reported by one participant. No significant changes in metabolic profile laboratory values were observed
[71]	Nine healthy subjects with no evidence of systemic or oral pathology	Oral antibacterial effect of MBE	Three pieces of compressed mints (corresponding to a total of 12.6 mg of MBE) were consumed or two pieces of chewing gums (corresponding to 4 mg of MBE) were chewed for 20 min	No general problems related to MBE ingestion were reported
[72]	111 healthy adults	Effect of MBE on the formation of oral volatile sulfur-containing compounds	1 piece of chewing gum containing 0.012% (w/w) zinc acetate and 0.15% (w/w) MBE (equivalent to 0.14% (w/w) magnolol) was chewed for 10 min up to 1 h	No general problems related to MBE ingestion was reported
[73]	111 healthy adults	Effect of MBE on the formation of oral volatile sulfur-containing compounds	Two tablets containing 0.17 mg of zinc lactate and 0.84 mg MBE were ingested (corresponding to 1.64 mg of MBE)	No general problems related to MBE ingestion was reported

Possible Side Effects in Humans

Although the use of MBE has a long history, especially in traditional Chinese and Japanese medicine, and it is found in many dietary supplements currently consumed worldwide, no epidemiological studies have examined the toxicity of MBE or its derivatives. This may partially be ascribed to the fact that the preparations frequently contain other substances besides *M. officinalis* and the magnolol and honokiol contents in formulations are often not specified. Thus, it is hard to calculate precisely the doses of neolignans consumed or to conduct adequate clinical-epidemiological studies evaluating any toxic effects.

Products containing *M. officinalis* have recently been marketed as anti-aging cosmetics [67]. Similar to other *Magnolia* extracts, for MBE, too, allergic contact dermatitis has been reported, so this ingredient might be a rare allergen.

MBE has been employed as functional ingredient in a series of human studies, either alone or with other herbal ingredients (► **Table 9**). In a clinical study with a food supplement comparing MBE and phellodendron with placebo, volunteers were given 11.25 mg/d of honokiol and 0.75 mg/d of berberine [68]. Forty-

two overweight female volunteers entered the study, and two subjects in the study group and one in the placebo arm withdrew because of adverse effects. The two volunteers in the study group anecdotally reported heartburn, trembling hands, perilabial numbness, sexual dysfunction, thyroid dysfunction, fatigue, and headaches. During the study, however, no serious adverse events related to treatment were recorded and no significant differences in biochemical markers were found between the two groups [68].

In another study which used the same supplement, the same symptoms were reported by two volunteers, although these were judged unrelated to treatment [69]. Furthermore, the blended nature of the supplement makes it impossible to relate any effect to a single constituent.

M. officinalis-based products have recently gained popularity as sedatives, too, on the basis of the ability of magnolol and honokiol to exert GABA-ergic and cannabimimetic activities, alongside the customer's perception of a product which is “natural” and hence somehow “safe” [70]. However, no peer-reviewed papers proving the abuse, misuse, or dependence on or addiction to magnolia-based products have been retrieved yet [70].

Concentrated MBE was employed in clinical studies where volunteers ingested a single dose of the extract presented in chewing gum or tablets [71–73]. Considering that about 50% of MBE was released by chewing gum during the chewing, it can be calculated that, in the study by Greenberg et al. and involving volunteers, 1–4 mg of magnolol plus honokiol was ingested [71]. A similar amount was taken by volunteers enrolled by Porciani et al., in which chewing gum or tablets, releasing respectively 1.5 mg and 3 mg of magnolol plus honokiol, were used [72, 73], and no side effects were reported.

In a clinical study involving 40 volunteers, five pieces of chewing gum per d, equivalent to 11.9 mg/d of total magnolol and honokiol, were chewed for 30 d. It was calculated that this corresponded to a daily intake of about 6 mg neolignans, and again, no volunteer reported side effects [74]. Overall, human studies investigating the efficacy of MBE have encompassed dosages from 1 to about 10 mg of magnolol and honokiol for periods that span from single doses to 1 y and have found no side effects related to the ingestion of the two neolignans.

