Alkaloids from Lotus (*Nelumbo nucifera*): Recent Advances in Biosynthesis, Pharmacokinetics, Bioactivity, Safety, and Industrial Applications

Zhenyu Wang[a, b, 1], Yong Li[a, b, 1], Dandan Ma[a, b], Maomao Zeng[a, b], Zhaojun Wang[a, b], Fang Qin[a, b], Jie Chen [a, b], Mark Christian[*, c], Zhiyong He[*, a, b]

[a]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China

[b]International Joint Laboratory on Food Safety, Jiangnan University, Wuxi, Jiangsu 214122, China

[c]School of Science and Technology, Nottingham Trent University, Clifton, Nottingham, NG11 8NS, United Kingdom

[*]Corresponding author. Tel: +86 510 8591 9065; fax: +86 510 8591 9065.

E-mail address: zyhe@jiangnan.edu.cn (He Z.), mark.christian@ntu.ac.uk (M. Christian)

[1] Contributed equally to the manuscript

Abstract: Different parts of lotus (*Nelumbo nucifera* Gaertn.) including the seeds, rhizomes, leaves, and flowers, are used for medicinal purposes with health promoting and illness preventing benefits. The presence of active chemicals such as alkaloids, phenolic acids, flavonoids, and terpenoids (particularly alkaloids) may account for this plant's pharmacological effects. In this review, we provide a comprehensive overview and summarize up-to-date research on the biosynthesis, pharmacokinetics, and bioactivity of lotus alkaloids as well as their safety. Moreover, the potential uses of lotus alkaloids in the food, pharmaceutical, and cosmetic sectors are explored. Current evidence shows that alkaloids, mainly consisting of aporphines, 1-benzylisoquinolines, and bisbenzylisoquinolines, are present in different parts of lotus. The bioavailability of these alkaloids is relatively low *in vivo* but can be enhanced by technological modification using nanoliposomes, liposomes, microcapsules, and emulsions. Available data highlights their therapeutic and preventive effects on obesity, diabetes, neurodegeneration, cancer, cardiovascular disease, etc. Additionally, industrial applications of lotus alkaloids include their use as food, medical, and cosmetic ingredients in tea, other beverages, and healthcare products; as lipid-lowering, anticancer, and antipsychotic drugs; and in facial masks, toothpastes, and shower gels. However, their clinical efficacy and safety remains unclear; hence, larger and longer human trials are needed to achieve their safe and effective use with minimal side effects.

Keywords: Lotus alkaloids, Biosynthesis, Bioavailability, Bioactivity, Safety, Industrial applications

1. Introduction

Lotus is a perennial aquatic herb with two living species, *Nelumbo nucifera* Gaertn. (distributed in Asia, Australia, and Russia) and *Nelumbo lutea* willd. (restricted to eastern and southern North America) (Wang, Cheng et al. 2021; Yang et al. 2012). Asia is the main production area of lotus, and the total areas for its agriculture in China, Japan, and India (Yamuna River) are estimated to be more than 330,000, 4,000, and 400 hectares, respectively (Verma and Prakash 2016; Wang, Cheng et al. 2021). Over 608 cultivars, categorized by morphological features, are extensively dispersed throughout various areas of China, especially in southern China (e.g., Hunan, Hubei, Jiangsu, Jiangxi, and Fujian provinces) (Li, Liu et al. 2010). These *Nelumbo* accessions possess high genetic diversity (~96.4%) due to long-term geographical isolation and a lack of gene exchange (Li, Liu et al. 2010).

All parts of lotus (Fig. 1), including the roots (rhizomes), leaves, seeds, embryos, and flowers, are edible, with the rhizomes and seeds being the most commonly consumed. Importantly, the lotus is recognised not only as a food but also as a medicinal product, as recorded in the “Chinese Pharmacopeia” (Limwachiranon et al. 2018). More than 130 chemical components, including alkaloids, phenolic acids, flavonoids, polysaccharides, sterols, and volatile oil have been isolated and identified in lotus and exert multiple pharmacological effects (Chen et al. 2021; Lin, Wang et al. 2019; Wang et al. 2019).

Alkaloids represent an important group of nitrogen-containing secondary compounds that are mainly present in the leaves and embryos of lotus, with total concentrations of

1.41% and 2.43% (dry-weight basis), respectively (Deng et al. 2016; Do et al. 2013). The major alkaloids in lotus embryo extracts are neferine and isoliensinine, which account for 2~3% and 0.7~1.2%, respectively (Chen et al. 2021). By comparing 92 different lotus cultivars, nuciferine and *O*-nornuciferine were found to be the most abundant alkaloids in the seeds and certain flowers. Nevertheless, anonaine, roemerine, and *N*-nornuciferine are prevalent exclusively in some flower-producing varieties (Chen, Zhang et al. 2013). Thus, different parts of lotus show large differences in alkaloid compounds and contents. The genetic background (origin and geographical distribution) and developmental stages are considered important factors that affect the temporal and spatial distributions of alkaloids in lotus (Table 1). For example, the amount of total alkaloids in “10-48” (a cultivar of *N. nucifera*) leaves reaches a maximum value (2060.7 mg/kg) at semi-maturity, and “Luming Lian” at maturity contains 4.6 times more alkaloids than “10-48” (Yang et al. 2017). Furthermore, a high-performance liquid chromatography (HPLC) analysis of raw rhizome samples (white lotus) in Korea showed that the alkaloid content in Muan, Korea, was 7.35 times that in Nigata, Japan, and the alkaloids in the samples (red lotus) varied in the two regions of Korea (Daegu and Haman) (Zhao et al. 2014). The biosynthesis and structural diversity of alkaloids in lotus may be linked to transcription factors, including the NCSI subfamily (especially the NnNCS7 gene), NnMYB6/12/113, NnCYP80A and NnCYP80G (Deng et al. 2018; Vimolmangkang et al. 2016). Recently, interest has increased in the research of alkaloids from lotus and their pharmacological activities (Fig. 2). Published evidence indicates that

specific alkaloids exhibit "drug-like" potential, for instance, in B16 melanoma 4A5 cells, aporphine- and benzylisoquinoline-type alkaloids, including nuciferine, N-methylasimilobine, lirinidine, and 2-hydroxy-1-methoxy-6a,7-dehydroaporphine extracted from lotus, impair the transcription of tyrosinase and tyrosinase-related protein (TRP) 1/2, suggesting their inhibitory activity on melanogenesis (Nakamura et al. 2013). In MRL/MpJ-lpr/lpr mice with autoimmune disease, (S)-armepavine from lotus seeds prevented lymphadenopathy, as demonstrated by the reduction in T lymphocyte-mediated cytokines (e.g., interleukin [*IL*]-2, type II IFN [*IFN-γ*]) and anti-dsDNA autoantibody, with effects comparable to those of cyclosporin A (Liu et al. 2006). Moreover, considerable reductions of body mass and improvements of lipid metabolism by alkaloids from lotus leaves have been widely acknowledged in both cell culture and animal studies (Fan et al. 2013; Ma et al. 2014). Therefore, the alkaloids in lotus and their pharmacological efficacy are worth exploring in-depth. ~~However, to date, there has been no systematic overview of alkaloids in lotus plants.~~ Here, we discuss issues related to the biosynthesis and profile of alkaloids in lotus plants. Their pharmacokinetics and health-promoting activities are also described, along with an assessment of their safety and applications in the food and healthcare, pharmaceutical, and cosmetic industries.

2. Overview of alkaloid biosynthesis and their profiles in lotus

The biosynthesis of alkaloids in lotus begins with decarboxylation of tyrosine or L-dihydroxyphenylalanine. Then, the resulting 4-hydroxyphenylacetaldehyde (4-HPAA) and L-dopamine combine to produce (S)-norcoclaurine that is catalysed by

norcoclaurine synthase (NCS) enzymes, forming the first benzylisoquinoline alkaloid scaffold. Finally, a series of subsequent reactions, including internal carbon-carbon, carbon-oxygen phenol coupling, and functional group substitutions, takes place, resulting in the production of various kinds of benzylisoquinoline alkaloids (Fig. 3). Evidence suggests that 1-benzylisoquinoline alkaloids are present at high levels in various organs of lotus (Fig. 4), among which lotus embryos are considered to be the main site of 1-benzylisoquinoline alkaloid occurrence. Enzymatic modifications, such as intramolecular C-C phenol coupling and methylation/demethylation at specific locations by O-methyltransferases (OMT) and N-methyltransferase (NMT) have been identified as being involved in the biosynthesis of 1-benzylisoquinoline alkaloids, impacting their structural diversity (Deng et al. 2018; Menéndez-Perdomo and Facchini 2020). O- and N-methylation driven by norcoclaurine is required for the production of recognized 1-benzylisoquinoline alkaloids in lotus (Menéndez-Perdomo and Facchini 2020). For example, coclaurine generated by the 6-O-methylation of norcoclaurine, norarmepavine generated by the 7-O-methylation of coclaurine, N-methylcoclaurine generated by the N-methylation of coclaurine, and armepavine generated by the 7-O and N-methylation of coclaurine have been identified in lotus flowers (Morikawa et al. 2016).

Aporphine alkaloids, including nuciferine, lirinidine, liriodenine, *N*-nornuciferine, *O*-nornuciferine, dehydronuciferine, lysicamine, anonaine, dehydroanonaine, roemerine, dehydroroemerine, pronuciferine, asimilobine, caaverine, *N*-methylasimilobine, and 7-hydroxydehydronuciferine, and cepharadione B have been identified mainly in the

flowers or/and leaves, with especially high accumulation in leaves (Fig. 4). These aporphine alkaloids are thought to be derived from an (S)-*N*-methylcoclaurine or (S)-reticuline precursor (Deng et al. 2018). Enzymatic processes (e.g., mediated by cytochrome P450[CYP]80B and 4′OMT) such as dehydroxylation and demethylation at the C-4′ and C-3′ position, respectively are responsible for converting (S)-*N*-methylcoclaurine or (S)-reticuline to aporphine-type alkaloids in different parts of lotus, especially the leaf (Deng et al. 2018). For example, the conversion of reticuline to nuciferine involves two processes, intramolecular C-C phenol coupling and 7-O-methylation mediated by CYP80G and 7-O-methyltransferase catalysis, respectively (Ikezawa, Iwasa, and Sato 2008). KEGG analysis revealed that NCS homologous genes, particularly NNU14334/03165/03166/ 23168/11880/08355/15801/25948/21373, are involved in O-methyltransferase and CYP monooxygenase synthesis in the aporphine alkaloid pathway (Yang et al. 2017). For example, the methylenedioxygen bridges in the structures of anonaine and roemerine can be synthetized by CYP719A genes (Nelson and Schuler 2013). It is noteworthy that all 1-benzylisoquinolines identified in lotus possess a 4'-hydroxyl or 4'-methoxyl moiety, but why aporphines do not have these modifications is unclear. Further research into the biosynthesis of 1-benzylisoquinolines is needed to gain insight into this curious phenomenon.

Bisbenzylisoquinoline alkaloids are composed of two benzylisoquinoline components connected *via* diphenyl ether, benzyl phenyl ether, or biphenyl linkages (Weber and Opatz 2019). In lotus, norcoclaurine-derived 1-benzylisoquinolines is the

representative route for biosynthesis of bisbenzylisoquinoline alkaloids (e.g., nelumboferine, liensinine, isoliensinine, neferine), with the embryo part having the most bisbenzylisoquinoline alkaloids (Fig. 4). A total of 57 alkaloids were initially identified in lotus plumules (embryos), at least half of which are bisbenzylisoquinoline alkaloids (Zhang et al. 2021). The primary alkaloids found in Plumula nelumbinis are neferine and liensinine, with the overall profile of bisbenzylisoquinolines differing considerably among the various genotypes (Deng et al. 2016; Zhou et al. 2013). Neoliensinine, a tribenzylisoquinoline alkaloid with a relatively complicated structure, was also recently identified from lotus embryos (Yang et al. 2018). Collectively, different O- and N-methylation patterns produce diverse bisbenzylisoquinolines and tribenzylisoquinolines. Nonetheless, the biosynthetic pathway of benzylisoquinoline alkaloids in lotus is flexible, and both structural and regulatory genes responsible for the biosynthesis remain poorly understood.

3. Extraction, isolation and analysis of alkaloids in lotus

Organic solvents (e.g., ethanol, methanol, and chloroform), sometimes coupled with acids (e.g., hydrochloric, sulfuric, and formic acids) have been mainly used to extract alkaloids from lotus plants (Liu et al. 2008; Yan et al. 2015; Zou et al. 2020). For example, a mixture of diethyl ether with 1% aqueous acetic acid was used to isolate alkaloids from lotus flower buds (Nakamura et al. 2013). In addition to conventional methods, mechanochemical treatments, including microwave- and ultrasound-assisted extraction, are performed for the extraction of alkaloids from lotus due to their

advantages of destroying cell walls and increasing the total contact surface area (Chen, Zhang et al. 2013; Liu et al. 2008; Luo et al. 2005; Ma et al. 2010). A high yield of alkaloids of 65.3~66.7% was obtained from lotus plumules using 65% ethanol and 220 W ultrasound at 30℃ (Liu et al. 2019). The combination of 65% methanol and microwave treatment at 200 W was applied to lotus plumules for 260 s, with a total alkaloid peak area of 1306.26, and the efficiency was higher than that of methanol treatment alone (<1000) (Xiong et al. 2016). A modified method using ionic liquid (1.0 M [C6MIM]Br)-based microwave-assisted extraction (280 W) was explored for the extraction of *N*-nornuciferine, *O*-nornuciferine, and nuciferine from lotus leaves (Ma et al. 2010). The results showed that the extraction efficiency exhibited a 0.9~43.7% improvement, and the extraction time was also dramatically shortened from 2 h to 2 min compared to conventional heat-reflux and microwave-assisted EtOH extraction. In addition, a novel method coupling microwave-assisted extraction with solid-phase microextraction was successfully developed for extracting and enriching target alkaloids of lotus leaves (Zou et al. 2020). In comparison to individual microwave treatments, the concentration of nuciferine, *O*-nornuciferine, norepavine and armepavin was increased 13-, 10-, 5- and 12-fold, respectively. Recently, supercritical fluid extraction has been frequently used to extract alkaloids from *N. nucifera* because the process is quicker and more efficient without residual organic solvents. The highest nuciferine yield of 325.54 μg/g in lotus leaves was obtained under the following conditions: time 2 h, temperature 70 °C, pressure 30

MPa, and modifier (10% diethylamine and 99% methanol) flow rate 1.2 mL/min (Xiao et al. 2010).

In further isolation and purification steps, a great deal of research has been conducted to enrich the alkaloid concentration in lotus crude extracts, including chemical and chromatographic methods. Using superparamagnetic Fe_3O_4-graphene oxide (MGO) can effectively enrich and isolate nuciferine in lotus leaf extract, and nuciferine rapidly attached to the MGO surface due to high adsorption capability of the nanocomposite (Fan et al. 2016). In the early days of this work, high-speed counter-current chromatography (HSCCC) combined with a D101 macroporous resin column was used to isolate anonaine (9.73%), pronuciferine (19.8%), *N*-nornuciferine (20.9%), nuciferine (14.7%), and armepavine (15.5%) from lotus leaf extracts with purities of 95.6, 88.2, 92.5, 94.3, and 92.1%, respectively (Hu et al. 2010). To subsequently enhance the polarity of organic solvents while also increasing the solubility of medium-polar substances, ionic liquid ([C4mim][BF4])-modified HSCCC was established and applied to separate aporphine alkaloids from *Nelumbinis folium* with higher purities (pronuciferine: 90.53%, *N*-nornuciferine, 92.25%, nuciferine: 99.86%, and roemerine: 98.63%) (Wu et al. 2018). Furthermore, pH-zone-refining counter-current chromatography (CCC) has been employed by Xu and collaborators for the isolation of alkaloids from lotus leaves (Xu et al. 2011). It was found that 7.4 mg of N-demethylarmepavine (purity: 90%), 45.3 mg of nuciferine (purity: 92%), and 26.6 mg of roemerine (purity: 96%) were successfully purified from crude extracts (500 mg). Subsequently, to improve the partitioning of *N*-

nornuciferine, nuciferine, and roemerine from leaves, and isolienssinine and neferinine, liensinine, and neferine from plumules, a general ionic liquid pH-zone-refining CCC method was further developed by Fang et al. (Fang et al. 2017). The addition of [C4mim][PF6] to organic solvents markedly increased the partition coefficients of the six alkaloids, and purities of 97.0%, 90.2%, 94.7%, 92.8%, 90.4%, and 95.9% were obtained *via* one-step separation. Another available approach was established by Wang et al. utilizing a modified K-targeted CCC strategy with lysine as the pH regulator to effectively isolate phenolic alkaloids (isolienine) from the crude ethanol extract of lotus embryos (Wang et al. 2017).

To identify alkaloids from *N. nucifera*, common analysis methods including colorimetric titration, ultraviolet spectrophotometry, thin layer chromatography (TLC), capillary zone electrophoresis (CZE), HPLC, and NMR have been employed to analyse isolated fractions obtained from lotus samples (Liu et al. 2009; Yang et al. 2012). For example, the presence of alkaloids in lotus was confirmed on TLC plates, which showed orange spots for alkaloids when sprayed with Dragendorff's reagent (Akinjogunla et al. 2020). (Ultra)-HPLC with suitable detection systems, such as a UV-diode array detector (DAD) or photodiode array detector (PDA) coupled with mass spectrometry (MS), is one of the most often used analytical techniques for the identification and quantification of alkaloids from lotus plants (Chen, Zhang et al. 2013; Du, Ren, and Wang 2011; Morikawa et al. 2016; Xiao et al. 2010; Zhou et al. 2013). Importantly, a combination of non-aqueous capillary electrophoresis and UV-MS has been used synergistically to detect alkaloids in lotus leaves, and the

quantitative results were accurate, repeatable, and comparable to those obtained by alternative approaches (e.g., HPLC-MS) (Do et al. 2013). Subsequently, a more efficient method combining HPLC-MS/MS and micro-SPE has been introduced for the simultaneous detection of five alkaloids (nunciferine, O-nornuciferin, liriodenine, armepavine, and pronuciferine) in the plasma of lotus leaf-treated rats with higher coefficients and a lower limit of quantification (Zou et al. 2019). With the evolution of these techniques over time, newer, better, and more efficient analytical approaches will be developed and applied.

4. The pharmacokinetics and bioavailability of alkaloids from lotus plants

Based on currently available data, a range of alkaloids are abundant in lotus plants, especially in the leaf and embryo. Thus, how they are absorbed, metabolized, distributed and eliminated throughout the body after ingestion warrant further investigation. As shown in Table 2, evidence involving their pharmacokinetic properties and bioavailability is summarized.

4.1. Absorption

The pharmacokinetics of alkaloids in lotus have been widely investigated in multiple animal models, including rat, mice, dog, rabbits, and humans. Evidence suggests that nuciferine is rapidly absorbed into the blood, reaching a mean maximum concentration (C_{max}) of 1.71 μg/mL at 0.9 h following oral administration (50 mg/kg) (Ye et al. 2018). Moreover, pharmacokinetic findings in lotus leaf extract (nunciferine, O-nornuciferin, liriodenine, armepavine, and pronuciferine were 28.8, 9.36, 3.6, 6.12,

and 9.41 mg/kg, respectively) treated rats showed that armepavine was rapidly absorbed and eliminated, and nunciferine and pronuciferine showed high plasma levels ($AUC_{0–24\ h}$ was 2069 and 2031 ng/ml, respectively) following oral gavage (Zou et al. 2019). However, a comparative analysis revealed that the time-to-maximum (T_{max}=3.50 h) and terminal half-life ($T_{1/2}$=6.18 h) of nunciferine in the study of Zou et al. (2019) were longer than the values obtained (0.9 and 1.65 h, respectively) in the study by Ye et al. (2018). This may be due to the synergistic effects of mixed components improving pharmacokinetics. The evaluation of the pharmacokinetic parameters of liensinine, isoliensinine, and neferine, after intravenous administration (5.0 mg/kg), by ultra-HPLC-MS/MS showed that their $AUC_{0\rightarrow\infty}$ values in rat plasma were 1164.09, 1695.52, and 3540.90 ng/mL•h, respectively, indicating abundant plasma levels (Hu et al. 2015). Interestingly, in contrast with the results of Hu et al. (2015), the $T_{1/2}$ and $AUC_{0\rightarrow\infty}$ of liensinine in rat plasma following intravenous administration at 5.0 mg/kg were 8.2 h and 1802.9 ng/ml•h, respectively (Lv et al. 2015). The specific reasons for the different phenomena are therefore worthy of in-depth investigation. Notably, in a rabbit model, higenamine (synonym: norcoclaurine, an alkaloid occurring naturally in lotus plumules) was rapidly absorbed to reach a peak concentration within 10 min, with the $T_{1/2}$ being approximately 20 min after oral administration (Lo and Chen 1996). However, in a dog model, the $T_{1/2}$ of higenamine was 8.6 minutes following intravenous infusion (Zheng et al. 2004). In a human trial, the mean $T_{1/2}$ detected after the intravenous injection of 22.5 g/kg higenamine was 8 minutes (Feng et al. 2012). Although numerous studies have described the $T_{1/2}$ of

higenamine, the results vary in different animal models, and knowledge of higenamine's metabolic characteristics is also limited. Thus, the pharmacokinetics of higenamine warrants further exploration.

4.2. Distribution

Understanding the tissue distribution of alkaloids upon intake is important to better understand their *in vivo* pharmacological effects. The analysis of the quantitative tissue distribution of lotus alkaloids in both female and male Wistar rats following oral gavage revealed that neferine (10 or 20 mg/kg) immediately diffused into multiple organ systems, with tissue concentrations in the order of liver>lung>kidney>heart>brain (Huang et al. 2007). According to transcellular transport studies in MDCK, MDCK-MDR1 and MDCK-MRP2 cells, ATP-binding cassette transporters (ABC transporters) may regulate the absorption and distribution of neferine and its analogues (liensinine and isoliensinine) (Yu et al. 2013). Furthermore, a study by Wang et al. (2018), in which male rats were orally administered nuciferine (10.0 mg/kg), supports the rapid and wide distribution of nuciferine, with the highest concentrations found in the kidneys, followed by the lungs, spleen, liver, brain, and heart (Wang et al. 2018). However, studies of the tissue distribution of lotus alkaloids in humans and experimental models are still extremely limited and more research needs to be carried out in the future.