Additional Information on the Authorization of MBE and Supporting Evidence

In addition to the studies derived from the structured search and following systematic review, it is important to consider that MBE has been the subject of a series of specific authorizations for use in food. MBE is listed among herbs allowed for used in different European countries such as Italy [75], France [76] and Belgium [77]. In particular, the Belgian decree reported a warning on the use of magnolia extracts in pregnancy or the simultaneous use of anticoagulant drugs. So far, numerous papers have been published on the effects of *M. officinalis* and its active ingredients honokiol and magnolol on hemostasis, and the biochemical mechanisms underlying their effects have been widely investigated *in vitro*. They were reported to act as inhibitors of thromboxane formation, platelet activation, and aggregation [78], and these effects were confirmed *in vivo* in rodents. Magnolol was reported to prolong the tail bleeding time of mice when injected i.p., starting from the dose of 10 mg/kg b.w., whereas no effects were observed at lower doses [79]. Honokiol has recently been reported to inhibit platelet activation and aggregation in rats when injected i.v. at 0.5 mg/kg b.w. [80]. On the basis of these observations, it is reasonable to establish that the lowest dose of magnolol and honokiol required for antihemostatic action in rodents is 0.5 mg/kg b.w. when administered i.v. Only two studies are available so far on the effect of magnolia extract in pregnancy and on fetal development. The first one investigated the effects of honokiol and magnolol extracted from *M. officinalis* on muscular contractile responses *ex vivo* in the nonpregnant rat uterus [81]. Both compounds exerted a spasmolytic effect on uterine muscle contraction and their potential use was envisaged in the treatment of some gynecological disturbances and dysfunction associated with an increase in uterine muscular activity, such as dysmenorrhea and premature delivery. The second study investigated the embryo-fetal developmental toxicity of honokiol microemulsion, a new dosage form of honokiol developed by the Chinese Peking

University [82]. In this study, done under Good Laboratory Practice regulations, honokiol was injected i.v. to pregnant rats at 0.2, 0.6, and 2.0 mg/kg b.w./d from d 6–15 of gestation, and body weights, clinical parameters, cesarian sections, and fetal morphology were recorded. The NOAEL of honokiol microemulsion was 0.6 mg/kg b.w./d [82], which is apparently comparable to the dose effective as antihemostatic in rodents [80]. However, in the same study Zhang et al. [82] concluded that the dose of honokiol microemulsion of 0.6 mg/kg b.w./d was about 75 times the dosage, which, they hypothesized, would be effective as an antihemostatic in humans.

On the base of these findings one can conclude that the warnings on the effect of magnolia extract in pregnancy are not justified so far. The warning about the use of MBE in pregnancy or simultaneous use of anticoagulant drugs recently issued by the Belgian authorities can be explained by the antihemostatic effect of the extract and the active compounds magnolol and honokiol.

Magnolol is also authorized as a flavoring agent. The specification reported a minimum of 92% magnolol and 3–7% honokiol, plus 1–2% eudesmol. The international organization of the flavor industry referred to an assessment by the joint FAO/WHO expert committee on food additives [76], which identified a NOAEL of 240 mg/kg b.w./d from the study by Liu et al. [19] and concluded that the intake as a flavoring would be 2400 times lower to ensure an adequate safety margin.

Conclusions

Magnolol and honokiol are two structurally related neolignans present as the main active compounds in MBE. The Chinese pharmacopeia indicates that extracts intended for traditional therapeutic use should contain at least 0.3% of neolignans by weight as the sum of the two main components [77]. However, this review focused on concentrated MBE containing more than 90% total magnolol and honokiol or on the pure compound, thus eliminating confounding effects due to any other constituents of the magnolia bark.

In vitro and *in vivo* studies have shown that MBE containing more than 92.5% of magnolol and up to 7.5% of honokiol do not give rise to any concern as regards mutagenicity or genotoxicity [2, 19, 20, 22, 23]. Their functional and structural similarity means that magnolol and honokiol have comparable pharmacodynamic and pharmacokinetic properties and are metabolized similarly. When administered orally, about 90% of both compounds are rapidly excreted in feces and urine [26]. The absorbed fraction is subsequently glucuronidated and only 1% of the oral dose remains circulating in the free form for 12–16 h [1, 28, 38, 39, 51]. Although magnolol and honokiol can be subject to enterohepatic circulation, they do not cause any specific hepatic side effects [2, 27]. In rats, the LD₅₀ for MBE was as high as 50 g/kg b.w. or more for oral intake but was significantly lower when injected i.p. [45]. The NOAEL based on a 90-d oral study was set at 240 mg/kg b.w. [49].

No troublesome side effects related to the ingestion of herbal formulations containing MBE or magnolol/honokiol have been reported so far in humans, and *M. officinalis* and *obovata* have been used for a long time in traditional oriental medicines.

In recent years the popularity of *Magnolia* has grown substantially because, similarly to other herbal-derived products, customers perceive it totally “natural” and hence “safe”. Concentrated formulations containing magnolia extracts can be easily purchased on the Internet today [70], facilitating self-administration. This raises important questions on the abuse/misuse of magnolia extract and its main constituents and on their ability to interfere with pharmacological treatments, particularly those required for chronic disease management. Nevertheless, the intake of magnolol and honokiol has been modelled for use as a flavoring substance and as a functional ingredient in mints and chewing gum and is considered safe against the reported NOAEL.

Supporting Information

Additional details on the bibliographic strategy are provided as Supplementary Information.

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Conflict of Interest

AS and NK are employees of Perfetti Van Melle SpA. The Department of Molecular Biochemistry and Pharmacology of the IRCCS – Istituto di Ricerche Farmacologiche “Mario Negri” has received scientific consultancy fees from Perfetti Van Melle SpA Company within the last 5 y.

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