4.3. Metabolism

An investigation of Caco-2 cells showed that the key metabolic pathways of bisbenzylisoquinoline alkaloids are methylation, demethylation, and glucuronidation. For example, norcolaurine is metabolized into methylnorcoclaurine and norcoclaurine-glucuronide, liensinine is metabolized into demethyl-liensinine, and neferine is metabolized into liensinine, isoliensinine, and their further demethylation products (Lin et al. 2015). Dog hepatic microsomal incubation experiments indicated that N- and O-demethylation are the two main metabolic pathways of isoliensinine and its analogue neferine (Zhou et al. 2007; Zhou et al. 2012). Both CYP3A and CYP2B are thought to be responsible for the metabolism of neferine, with CYP3A playing a major role (Jiang, Liang, and Xiong 2006). In human liver microsomes, CYP3A, 2C8, and 2D6 were found to catalyse N- and O-demethylation reactions, converting neferine to liensinine, isoliensinine, and other demethylated metabolites (Huang et al. 2007; Shen et al. 2014). Another investigation on higenamine in human liver microsomes showed that uridine diphosphate glucuronosyltransferase (UGT)1A9 is the major isoenzyme that regulates the glucuronidation of alkaloids (Liang et al. 2017). Furthermore, pharmacokinetic analysis of nuciferine in rabbits showed that the isozymes CYP3A4/1A2/2A6/2C8 were involved in the P450-mediated metabolism of nuciferine, and UGT1A4 was the most potent isozyme engaged in the N-glucuronidation of nuciferine (Lu et al. 2010). The metabolic pathways of nuciferine may be involved in glycine, serine, and threonine metabolism, the synthesis and degradation of ketone bodies, butanoate metabolism, pyruvate metabolism, the citrate cycle, glycolysis/gluconeogenesis, and glyoxylate and

dicarboxylate metabolism (Wang, Wang et al. 2020). An *in vivo* (mice) and *in vitro* study (recombinant enzyme incubation system) carried out by Cao et al. (2020) showed that multiple CYPs, two UDP-glucuronosyltransferases (UGT1A4, UGT1A9), and sulfotransferases were involved in the formation of phase I and II metabolites of nuciferine (Gao et al. 2020). However, Liu and colleagues argued that rabbits, whose nuciferine metabolism is most comparable to that of humans, might be suitable for further pharmacokinetic investigations *in vivo*. Other animals, such as rats, mice, dogs, and even monkeys, are considered inappropriate for such investigations of nuciferine because they lack the critical phase II metabolism that occurs in humans (Lu et al. 2010).

4.4. Excretion

A recent study conducted in rats demonstrated that nuciferine was excreted through the kidneys without liver metabolism, and the cumulative percentages of excreted nuciferine in urine and faeces were 50.7% and 12.9%, respectively, after oral gavage of 10.0 mg/kg (Wang et al. 2018). In addition, a human research study addressing the pharmacokinetics of higenamine in healthy Chinese subjects showed that the amount of higenamine excreted in urine within 8h was 9.3%, and the mean and renal clearance was 249 and 22.9 $L \cdot h^{-1}$, respectively, following intravenous administration at 22.5 μg/kg (Feng et al. 2012). These findings imply that the kidney was not the primary route of higenamine elimination, and that the liver also plays a critical role in the process. Although the level of research investigating the pharmacokinetics of lotus

alkaloids has been increasing, information on their excretion characteristics is still lacking.

4.5. Bioavailability

The bioactivity of alkaloids is restricted by the efficiency with which they are absorbed and transported to target tissues as fully physiologically active metabolites; consequently, their bioavailability becomes a major concern when they are used as therapeutic agents for critical diseases. Alkaloids are prone to absorption and biotransformation when administered orally, resulting in low bioavailability in target tissues such as the liver, kidney, brain, lung, heart, and spleen (Wang et al. 2018; Zou et al. 2019). The $AUC_{0-\infty}$ (81.92 vs. 1369.09 ng h/mL) and C_{max} values (6.70 vs. 668.4 ng/mL) following the intragastric injection of liensinine are much lower than those after intravenous administration, showing that liensinine has a relatively low bioavailability *in vivo* (Wei, Gou et al. 2021). The lower bioavailability of liensinine was further confirmed by the research of Tong et al. (2021). A pharmacokinetic study on O-demethyl nuciferine showed that its bioavailability was 6.4% following sublingual injection and gavage (Wu et al. 2019). Generally, the poor solubility of alkaloids in lotus, including neferine, higenamine, nunciferine, and N-nunciferine leads to their relatively low *in vivo* bioavailability (Table 2), thereby limiting their further clinical application.

In this context, encapsulation in liposomes, emulsions, micelles, and polymeric nanoparticles has been implemented to increase the bioavailability of alkaloids and

tested to improve the solubility of these compounds at absorption sites without losing their physical structure. After preparing liposomes carrying total lotus leaf alkaloids at an oil-water ratio of 4:1 with 250 mg soybean lecithin, the encapsulation efficiency was shown to be higher and reached 84.9% (Zhao and Chen 2009). Compared with the same dose of injected neferine, neferine nanoliposomes significantly prolonged survival time of $NaNO_2$-induced anoxia mice and increased protection against cerebral embolism in mice (Tan and Wen 2010). This indicates that the use of nanoliposomes as carriers of neferine can further improve its efficacy. In parallel, alkaloid-rich *Plumula nelumbinis* microcapsules prepared by Wu et al. (2006) using gelatine as the capsule material greatly reduced the duration of arrhythmia (Wu and Yuan 2006). Additionally, nuciferine nanoparticles were prepared by polylactic-co-glycolic acid to enhance oral delivery. After encapsulation, the relative bioavailability of nuciferine was increased 3.3 times, resulting in lower lipogenesis due to the prolonged controlled release of neciferine (Liu et al. 2017). Thus, improving alkaloid bioavailability is critical for increasing the therapeutic potential of alkaloids against chronic illnesses in targeted tissues.

5. Bioactivities

Based on the satisfactory pharmacokinetic properties of alkaloids from lotus, mounting evidence from cell and animal experiments indicates that their frequent consumption results in broad bioactivity and may therefore be beneficial to human health, as summarized in Table 3.

5.1. Antioxidant and antioxidative stress activity

Secondary ion mass spectrograms of 30 alkaloids identified in lotus embryos from 13 different habitats with alkaloid contents of 119.84~541.54 μg/mg showed strong antioxidant activities, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), and oxygen radical absorbance capacity (ORAC) (Tian et al. 2018b). Aporphine alkaloids (N-methylasimilobine, lysicamine, and nuciferine) from lotus leaves showed moderate antioxidant values in DPPH, ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid), chelating, and/or reducing power assays (Liu et al. 2014). Isoliensinine, a bisbenzylisoquinoline alkaloid from the lotus seed embryo, showed strong free radical scavenging activity, and the drug concentrations eliciting 50% of the maximum stimulation (SC_{50}) values of ABTS and DPPH were 12.07 and 25.26 μM, respectively (Jiang et al. 2018). An imbalance between oxidants and antioxidants causes oxidative stress, leading to molecular damage (Sies 2020). The alkaloid-rich fractions from lotus possessed the strongest antioxidant activities, as they minimized oxidative stress reactions (Baskaran et al. 2015; Wang, Wang et al. 2021). The antioxidative stress activity of alkaloids investigated in cell and/or rat models demonstrated that neferine and liensinine treatment were capable of increasing the activity of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and catalase (CAT), but lowered malondialdehyde (MDA) levels, suggesting an improvement in the body's oxidative stress level (Pang et al. 2015; Tang et al. 2019; Xie et al. 2013). The antioxidative mechanisms of nuciferine in liver may be involved in its inhibition of

the expression of CYP enzymes (Cui et al. 2020). Intriguingly, in the hippocampus of diabetic mice, nuciferine was found to reduce MDA levels, and enhanced SOD and GSH-Px values, which can be regulated by the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome pathway (Wu et al. 2020).

5.2. Anti-inflammatory activity

The liver, lung, and kidney are considered important sources of circulating inflammatory mediators involved in systemic inflammatory response syndrome. Multiple evidence has emerged to support the considerable anti-inflammatory effects of benzylisoquinoline alkaloids (higenamine, isoliensinine, lotusine, liensinine, neferine, nuciferine, armepavine, *O*-nuciferine) from lotus (Jun et al. 2021; Meng et al. 2019; Zhang et al. 2018b; Zhou et al. 2013). Through the inhibition of Toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/nuclear factor-κB (NF-κB) signalling and NLRP3 inflammasome activation, nuciferine (10, 20, or 40 mg/kg) was able to reduce IL-1β levels in the serum and kidney of hyperuricaemic mice (Wang et al. 2015). Studies with rodents demonstrated that oral gavage of neferine (20 mg/kg) downregulated inflammatory cytokines (IL-1β, IL-6, tumor necrosis factor [TNF]-α, and IL-10) by inhibiting the activation of the NF-κB signalling pathway in LPS-induced acute respiratory distress syndrome (ARDS) mice (Liu et al. 2018). The reduced inflammatory levels by blocking mitogen-activated protein kinase (MAPK) and NF-κB/IκBα signalling was also observed in carbon tetrachloride (CCl_4)-induced liver fibrosis rats after 6-week intraperitoneal injection of neferine (5 and 10 mg/kg) (Wang, Wang et al. 2021). Proinflammatory cytokines could lead to other

inflammatory diseases, such as osteoarthritis, dermatitis, and mastitis. A recent report by Ni and collaborators showed neferine (1, 5, and 10 μM) inhibited inflammatory regulators (inducible NOS [iNOS] and cyclooxygenase [Cox]-2) and catabolic enzymes (matrix metalloproteinase [Mmp]3, Mmp13, and adamalysin with thrombospondin type 1 motifs [Adamts] 5) in IL-1β-stimulated rat chondrocytes *via* the inactivation of MAPK and NF-κB pathways (Ni et al. 2020). An inhibitory effect of lotus leaf extract on atopic dermatitis-like skin lesions has been found in NC/Nga mice (Karki et al. 2011), and the activation of MAPK and NF-κB pathways may be responsible for its mechanism of action (Yang et al. 2021). Furthermore, data from LPS-induced mouse mammary epithelial cells indicates that nuciferine was able to alleviate mastitis by inhibiting TLR4/NF-κB signalling (Chen et al. 2018).

Of note, alkaloids can pass through the blood-brain barrier (BBB), thereby modulating neuroinflammation and neuroregulatory factors in the brain. It was reported that neferine (20~50 mg/kg) decreased the levels of inflammatory factors (e.g., TNF-α, IL-6, IL-1β, and IL-18) in the hippocampus of db/db mice (Wu et al. 2020) and decreased proinflammatory cytokine expression in the substantia nigra of Parkinson's disease mice (Jing et al. 2021). Additionally, nuciferine (5, 10, or 20μM) elicited the downregulation of proinflammatory markers (e.g., TNF-α, IL-1β, prostaglandin E2 [PGE2], and NO) in LPS-induced BV2 cells by activating peroxisome proliferator-activated receptor (PPAR)γ, suggesting important roles of nuciferine in the treatment of neurodegenerative diseases (Zhang, Gao et al. 2018). Unfortunately, to our knowledge, investigations on the effects of lotus alkaloids on

intestinal inflammation, including chronic enteritis, acute enteritis, colitis, chronic colitis, and ulcerative colitis are currently lacking and warrant further research.

5.3. Anti-obesity and anti-diabetic activity

Total alkaloids from lotus leaves have been reported to ameliorate obesity-related body indicators *in vivo* and *in vitro* (Fan et al. 2013). Lipase inhibitors can cause lipase to partially lose its decomposition capacity and restrict fat entry into blood, resulting in a lipid-lowering effect, and are therefore frequently employed as targets for treating obese individuals (Liu, Liu et al. 2020). In this regard, there is broad agreement that roemerine, nuciferine, 2-hydroxy-1-methoxyaporphin, armepavine, N-norarmepavine, and N-methylcoclaurine/N-methylisococlaurine from lotus leaves contribute to enhancing total lipase inhibitory activity (Zhang et al. 2017). Molecular simulation docking revealed nuciferine maintained its interaction with lipase by hydrogen bonding to Tyr195, P194, Arg169 and Asp238 (Liu, Liu et al. 2020). Aldose reductase enzyme inhibition is one of the treatment methods being explored to prevent or improve long-term diabetes problems. Interestingly, higher aldose reductase inhibitory activity was observed in *N. nucifera* alkaloidal extracts (IC_{50} =28.82 μg/mL) than in those of *P. nigrum* (IC_{50}=30.21 μg/mL) and *M. koenigii* (IC_{50} =35.66 μg/mL) (Gupta, Singh, and Jaggi 2013).

The ability of alkaloids to reduce fat deposition by regulating lipid metabolism was shown to be associated with the balance of triglyceride (TC) and total cholesterol (TG) concentrations (Zhou et al. 2020). In an *in vitro* 3T3-L1 cells model, nuciferine (5~20

μM) inhibited the differentiation of preadipocytes by downregulating PPARγ, CCAAT/enhancer binding protein (C/EBP)α/β and reducing intracellular lipid accumulation by inactivating critical lipogenic enzymes (e.g., fatty acid synthase [FAS]) (Xu et al. 2021). The inhibitory effects of nuciferine on adipocyte differentiation may be attributed to its suppression of the protein kinase B (AKT)-mechanistic target of the rapamycin complex (mTORC1) pathway (Yoo et al. 2019). With regard to glucose metabolism, 2-hydroxy-1-methoxyaporphine and pronuciferine, both derived from lotus leaves, significantly enhanced glucose consumption in 3T3-L1 adipocytes (Ma et al. 2014). The amelioration of glycolipid metabolism by pronuciferine (10 or 20 mg/L) and nuciferine (10 or 20 mg/L) in insulin-resistant 3T3-L1 adipocytes was found to occur *via* the activation of the adenosine 5‘-monophosphate-activated protein kinase (AMPK) signalling pathway (Ma et al. 2015). Furthermore, in INS-1E cells, nuciferine (10 or 20 μM) induced insulin secretion by closing potassium-adenosine triphosphate channels, suggesting the potential antidiabetic effects of lotus alkaloids (Nguyen et al. 2012).

In *in vivo* animal models, some evidence of the glucose- and lipid-lowering effects of alkaloids from lotus has emerged. Oral infusion of lotus leaf total alkaloids (20 , 40, or 80 mg/kg) into hyperlipidaemic rats for 40 days helped to lower serum lipid levels (e.g., reduce TC and TG) and control body mass (Fan et al. 2013). In diabetic db/db rats, oral administration of neferine (25 or 50mg) for 12 weeks ameliorated fasting glucose and insulin resistance while improving lipid metabolism (Wu et al. 2020), which might be closely related to the activation of PPARα/PPARγ coactivator-1α

(PGC-1α), G protein-phospholipase C (PLC)/protein kinase C (PKC) and AMPK signalling pathways (Zhang et al. 2018a; Zhao et al. 2019). High-throughput sequencing analysis revealed that endogenous metabolic disorders, particularly abnormal lipid metabolism caused by excessive high-fat diet (HFD) consumption, were effectively improved by nuciferine (10 mg/kg), which may involve alteration of the gut microbiota profile (e.g., *Firmicutes/Bacteroidetes*, *Desulfovibrio*, short-chain fatty acid [SCFA]-producing bacteria) in obese rats/mice (Wang, Yao et al. 2020; Yu et al. 2021). Additionally, the central nervous system is considered to be an important target for lotus alkaloids to combat obesity. Dopamine D1 and D2 receptors serve as regulators or balancers in food intake and weight management (Wang et al. 2009), which can be antagonized by coclaurine, *N*-norarmepavine, armepavine, asimilobine, *O*-nornuciferine, *N*-nornuciferine, and nuciferine isolated from lotus leaves, among which O-nornuciferine is the most potent. Therefore, a potential anti-obesity action of these compounds could be mediated by interacting with dopamine D1/D2 receptors (Zhou et al. 2021). Significantly, energy-burning brown adipose tissues (BAT) may be a potential therapy against obesity and diabetes (Hu et al. 2020; Wang, Zeng et al. 2021). To date, there is no evidence of the effects of lotus alkaloids on BAT activity; thus, further study is required in this field. Particular attention should be paid to the three phenolic alkaloids (tail-to-head phenolic benzyltetrahydroisoquinoline alkaloid dimers), neferine and its analogues, liensinine and isoliensinine due to their possible browning-promoting activity similar to polyphenols. Together, these findings support

the application of lotus alkaloids as dietary functional factors for combating obesity and diabetes.

5.4. Hepatoprotective, renoprotective, and lung-protective activity

Alkaloids, such as nuciferine, can be metabolized in the liver by the isozymes uridine diphosphate-glucuronosyltransferase (UGT) 1A4, CYP 3A4, 1A2, 2A6, and 2C8, and their metabolites may have biological functions after being absorbed and metabolized (Lu et al. 2010). Data from an alcohol-induced mouse model indicates that intraperitoneal injection of nuciferine (3 and 10 mg/kg) was able to relieve liver oxidative stress and inflammation, in which an important pathway involved the suppression of miR-144 and activation of the nuclear factor E2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) axis, suggesting hepatoprotective activity of nuciferine (Shu et al. 2019). In hamsters, oral gavage of nuciferine (10 and 15 mg/kg) can exert a strong protective effect on liver steatosis and injury induced by HFD consumption by regulating the expression of factors related to lipid metabolism (e.g., sterol-regulatory element binding protein [SREBP] 1, PPARα, cluster of differentiation 36 [CD36], and FAS) and inflammation (TNF-α and IL-6) (Guo et al. 2013). Metabolomic analysis indicates that the gene expression of key enzymes related to glycerophospholipid, linoleic acid, and α-linolenic acid metabolism play a key role in the exacerbation of lipid accumulation in the liver (Cui et al. 2020). Additionally, intraperitoneal injection of neferine (5, 10, and 20 mg/kg) into CCl_4-induced mice resulted in the regression of fibrosis by inhibiting transforming growth factor-β1 (TGF-β1) (Chen et al. 2015).

Regarding the renoprotective activity of alkaloids from lotus, there is increasing evidence that regular intake of nuciferine is inversely corelated with kidney damage (Li et al. 2021; Wang et al. 2015; Wang et al. 2016). One of the possible pathways involved is the inhibition of ferroptosis. Another potential mechanism is the inactivation of TLR4/phosphoinositide 3-kinase (PI3K)/MyD88/NF-κB and NLRP3 inflammasome signalling. In agreement with these findings, neferine (2~25μM) was found to induce the apoptosis of renal cancer cells by downregulating B-cell lymphoma-2 (Bcl-2) and p65 expression by repressing the NF-κB pathway in Caki-1 cells or inactivating reactive oxygen species (ROS)/NLRP3/caspase-1 signalling to counteract endothelial pyroptosis in LPS-ATP-induced HUVECs cells, suggesting the therapeutic effect of neferine in kidney disease (Kim et al. 2019; Tang et al. 2019).

The lung-protective activity of alkaloids in lotus was demonstrated by Zhao et al. (2010), who showed the pulmonary-protective activity of neferine (20 mg/kg), and a possible mechanism associated with the NF-κB/TGF-β inflammation pathways (NF-κB, TNF-α, IL-6 and endothelin-1) (Zhao et al. 2010). In line with this finding, the therapeutic effects of nuciferine (10 and 20 mg/kg) on LPS-induced acute lung injury were confirmed by reduced myeloperoxidase activity and the wet-to-dry weight ratio of lung, which may be attributed to its suppression of TLR4/NF-κB signalling (Wu et al. 2017). In addition to the anti-inflammatory mechanism, the beneficial effect of neferine (20 mg/kg) on the lung might also be associated with its ability to reduce surfactant protein D (SP-D), reverse T helper 1 (Th1)/Th2 imbalance and restore CD4+CD25+ Tregs (Niu et al. 2013). However, it is important to carry out further

research, especially toxicity assays on other alkaloids, with the intention of ensuring their safe use.

5.5. Antitumour and anticancer activity

The development of cancer is dependent on the interaction between proliferation and apoptosis signalling. There is evidence that alkaloids from lotus seeds, embryos, and petals exert anticancer (e.g., against gastric cancer, hepatoma, cervical cancer, and lung cancer) and antitumour activity (e.g., against osteosarcoma) *in vitro* and *in vivo* (Maneenet et al. 2021; Peng et al. 2017; Sivalingam et al. 2019; Yang,Yu et al. 2019; Zhang et al. 2012; Zhao et al. 2016). The proposed mechanisms of the anticancer effects of alkaloids from lotus involve proliferation, apoptosis, autophagy, and migration (Fig. 5). Study of the effect of neferine on the migration and invasion abilities of hepatocellular carcinoma cells found that it upregulated E-cadherin and downregulated vimentin, Snail, and N-cadherin, suggesting roles in the prevention and treatment of liver cancer (Deng et al. 2017). Evidence of increased protein expression of BAX, BAD, cleaved caspase-9, cleaved caspase-3, and poly (ADP) ribose polymerase (PARP) accompanied by the downregulation of BCL2 and p-AKT suggests that changes associated with neferine (6 and 10 μM) treatment were driven by mitochondrial membrane damage to induce cell death (Poornima, Quency, and Padma. 2013). A recent study by Shu and collaborators showed that isoliensinine triggered cell apoptosis by inhibiting p65 phosphorylation at Ser536 and NF-κB activation in hepatoma cell lines (3 and 10 μg/mL) and tumor xenograft nude mice (3 and 10 mg/kg) (Shu et al. 2015). Through a subsequent in-depth investigation, they

confirmed that the suppression of NF-κB by isoliensinine in hepatoma cells was attributed to its impairment of the PP2A/I2PP2A interaction and promotion of PP2A-dependent p65 dephosphorylation (Shu et al. 2016). Furthermore, there is evidence that the consumption of isoliensinine, liensinine, and neferine also results in positive anti-breast cancer effects (Zhang et al. 2015). The considerable inhibitory capacity was considered to be attributed to the induction of apoptosis and inhibition of proliferation by reducing the Bcl-2/Bcl-2 associated X (Bax) ratio, activating caspase-3, and subsequently cleaving PARP (Kang et al. 2017; Zhang et al. 2015). In tumor xenograft nude mice, nuciferine (50 mg/kg) can function as an “apoptosis promoter” by downregulating the expression levels of β-catenin and its downstream targets (e.g., c-myc, cyclin D, and VEGF-A) and decreasing the Bcl-2/Bax ratio *via* Wnt/β-catenin (beta-catenin-dependent Wnt) signalling, which may explain the anti-tumor effect of nuciferine (Liu et al. 2015). By using a network pharmacology approach, nuciferine was identified as an antitumour agent against neuroblastoma and colorectal cancer that acts by inhibiting PI3K-AKT signalling (Qi et al. 2016). Additionally, a significant inhibition of the progression of glioblastoma by *Nelumbinis Plumula* alkaloids containing liensinine, isoliensinine and neferine (50~200 μg/mL) was found to be mediated by the induction of apoptosis and cell cycle change *via* the sex determining region Y (SRY)-box 2 (SOX2)-AKT/signal transducer and activator of transcription-3 (STAT3)-Slug signalling pathway (Li, Wo et al. 2019).

In addition to apoptosis, autophagy is recognized as another cell death mechanism. Neferine, liensinine, and isoliensinine induce apoptosis and autophagy in LNCaP cells

by inhibiting 5-α-reductase and androgen receptor (AR) expression through the PI3K/AKT signalling pathway (Liu, Wu et al. 2021). Similarly, neferine may serve as a therapeutic target in combating ovarian cancer, as indicated by the induction of A549 cell autophagy *via* the inhibition of the PI3K/AKT/mammalian target of rapamycin (mTOR) and activation of p38 MAPK/c-Jun N-terminal kinase (JNK) signalling (Poornima, Weng, and Padma 2013; Xu et al. 2016). The contribution of 7-hydroxydehydronuciferine (10, 25, 50, or 100μM) to stimulating G2/M arrest, autophagy, and migratory inhibition, as evidenced by enhanced expression of autophagy protein (ATG)-6, ATG-3, ATG-7, ATG-16, and ATG-5, indicates that this component is applicable as a target for malignant skin tumour treatment (Wu et al. 2015). These studies elucidated the functional roles of alkaloids from lotus against the migration, invasion, autophagy, and apoptosis of cancer cells and may provide evidence of antitumour and cancer properties.

5.6. Cardiovascular protective activity

Cardiovascular illnesses are prevalent diseases that cause significant mortality and morbidity worldwide, and they are now regarded as one of the leading causes of death in humans. Bisbenzylisoquinoline alkaloid derivatives are useful for preventing the progression of reentrant ventricular tachyarrhythmias (Cheng, Li et al. 2021; Jia-Qing 2002; Sharma et al. 2017). Neferine shares a similar chemical backbone with liensinine. Mechanistic studies showed the anti-arrhythmic actions of neferine and liensinine are linked to blocking the human Ether-a-go-go Related Gene (hERG) K^+ channel, decreasing Na^+, Ca^{2+}, and K^+ transmembrane transport, and altering the

pharmacokinetics of amiodarone (Chen et al. 2021). Evidence is growing regarding a cardio-protective effect of alkaloids from lotus embryo *via* possible regulation of RhoA/ROCK signaling (Li et al. 2019). Subsequently, the docking data of 5 bisbenzylisoquinolines (isoliensinine, liensinine, methyl neferine, neferine, nelumboferine) and 1 tribenzylisoquinoline (neoliensinine) showed they were effective inhibitors with strong binding affinity for both A and B chains of ROCK1 *via* hydrogen bonding and hydrophobic interaction, suggesting their possible roles in cardiovascular protection (Liu, Lu et al. 2021).

Additionally, *in vitro* cell experiments demonstrated that alkaloid rich fractions of lotus and neferine have potent inhibitory effects on vascular smooth muscle cell (VSMC) proliferation and migration by abolishing PDGF-Rb activation, dephosphorylation of extracellular signal-regulated kinase (ERK)1/2, inactivation of JNK/P38 MAPK, and downregulation of fractalkine gene expression (Jun et al. 2016; Li, Tong, et al. 2010; Zheng et al. 2014). Through iTRAQ proteomics analysis, blocking phospholipase Cβ (PLCβ) and 145 multidomain guanine nucleotide exchange factor 12 (RhoGEF12) expression by activation of G protein-coupled receptor (GPCR) signaling may be responsible for neoliensinine-dependent vascular smooth muscle relaxation (Yang,Yan et al. 2019). The protective effect of neferine (10 μM) on H9c2 cardiomyoblasts was demonstrated by reduced doxorubicin-induced apoptosis mediated by the regulation of the NADPH oxidase/ROS-mediated NF-κB redox and insulin-like growth factor (IGF)/Nrf2 signalling pathways (Bharathi Priya et al. 2018; Priya et al. 2017). Lotus embryo and its active alkaloids may also suppress

TLR2/4/MyD88/interleukin-1 receptor-associated kinase (IRAK)/NF-κB signaling to attenuate vascular inflammation, implying cardiovascular benefits (Chen et al. 2021). For example, neferine (3, 10, or 30 μM) has been shown to inhibit the permeability and adhesion of IL-1β induced vascular endothelial cells, with the potential mechanism being correlated with IκB degradation and p65 phosphorylation, which is regulated by the NF-κB signaling pathway (Zhong et al. 2020). An *ex vivo* study found neferine (0.1, 1, or 10 μmol/L) relaxed corpus cavernosum smooth muscle cells isolated from adult males by blocking voltage-dependent calcium channels, alpha 1 adrenoceptor-operated calcium channels, and calcium release from the intracellular calcium pool (Chen et al. 2007).

In vivo, isoliensinine (1, 2.5, or 5 μmol/L) showed the potential to antagonize stretch-induced arrhythmia in rats and guinea pigs, and this effect may be due to disruption of ion homeostasis (Chen et al. 2006), for example, potential inhibition of late sodium and L-type calcium currents (Liu, Hu et al. 2021). Moreover, experimental data from isoproterenol-stimulated rats indicates that oral administration of neferine (10 mg/kg) for 30 days may confer cardiovascular benefits against myocardial infarction by relieving oxidative stress in heart tissue (Lalitha et al. 2013). However, in human induced pluripotent stem cell-derived cardiomyocytes, both liensinine and neferine (>10 μM) were found to cause cardiotoxicity by disrupting calcium homeostasis (Yu et al. 2016). Research by Chen and coworkers showed that higenamine (0.5, 5, or 50 μM) suppressed doxorubicin-induced cardiomyocytes in *in vitro* neonatal rat cardiomyocytes and H9c2 cell line models, which may involve the attenuation of

antioxidative stress and antiapoptotic effects regulated by PI3K/Akt signalling (Chen, Zhuang et al. 2013). Consistent with this work, in primary neonatal rat cardiomyocytes (100 μM) and mice treated with higenamine (10 mg/kg), activation of β2-adrenergic receptor (β2-AR)/PI3K/AKT signalling was considered the key pathway restricting apoptosis and ischaemia/reperfusion injury (Wu et al. 2016).

These results suggest that benzylisoquinoline alkaloids from lotus offer therapeutic potential for cardiovascular diseases and that the possible mechanisms of action involve inactivating Ca^{2+} and K^{+} channels, interacting with IGF-1 receptor (IGF-1R), TLR and GPCRs, and inhibiting the RhoA/ROCK and TGFβ1 signalling pathways, as shown in Fig. 6.

5.7. Neuromodulatory activity

Alkaloids are able to cross the BBB and show a broad distribution in the brain, implying neuropharmacological benefits (Manogaran, Beeraka, and Padma 2019; Zhang, Yang et al. 2018). In an *in vitro* enzyme assay, lotus embryos showed potential for Alzheimer's disease treatment, as evidenced by inhibition of acetylcholinesterase (AChE), β-site amyloid precursor protein-cleaving enzyme 1 (BACE1), and butyrylcholinesterase (BChE), which is closely related to the presence of neferine, liensinine, and northalifoline (Jung et al. 2015). In an *in vitro* cell model, BBB-permeable aporphine-type alkaloids in lotus flowers were found to accelerate neurite outgrowth in PC-12 cells (Yano et al. 2020). In BV2 microglia, the total alkaloids of lotus plumules (50 μg/mL) and higenamine (20 μM) significantly

inhibited LPS-stimulated depression-like behaviour by increasing BDNF levels and suppressing ER stress-related proteins (BIP, p-JNK/JNK and p-eukaryotic translation initiation factor 2α (eIF2α)/eIF2α) while promoting autophagy (Chen et al. 2019).

In an *in vivo* animal study, a lotus leaf alkaloid extract (27.76% nuciferine, 13.23% *N*-nornuciferine, and 4.23% 2-hydroxy-1-methoxyaporphine) exerted sedative, hypnotic and anxiolytic actions through binding to the γ-amino butyric acid (GABA) receptor and activating the monoaminergic system (Yan et al. 2015). The presence of a hydroxyl group at C-12 or C-7' may be responsible for the enhancement of sedative activity (Nishimura et al. 2012). Lotus alkaloids also exhibited atypical antipsychotic-like actions, as evidenced by the observation that injection of nuciferine blocked head-twitch responses, controlled locomotor activity, and rescued prepulse inhibition (Farrell et al. 2016). In addition, neferine improved memory and cognitive performance in diabetic mice by modulating the NLRP3 inflammasome pathway, and it greatly protected Parkinson's disease-induced animals by boosting dopamine and tyrosine hydroxylase protein production (Jing et al. 2021; Wu et al. 2020). The central effects of neferine, for example, antidepressive-like activity, are possibly linked to the 5-hydroxytryptamine 1A (5-$HT_{1A,}$ also serotonin-1A) receptor. The docking interaction of neferine with $5HT_{1A}$ indicated the aromatic rings in the structure of neferine were mostly responsible for the ligand-receptor interactions (Yeh et al. 2020). In agreement with this result, the antidepressant effects in forced swimming mice exerted by neferine and its analogues (liensinine and isoliensinine) (intraperitoneal injection of 25 or 50 mg/kg) were shown to potentially be related to serotonergic

mechanisms (Sugimoto et al. 2015). Higenamine (intraperitoneal injection of 10 mg/kg) may be beneficial for ischaemic injuries, as manifested by the reduction of brain infarct size, mortality rate, and myeloperoxidase (MPO) activity in middle cerebral artery occlusion (MCAO) rats by inducing heme oxygenase-1 (HO-1) *via* PI3K/Akt/Nrf2 signalling pathways, suggesting its possible role in stroke patient therapy (Ha et al. 2012). The proposed mechanism underlying the neuroprotective effect of alkaloids from lotus is shown in Fig. 7.

5.8. Antibacterial and antiviral activity

Lotus extracts were found to show antibacterial action against certain gram-negative fish pathogenic bacteria (*Escherichia coli* and *Vibrio anguillarum)* and wound pathogens, including both gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), which may be correlated with the presence of alkaloids (Adelakun et al. 2015; Akinjogunla et al. 2009). In addition to their antibacterial effects, lotus alkaloids have also been identified to exhibit anti-HIV properties, as evidenced by lower EC_{50} values (concentration of the treatment that was able to suppress HIV replication by 50%) of coclaurine and norcoclaurine (0.8 and <0.8 μg/mL, respectively), which were lower than that of the quercetin 3-O-β-D-glucuronide (EC_{50}=2 μg/mL). Other powerful anti-HIV bisbenzylisoquinoline alkaloids identified from lotus leaves include nuciferine, liensinine, negferine, and isoliensinine, which had EC_{50} values of less than 0.8 g/mL and therapeutic index values of 36, >9.9, >8.6, and >6.5, respectively (Kashiwada et

al. 2005). By analysis of fragmentation patterns of the neferine and its phase I metabolites in dog hepatic microsome incubation, it was observed that the metabolites produced from neferine shared a similar bisbenzyltetrahydroisoquinoline alkaloid moiety, potentially possessing anti-HIV activities (Zhou et al. 2007). The antiviral and antibacterial properties of alkaloids are thought to be enhanced by quaternary nitrogen and methylenedioxy at C-2 and C-3 (Qing et al. 2017). Structural modification of alkaloids, especially phenolic alkaloids, based on computational chemistry techniques may be a novel approach for producing more effective lotus-based antibacterial and antiviral drugs.

5.9. Intestinal activity

Evidence obtained from Caco-2 cells suggests that liensinine, neferine, and isoliensinine can be absorbed and distributed by P-glycoprotein, which may benefit intestinal health (Yu et al. 2013). Several lines of evidence indicate the importance of lotus alkaloids in gut microbial ecology. The gut histomorphology of *Nile tilapia* revealed that the villous heights, goblet cell numbers and intraepithelial lymphocytes in all fish fed lotus leaf powder (50.18% nuciferine, 16.38% nornuciferine, and 4.18% roemerine) were remarkably increased (Abdel Rahman, Hassanin, and ElHady 2019). Data from Caco-2/HT-29 cells and HFD-fed mice indicate that nuciferine treatment (dose: 0~200μM *in vitro* and 0.3% *in vivo*) attenuated intestinal permeability by improving autophagy and altered the diversity and composition of the gut microbiota (reduced *Butyricimonas* and increased *Akkermansia*) (Shi et al. 2021). These findings

show that alkaloid-rich lotus may be an excellent source of prebiotics, contributing to enhancing the immune response and facilitating the growth of beneficial bacteria.

5.10. Other activities

Recent *in vivo* evidence suggests that oral gavage of nuciferine (4 mg/kg) for 7 days exhibits an anticoagulation effect, as confirmed by an extended activated partial thromboplastin time (APTT) and thrombin time and decreased fibrinogen content in rats (Chen et al. 2020). Moreover, the injury repair action of neferine (10 and 20%) has also been identified in experimental diabetic rats *via* inhibitors of inflammatory cytokine and Nrf2 pathways (Li et al. 2020). Neferine (5 μM) may be a viable candidate molecule for proliferative vitreoretinopathy therapy, as evidenced by the inhibition of proliferation and migration of retinal pigment epithelium through downregulating p38 MAPK and PI3K/AKT signalling in epidermal growth factor (EGF)-induced RPE cells (Ozal et al. 2020). A recent study by Wei and collaborators demonstrated that orally administered higenamine (30, 60, or 120 mg/kg) dramatically alleviated rubbing and sneezing and improved the pathology of lung and nasal tissues in ovalbumin-induced mice, which may be ascribed to its activation of AKT1 signalling and repression of epidermal growth factor receptor (EGFR)/tyrosine-protein kinase JAK2/c-Jun proto-oncogene signalling, suggesting its therapeutic potential for allergic rhinitis (Wei, Zhang et al. 2021). Additionally, a significant inhibitory effect of higenamine (intraperitoneal injection of 10 mg/kg) on diabetic gastroparesis (DGP) mediated by the activation of the β2-AR/PI3K/AKT pathway has also been observed (e.g., increased cell proliferation and diminished apoptosis), which

provides new insight into the targeting of DGP by lotus alkaloids (An et al. 2017). However, the pharmacological efficacy of most alkaloids in lotus remains unknown; thus, in future investigations, methods including omics technologies (genomics, proteomics, metabolomics, transcriptomics, lipidomics, immunomics, and glycomics) and computer molecular target prediction can be utilized to find new potential targets that are not yet documented.

6. Safety

Few studies have focused on the safe consumption of alkaloids for disease prevention and treatment, although they are critical for using lotus alkaloids to promote human health. According to the Chinese Pharmacopoeia, daily dose of lotus leaf intake for an adult is 3~10g (total raw material), containing about 17.9~76.1 mg nuciferine (Wang et al. 2015). A lotus leaf extract at a dose of 2 g/kg caused no signs of toxicity or changes in blood cells, liver enzymes or kidney function, indicating the safety of the plant in rats (Deepa et al. 2021). Moreover, the acute toxicity (LD_{50}) of the lotus rhizome methanolic extract (166 mg/g alkaloids) was determined to be more than 5,000 mg/kg and is declared safe for use (Murtala et al. 2019). Additionally, an acute toxicity study revealed that the LD_{50} value of the aqueous extract of lotus flowers (20 mg/mL aqueous extract containing 72.37 mM nitrites) was greater than 5,000 mg/kg, and no signs of neurobehavioural changes were found (Kameni Poumeni et al. 2017). Modern toxicology investigations revealed that total alkaloids from lotus embryos at 400 mg/kg did not induce organ damage in rats, while the maximum tolerable dose investigated in mice was 5,000 mg/kg (Yang et al. 2015). Nevertheless,

if non-standardized lotus, especially the leaf and embryo, containing products are ingested in excess or by individuals who already have cardiac issues, the existing hERG toxic alkaloids in lotus may raise the risk. The recommended dose of hERG-blocking alkaloids in the daily diet is up to 992 μg (Grienke et al. 2015). As such, an *in vitro* and *ex vivo* investigation of liensinine and neferine by Yu et al. using a real-time cellular analysis (RTCA) cardio system showed potential adverse effects when the concentration exceeded 10 μM, which was attributed to the disruption of calcium homeostasis by blocking calcium transients and restraining rhythmic calcium exchange (Yu et al. 2016).

Notably, higenamine has been included as an ingredient in a non-prescription fat-burner and sports supplements available in the market of U.S. (Cohen et al. 2019). However, because of accidental higenamine doping, safety concerns are on the rise, necessitating increased attention. A report from MedWatch stated that consumers ingesting OxyELITE Pro products (aegeline: 33.2~112.0 mg/capsule, higenamine: 15.5~41.6 mg/capsule, yohimbine: 1.6~3.2 mg/capsule) presented signs of liver damage (Klontz et al. 2015). According to the oral pharmacokinetics of higenamine (>0.001 mg/kg), the higenamine level in the urine of rat exceed the safety threshold (10 ng/mL) permitted by WADA (World Anti-Doping Agency) (Wang, Xiong et al. 2020). The pharmacokinetics of higenamine in human volunteers orally consuming Plumula nelumbinis capsules (6×0.34 g per day) or tablets (3×5 mg per day) for one week showed that the higenamine concentration in the urine of most samples exceeded the limit specified in WADA, and the maximum concentration detected

reached 500 ng/mL (Yan et al. 2019). Additionally, an analysis of the urinary higenamine concentration in human subjects who consumed 0.8 g of lotus plumule (equal to 679.6 μg of higenamine) 3 times a day for 3 days showed that the detected value was above the maximum (10.0 ng/mL) permitted by WADA (Yen et al. 2020). Thus, safe assessment of the absorption and metabolism of higenamine and higenamine-rich products *in vivo* is very important. Taken together, to make better use of lotus alkaloids, their toxicity and safe doses in humans should be more fully and thoroughly investigated.

7. Industrial applications

Various parts of lotus are edible and possess many potential health benefits (Paudel and Panth 2015). Benzylisoquinoline alkaloids are one of the main types of compounds in lotus that exert multiple effects, making them key functional factors used in the food and healthcare, medicine, and cosmetic industries, as shown in Fig. 8.

7.1. Food and healthcare industry application

Recently, a range of healthy and functional products, including tea and other beverages, containing lotus alkaloids have been introduced into daily life. There are several varieties of slimming teas on the market that are made from alkaloid-rich lotus leaves and embryo, for example, Mei-Yun-Xiu tea (Le Shu Yuan Health Products Co., Ltd, China), Lian-Xin-Bao-Jian tea and Lian-Xin juice (neferine or nuciferine is considered as the important component) (Chen et al. 2021). As experimentally observed, a lipid-lowering granulated tea (7.12 mg alkaloids/g and 3.17 mg

polyphenols/g) prepared with raw materials containing lotus leaves exerted fat-reducing effects and modulated the intestinal microbiota (Ding, Pu, and Kan 2017). In parallel, a lotus seed tea, produced by roasting and hot water infusion, can effectively enhance protection against UVB exposure, thus demonstrating the potential of adding alkaloids as functional compounds in beverages for skin benefits (Kim and Moon 2015). Furthermore, a complex fruit and vegetable drink named “72 variations cocktail” infused with nuciferine is popular in the Taiwanese market (http://china.makepolo.com/product-detail/100083027238.html). It is claimed to contribute to fat loss but clinical studies are required to fully substantiate any clinical benefit. In addition, the use of lotus as the principal material in weight loss products in the form of capsules and tablets has grown in popularity. Lv-ting capsule (China Health Food Approval number: G20050386) mainly composed of lotus leaf is available in drugstores with claims of a lipid-reducing effect, and nuciferine could account for its potential action.

7.2. Pharmaceutical industry applications

Lotus and its active alkaloids with a range of biopharmaceutical properties have often been utilized in traditional Chinese and Western medicine and as stimulants, pesticides, aphrodisiacs, and narcotics for humans and other species (Chen and Lin 2019; Chen et al. 2021; Funayama and Cordell 2014). Clinically, Xin-Nao-Jing tablets, a traditional Chinese medicine composed of Plumula Nelumbinis and another 14 types of herbs has been used to treat hypertension combined with sleep disorder (Chen et al. 2021). Regarding the application in slimming drugs, Hugan Qingzhi

tablets (whose major ingredients are nuciferine and alisol A 24-acetate), a lipid-lowering and inflammation-inhibiting pharmaceutical formulation (Tang et al. 2015), have been employed to prevent and treat fatty liver by shifting the gut microbiota profile (Tang et al. 2018). Another traditional Chinese medicine lipid-lowering tea (national medicine permission number: Z20026881) named HeYe TiaoZhi Cha (1.5g per sachet) which is composed of lotus leaf, Plantain, and Senna also benefits hyperlipidemia and obesity subjects (https://www.zhzyw.com), of which nuciferine (0.3589~0.4022 mg/g) may act as the critical agent (Jian et al. 2012). Moreover, higenamine capsules (Swft Stims®) containing 30 mg of higenamine HCl (without any other commonly recognized medications) are prevalently used by competitive athletes (Talay Pinar, Yardim, and Senturk 2020). Notably, 2-hydroxy-1-methoxyaporphin, identified as an alkaloid in lotus, is employed as an ingredient in the traditional Chinese medicine Zhi-Jiang-Ning, which is responsible for the major pharmacodynamic action (Chen, Rao, and Wen 2012). Additionally, nuciferine, which has a receptor profile similar to that of aripiprazole-like antipsychotics, such as antagonists of 5-HT2A, 5-HT2B, and 5-HT2C, shows potential as an antipsychotic drug (Farrell et al. 2016). Nuciferine also has the potential to overcome resistance to several drugs (doxorubicin, paclitaxel, daunorubicin, and docetaxel) in cancer cell therapy, thereby improving chemotherapy efficacy (Liu, Xu et al. 2020).

7.3. Cosmetic industry applications

Different parts of lotus are claimed to act as anti-wrinkling agents and are thus extensively used in cosmetic anti-ageing products (Sable et al. 2013). Multiple lines

of compelling evidence support the antioxidant and free radical-scavenging effects of lotus alkaloids. Moreover, lotus alkaloids, especially neferine, is capable of preventing UV-induced oxidative damage, making it a viable agent for the prevention of skin damage and photoaging (Bai et al. 2018; Khan et al. 2017). In this context, a neferine moisture base cream composed of 0.1% neferine, 58.5% ethanol, 20.0% propanediol, 20% water, and 1.5% hydroxypropyl methyl cellulose with properties of moisturizing and preventing collagen loss can effectively mitigate UV-induced skin photoaging and photodamage (Khan et al. 2020). Importantly, the antibacterial and anti-inflammatory effects of alkaloids in lotus indicate possible applications in oral health. It is generally accepted that liensinine is functional chemical found in the leaves, flowers, and embryo that can be used to treat periodontitis, similar to metronidazole drugs (Pang et al. 2015). Accordingly, a toothpaste has been formulated based on lotus leaves and flowers (https://item.jd.com/1215918.html). Alkaloid-rich lotus extracts also function as an inhibitor of sebum production (Koch et al. 2019), and it can therefore be used in the production of skincare and haircare products. On the market, lotus plumules and flowers have been used in shower gels for the control of casual sebum secretions and toxin removal from the skin (https://item.jd.com/10028670631133.html). They are also used as an important raw material in facial cleansers and face masks for improving acne skin problems (https://www.inoherb.com/). In summary, these multiple potential effects of alkaloids in lotus make different parts of lotus useful raw materials for cosmetic products.

8. Conclusion and perspectives

Currently, there are 154 lotus cultivars being investigated, of which 123 are flower-producing cultivars, 21 are seed-producing cultivars and 10 are rhizome-producing cultivars (Limwachiranon et al. 2018). These edible parts have been well recognized as appetising foods and beneficial traditional herbal remedies against a variety of illnesses, which are largely conferred by the presence of benzylisoquinoline alkaloids. In-depth research of biosynthesis routes of lotus alkaloid has shown several chemical processes involving alkaloid transformations, including glycosylation, acylation, reduction, oxidation and methylation pathways. The available draft genome of sacred lotus has facilitated the initial isolation of biosynthesis genes and enzymes of benzylisoquinoline alkaloids. Nevertheless, a comprehensive biochemical and physiological evaluation of potential genes and enzymes is required for the conclusive identification of biosynthetic pathways. Using immunoblot and quantitative PCR assays can provide helpful information on the accurate localisation of biosynthetic enzymes and corresponding gene transcripts. Alkaloids, especially nuciferine, neferine, liensinine, and isoliensinine, have risen to prominence as a result of increasing pharmacological data indicating their contribution to reducing health risks, such as cardiovascular disease. The cardioprotective actions of lotus alkaloids have been demonstrated to be mediated by multiple mechanisms and pathways. However, other effects on membrane receptors, especially α-adrenoceptors, epidermal growth factor receptor (EGFR), farnesoid X receptor (FXR), and G protein-coupled bile acid receptor (TGR5), should be emphasized among the vasodilatory and antiarrhythmic activities of lotus alkaloids. The most important use of nuciferine and neferine has

been the treatment of Alzheimer's disease. These alkaloids not only display remarkable inhibition of BACE1 and BChE activity but also stimulate GABA and serotonin receptors and increase the release of neurotransmitters, thus impacting neuronal function. However, despite the effectiveness of lotus alkaloids in Alzheimer's disease therapy, the potential mechanisms are still not conclusively and comprehensively understood. For example, when the aqueous extract of lotus flowers was orally administered to rats for 28 days, no evidence of neurobehavioural alterations was observed in male rats, while female rats showed a dose-dependent response in the open field test, implying sex- and dose-related psychotropic effects of alkaloids (Kameni Poumeni et al. 2017). Moreover, in *in vitro* and *in vivo* studies, the anticancer effects of lotus alkaloids are closely associated with their effects on apoptosis, DNA fragmentation, Ca^{2+} accumulation, mitochondrial membrane potential, oxidative stress and the antioxidant pool of tumour/cancer cells. Their synergetic action with other ingredients remains an area of potential interest and importance. Notably, the cotreatment of alkaloid derivatives of lotus with antineoplastic agents, including adriamycin, doxorubicin, cisplatin, vincristine, etc., may potentiate the pharmaceutical efficacy of the drugs and prevent chemoresistance in cancer cells. For example, when the anticancer drugs taxol, oxaliplatin, and doxorubicin were combined with neferine, their IC_{50} values were significantly reduced (Marthandam Asokan et al. 2018). However, the effects of isoliensinine, isoliensinine, liensinine, neferine, and nuciferine as therapeutic and preventative chemical candidates on

cancers such as bladder, oesophageal, colon, thyroid, and brain cancers have not been investigated and therefore further research is needed.

Although there has been an increase in studies on lotus alkaloids, it is still important to highlight that some issues must be addressed immediately. First, most alkaloids in lotus possess poor water solubility, impacting their absorption rate in the body and, as a result, their therapeutic effectiveness. The use of nanodelivery systems may be an effective strategy to increase their bioavailability. Second, the activities of many enzymes and transporters can be altered by pathological conditions, which can in turn influence drug pharmacokinetics *in vivo*. Therefore, the pharmacokinetic abilities of alkaloids in lotus in diverse disease models should be a focus of future research. Third, comprehensive evaluation of the toxicity of these alkaloids in multiple animal models (mice, rats, rabbits, and monkeys) and undesirable effects in humans need to be undertaken to better understand their pharmacological activity and the safety approval of therapeutic applications. Fourth, further research on the activity of additional alkaloid chemicals in this family is also needed, as significant amounts of these chemicals have been isolated but not biologically evaluated. Identifying novel alkaloids in lotus and dissecting the molecular mechanisms of their pharmacological effects should certainly be prioritized in future research.

There are numerous configurations and conformations of bisbenzylisoquinoline alkaloids, and their structure-activity relationships should be investigated to clarify the pharmacological effects of their mechanisms of action and ensure the stability and safety of their clinical use. These pioneer molecules can be chemically modified to

produce pharmacologically selective and therapeutically powerful medications in the future. Additionally, lotus alkaloids have been developed in the food, health, and cosmetics industries, and a detailed exploitation of these promising active components with safe and efficient application will undoubtedly be the focus of future research.

Declarations of competing interest

The authors declare no conflicts of interest

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Table 1. The different contents of alkaloids in lotus from distinctive origin, geographical distribution and developmental stages.

Lotus plant parts	Origin	Treatment of Samples	Contents	Unit	References
Seeds	Muan, Korea	Dry lotus was extracted by 70% ethanol and partitioned by hexane	24.24 (white lotus)	mg/kg	Zhao et al. (2014)
	Ho Chi Minh City, Vietnam		51.64 (red lotus)	mg/kg	
	Lamphun, Thailand		23.20 (red lotus)	mg/kg	
	Guangzhou, China		45.48 (red lotus)	mg/kg	
	Hubei, China		49.29 (red lotus)	mg/kg	
Hull of lotus seed	Zhuhai, China	Dry sample was extracted by microwave (200 W) with 60% methanol	Isoliensinine (0.08), Nuciferine (0.02)	mg/kg	Xiong et al. (2016)
	Macao, China		Isoliensinine (0.10), Nuciferine (0.02)	mg/g	
Embryo (Plumula Nelumbinis)	Hunanxiangtan, China	Dry sample was extracted by 80% EtOH and then isolated by D001 cation exchange resin chromatography	232.05	μg/mg	Tian et al. (2018b)
	Jiangxiguangchang, China		189.66	μg/mg	
	Jiangxishicheng, China		295.66	μg/mg	
	Shandongweihu, China		177.31	μg/mg	

Shandongheze, China		286.98	μg/mg	
Zhejianghangzhou, China		463.49	μg/mg	
Fujiannanping, China		175.04	μg/mg	
Shanxiweinan, China		126.32	μg/mg	
Shanxiweinan, China		119.84	μg/mg	
Anhuibozhou, China		222.75	μg/mg	
Yunnanwenshan, China		541.54	μg/mg	
Zhejiangwuyi, China		128.48	μg/mg	
Fujianfuzhou, China		128.48	μg/mg	
Hunan, China	Dry sample was extracted by ultrasound (220w) with 65% ethanol	65.3	%	Liu et al. (2019)
Jiangxi, China		66.7	%	
Hubei, China		66.2	%	
Fujian, China	Dry sample was extracted with methanol by sonicating	3022.19	mg/g	Deng et al. (2016)

Yunnanwenshan, China	Dry sample was extracted with 80% EtOH loaded onto the pretreated D001 strongly acidic cation exchange resin	541.54	μg/g	Tian et al. (2018a)
Zhejianghangzhou, China		463.49	μg/g	
Jiangxishicheng, China		295.66	μg/g	
Shandongheze, China		286.98	μg/g	
Fujianfuzhou, China		235.36	μg/g	
Hunanxiangtan, China		232.05	μg/g	
Anhuibozhou, China		119.84	μg/g	
Shanxiweinan, China		126.32	μg/g	
Zhejiangwuyi, China		128.48	μg/g	
Zhejiang, China	Dry sample was extracted by microwave (200 W) with 60% methanol	Liensinine (1.38~1.72), Isoliensinine (1.81~2.02), Neferine (4.68~4.93), Nuciferine (0.22)	mg/g	Xiong et al. (2016)
Zhuhai, China		Liensinine (0.36), Isoliensinine(4.36), Neferine(11.70), Nuciferine (0.02)	mg/g	
Hubei, China		Liensinine (1.70~2.12), Isoliensinine (1.96~2.95), Neferine (5.23~6.20), Nuciferine (0.11)	mg/g	
Shandong, China	Dry sample was extracted with methanol and	Lotusine (0.423), Liensinine (0.151), Isoliensinine (0.246), Neferine (0.694)	%	Yang et al. (2012)

	Jiangsu, China	reflux	Lotusine (0.406), Liensinine (0.226), Isoliensinine (0.224), Neferine (0. 0.56)	%	
Leaf (Folia nelumbinis)	Jiande, Zhejiang	Dry sample was extracted with methanol by sonicating	318.45	μg/g	Fan et al. (2013)
	Hangzhou, China	Dry sample was extracted ionic liquids based microwave-assisted method	*N*-nornuciferine (0.677~0.973), *O*-nornuciferine (1.590~1.701), Nuciferine (4.763~ 5.945)	mg/g	Ma et al. (2010)
	Wuxi, China	Dry sample was extracted with methanol by sonicating	Stage 1: 945.90, Stage 2: 1158.93, Stage 3:1244.56, Stage 4: 1466.55 Stage 1-4: *N*-nornuciferine (127.65~324.21), Stage 1-4: *O*-nornuciferine (5.60~38.90); Stage 1-4: anonanine (25.92~44.31), Stage 1-4: nuciferine (698.52~1049.47), Stage 1-4: roemerine (945.90~1446.55)	μg/g	Liu, Shi et al. (2021)
	Wuhan, China	Dry sample was extracted with methanol by sonicating	*N*-nornuciferine (15.00), *O*-nornuciferine (2.13), Anonaine (2.50), Nuciferine (27.09); Roemerine (7.43)	mg/mL	Chen, Zhang et al. (2013)
	Fujian, China	Dry sample was extracted with methanol by sonicating	10281.86	μg/g	Deng et al. (2016)
	Wisconsin, American	Raw sample was extracted with methanol	Non-phenolic base fraction: nuciferine (0.9466) and *N*-nornuciferine (0.0134); Phenolic base fraction: N-norarmepavine (0.0554) and armepavine (0.0046).	%	Kupchan et al. (1963)

	Jiande, zhejiang (China)	Dry sample was extracted with supercritical CO_2 fluid	318.45	μg/g	Wang et al. (2011)
	“Ruixue” Cultivar, China	Dry sample was extracted with methanol by sonicating	*N*-nornuciferine (15.00), *O*-nornuciferine(2.13), Anonaine (2.50), Nuciferine (27.09), Roemerine (7.43)	mg/mL	Chen, Zhang et al. (2013)
	Hubei, China	Dry sample was extracted by methanol in an ultrasonic bath	11.33~21.20	%	Xiao et al. (2010)
		Dry sample was extracted by supercritical fluid	20.86~49.31	%	
	Hunan, China	Dry sample was extracted by 0.1% HCl and loaded onto a D001 resin column for enrichment	Nuciferine (27.76), *N*-nornuciferine (13.23), 2-hydroxy-1-methoxyaporphine (4.23)	%	Yan et al. (2015)
Rhizome/Root	Fujian, China	Dry sample was extracted with methanol by sonicating	14.62	μg/g	Deng et al. (2016)
	Siheung, Korea (red)	Raw rhizome was extracted by 70% ethanol and partitioned by hexane	4.46 (Dauricine (2.87), Neferine (1.59));	mg/g	Zhao et al. (2014)
	Daegu, Korea (red)		5.60 (Nuciferine (0.25), Dauricine (1.64), Isoliensinine (1.49), Neferine (2.21))	mg/g	

	Haman, Korea (red)		4.44 (Dauricine (1.37), Neferine (3.07))	mg/g	
	Muan, Korea (white)		1.33 (Dauricine (1.33))	mg/g	
	Nigata, Japan (white)		9.78 (nuciferine (0.26), Dauricine (5.43), Neferine (4.09))	mg/g	
	Siheung, Korea (red)	Dry rhizome was extracted by 70% ethanol and partitioned by hexane	4.91 (nuciferine (0.15), Dauricine (2.60), Neferine (2.16))	mg/g	Zhao et al. (2014)
	Daegu, Korea (red)		6.57 (nuciferine (0.22), Dauricine (2.13), Isoliensinine (1.53), Neferine (2.69))	mg/g	
	Haman, Korea (red)		4.80 (Dauricine (2.36), Neferine (2.54))	mg/g	
	Guangxi, China (red)		7.43 (nuciferine (0.48), Dauricine (1.77), Isoliensinine (1.40), Neferine (3.78))	mg/g	
	Ho Chi Minh City, Vietnam (red)		14.48 (nuciferine (0.29), Dauricine (2.37), Isoliensinine (4.69), Neferine (7.13))	mg/g	
	Muan, Korea (white)		3.36 (Dauricine (1.44), Neferine (1.93))	mg/g	
	Nigata, Japan (white)		9.50 (nuciferine (0.27), Dauricine (6.00), Neferine (3.23))	mg/g	
Flowers	Nakhon Ratchasima, Thailand (the whole)	Dry sample was extracted with methanol and reflux	14.96	mg/g	Morikawa et al. (2016)
		Dry sample was extracted with 50% methanol	11.02	mg/g	

	and reflux				
	Dry sample was extracted with H_2O and reflux	5.66		mg/g	
	Dry sample was extracted with methanol combined with sonication	8.07		mg/g	
	Dry sample was extracted with 50% methanol combined with sonication	10.92		mg/g	
	Dry sample was extracted with H_2O combined with sonication	3.91		mg/g	
Taiwan (the whole)	Dry sample was extracted two times with methanol under reflux for 120 min	3.53		mg/g	Morikawa et al. (2016)
Zhuhai, China (the whole)	Dry sample was extracted by microwave (200 W) with 60% methanol	Liensinine (0.19), Isoliensinine (0.14), Neferine (0.39), Nuciferine (0.41)		mg/g	Xiong et al. (2016)
Macao, China (the whole)		Liensinine (0.41), Isoliensinine (0.32), Neferine (0.38), Nuciferine (0.61)		mg/g	
Fujian, China (Petal)	Dry sample was extracted with methanol by sonicating	538.67		μg/g	Deng et al. (2016)

Tamil Nadu, India (Petal)	Dry petals were extracted in 70% ethanol by the continuous hot extraction method at 50 °C	4.55		%	Rv and Shoba (2015)
Taiwan (Petal)	Dry sample was extracted two times with methanol under reflux for 120 min	5.76		mg/g	Morikawa et al. (2016)
Nakhon Ratchasima, Thailand (Petal)		17.04		mg/g	

Table 2. The pharmacokinetics and bioavailability of alkaloids from lotus after oral or/and intravenous administration.

Source	Subjects	Dose and Design	Sampling and Units	C_{max} (μM)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (ng/mL*h)	Excretion (%)	Bioavailability (%)	References
Lotus leaf Extract	Sprague Dawley rats	Oral administration of 2.4 g/kg	Blood was collected at 0, 0.083, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 24 h	317.58 (Nunciferine)	3.50 (Nunciferine)	6.18 (Nunciferine)	2142 (Nunciferine)	ND	ND	Zou et al. (2019)
				42.08 (O-nornuciferin)	2.60 (O-nornuciferin)	6.67 (O-nornuciferin)	335.6 (O-nornuciferin)	ND	ND	
				54.69 (Liriodenine)	5.70 (Liriodenine)	3.77 (Liriodenine)	429.0 (Liriodenine)	ND	ND	
				8.78 (Armepavine)	0.30 (Armepavine)	5.22 (Armepavine)	53.79 (Armepavine)	ND	ND	
				210 (Pronuciferine)	4.90 (Pronuciferine)	4.44 (Pronuciferine)	2096 (Pronuciferine)	ND	ND	
Nunciferine	Sprague Dawley rats	Oral administration of 50 mg/kg	Blood was collected at 0, 2, 5, 15, 30, 1, 2, 4, 6, 10, 12 and 24 h	1.71	0.90	2.48	6.13	ND	58.13	Ye et al. (2018)
		Intravenous administration of 10 mg/kg	Blood was collected at 0.08, 0.17, 0.25, 0.50, 1, 2, 4, 6, 8, 12, and 16 h	ND	0.00	2.09	2.09	ND	ND	

		Intravenous administration of 20 mg/kg	Brain dialysates were collected every 20 min time interval for 2 h and then every 30 min time interval for 4 h	0.32	0.89	1.24	0.78	ND	ND	
	Sprague Dawley rats	Oral administration of 10.0 mg/kg	Blood was collected from the ocular vein at 0, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h	45.3	ND	6.5	308.2	Urine:50.7; Feces:32..9	1.9	Wang et al. (2018)
		Intravenous administration of 0.2 mg/kg nuciferine	Blood was collected from the ocular vein at 0, 2 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 10 h, 12 h and 24 h	ND	ND	6.6	2026.3	ND	1.9	
N-Nunciferine	Sprague Dawley rats	Oral administration of 50 mg/kg	Plasma was collected at 0, 2, 5, 15, 30, 1, 2, 4, 6, 10, 12 and 24 h	0.57	1.65	2.94	3.32	ND	79.91	Ye et al. (2018)

		Intravenous administration of 10 mg/kg	Plasma was collected at 0.08, 0.17,0.25, 0.50, 1, 2, 4, 6, 8, 12, and 16 h	ND	0.00	3.84	0.74	ND	ND	
		Intravenous administration of 20 mg/kg	Brain dialysates were collected every 20 min time interval for 2 h and then every 30 min time interval for 4 h	0.16	1.22	1.39	0.46	ND	ND	
Liensinine	Sprague Dawley rats	Intravenous administration of 5.0 mg/kg	The blood was collected from tail vein at time-points of 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h,	ND	ND	9.81	1369.09	ND	ND	Hu et al. (2015)
Isoliensinine				ND	ND	7.88	1925.227	ND	ND	
Neferine				ND	ND	5.49	3755.40	ND	ND	

Liensinine	Sprague Dawley rats	Oral administration of 5, 12.5, and 25 mg/kg	Blood sampling was performed at the intervals of 0.00, 0.08, 0.25, 0.5, 1, 3, 6, 9, 12, and 24 h	6.70 (dose 5.0 mg/kg); 33.66 (dose 12.5 mg/kg); 154.74 (dose 255 mg/kg)	ND	12.72 (dose 5.0 mg/kg); 11.05 (dose 12.5 mg/kg); 11.08 (dose 255 mg/kg)	81.92 (dose 5.0 mg/kg); 279.25 (dose 12.5 mg/kg); 881.90 (dose 255 mg/kg)	ND	ND	Wei et al. (2021)
		Intragastric administration of 5 mg/kg		6.70	ND	12.72	81.92	ND	ND	
		Intravenous administration of 5 mg/kg		668.4	ND	9.81	1369.09	ND	ND	
Liensinine	ICR mice	Oral administration of 5 mg/kg	The blood samples were withdrawn from caudal vein after dosing at	5.3	ND	1.9	19.1	1.8	ND	Tong et al. (2021)

		Intravenous administration of 1 mg/kg	0.083, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 h,	169.5	ND	3.8	227.9	ND	ND	
	Sprague-Dawley (SD) rats	Intravenous administration of 5 mg/kg	Blood samples were collected from the tail vein at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h	668.4	ND	8.2	1802.9	ND	ND	Lv et al. (2015)
Neferine	Sprague-Dawley rats	Oral administration of 10 mg/kg	Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h	440.25	0.63	4.87	1976.92	ND	40.26	Hu et al. (2021)
		Intravenous administration of 5 mg/kg		ND	ND	6.06	2455.15	ND	ND	

Higenamine	Rabbit	10, 20, and 30mg/kg	Blood samples were withdrawn from ear vein after 1, 3, 5, 7, 9, 12, 15, 20, 30, 40, 60, 80, 100, 120, 121, 123, 125, 127, 129, 132, 135, 140, 150, 165, 180, and 210 min	ND	ND	20.18 (dose 10mg/kg); 23.73 (dose 20mg/kg); 23.03 (dose 30mg/kg)	79.05* (dose 10.0 mg/kg); 128.05* (dose 20.0 mg/kg); 239.28* (dose 30.0 mg/kg)	2.84	21.86	Lo and Chen (1996)
	Dog	Higenamine was added into plasma at 50.0, 500.0, 5000.0 ng/mL	Venous blood was collected at time-points of 1, 3, 5, 7, 9, 12, 15, 20, 30, 45, 60, 90, 120 and 150 min	ND	ND	8.60	$AUC_{(0–30min)}$= 0.076	ND	ND	Zheng et al. (2004)
	Healthy Chinese	Intravenous administration of 22.5 μg/kg	Blood was taken at 3, 6, 9, 12, 13, 15, 17, 19, 21, 24, 27, 32, 42, 72, and 102 min	31.3	ND	0.133	AUC_{last}:5.31	9.3	ND	Feng et al. (2012)

AUC, total area under the plasma concentration-time curve; C_{max}, peak concentration; ND, no detection; T_{max}, peak time; $T_{1/2}$, half-life time; *, μg·min mL^{-1}

Table 3. Health-promoting effects of alkaloids from lotus *in vitro, ex vivo* and *in vivo*.

Alkaloids from lotus	Model	Doses	Duration	Effects and mechanisms	References
Antioxidant and anti-oxidative stress activity					
Isoliensinine				DPPH and ABTS free radical removal capacity↑	Jiang et al. (2018)
N-methylasimilobine, lysicamine, and nuciferine				DPPH and ABTS free radical removal capacities↑; ferrous ion chelating↑, reducing power↑	Liu et al. (2014)
Neferine				ROS and MDA ↓; SOD↑	Tang et al. (2019)
	Tetrachloride-induced rat	5 and 10 mg/kg	6 weeks	SOD, GSH-PX, and CAT↑	Wang, Wang et al. (2021)
	Diabetic mice	25 and 50 mg/kg	12 weeks	MDA↓; SOD and GSH-Px↑	Wu et al. (2020)
	CoCl2-induced muscle cells	700 nM	24 h	SOD, CAT, GSH-Px, and GST↑	

Alkaloid from *Nelumbinis Plumula*	*tert*-butyl hydroperoxide-induced HepG2	6.25, 12.5, and 25 μg/mL	24 h	ROS, TBARS, and LDH↓; GSH↑	Xie et al. (2013)
Nuciferine	HFD-fed rat	20 mg/kg	8 weeks	SOD and GSH-Px↑; MDA↓	Cui et al. (2020)
Liensinine	Pathogens suspension were added into the ligation parts of mice	100 and 200 mg/kg	8 weeks	SOD, CAT, and GSH-Px↑	Pang et al. (2015)
Anti-inflammatory activity					
Neferine Liensinine Isoliensinine	LPS-induced RAW264.7 Cells	0.1–10 μM	24 h	LPS-induced iNOS, IL-1β, IL-6 and TNF-α↓	Meng et al. (2019)
	LPS-induced RAW264.7 Cells	10 μM	24 h	LPS-induced inflammatory cytokine IL-6 and TNF-α↓	Zhang et al. (2018b)
Nuciferine	Potassium oxonate-induced hyperuricemic Kun-Ming mice	10, 20 and 40 mg/kg	7 days	TLR4/MyD88/NF-κB signaling↓; NOD-like receptor family↓; NLRP3 inflammasome activation↓	Wang et al. (2015)
	HK-2 cells incubated with uric acid	10, 20 and 40 μM	24 h		

	LPS-induced mice mastitis	10, 15, and 20 mg/kg	18 h	TNF-α and IL-1β↓; NF-κB activation↓	Chen et al. (2018)
Neferine	Carbon tetrachloride-induced rat liver fibrosis	5 and 10 mg/kg	6 weeks	Cox-2, IL-1β, IL-6, and TNF-α ↓	Wang, Wang et al. (2021)
	LPS-induced mice ARDS	20 mg/kg/day	7 days	Lung-capillary permeability↓; IL-1β, IL-6, TNF-α, and IL-10 ↓; and NF-κB signaling pathway↓	Liu et al. (2018)
	Rat chondrocytes	1, 5, and 10 μM	24 h	MAPK and NF-κB signaling pathways↓	Ni et al. (2020)
	Tetrahydropyridine induced Parkinson's disease mouse	20 mg/kg	14 days	TNF-α, IL-1β and IL-6↓	Jing et al. (2021)
	BV2 microglia cells	20 μM	1 h	TNF-α, IL-1β, PGE2 and NO↓; NF-κB activation↓	Zhang, Gao et al. (2018)
	TNF-α/IFN-γ-simulated HaCaT Cells	1, 3, and 10 μM	20 min	Phosphorylation of MAPK and the NF-κB signaling pathway↓	Yang et al. (2021)
	DNCB-induced BALB/c mice	10 mg/kg		Phosphorylation of MAPK and the NF-κB signaling pathway↓; Ransepidermal water loss, erythema, blood flow, and ear thickness↓; surface skin hydration↑	
Liensinine	TNF-α-treated VSMC	1–30 μM	1 h	IL-6 production↓	Jun et al. (2021)

Anti-obesity and anti-diabetic activity					
2-hydroxy-1-methoxyaporphine	3T3-L1 cells	1 and 2μg/ml	2 days	Glucose Consumption↑	Ma et al. (2014)
Pronuciferine		2 mg/L	48 h	GLUT4 and phosphorylation of AMPK↑	Ma et al. (2015)
Neferine	Diabetic db/db mice	25 and 50 mg	12 weeks	Blood glucose and serum lipid↓	Wu et al. (2020)
Nuciferine	3T3-L1 preadipocytes	20 μM	48 h	mRNA levels of PPARγ, C/EBPα, C/EBPβ, FAS, ACC, HSL and ATGL↓	Xu et al. (2021)
		2 mg/L	48 h	GLUT4 and phosphorylation of AMPK↑	Ma et al. (2015)
	INS1-E cells	10 or 20 μM	1 h	Stimulate insulin secretion by closing potassium-adenosine triphosphate channels↑	Nguyen et al. (2012)
	HFD/streptozocin-induced diabetic mice	0.06% or 0.12% (w/w)	13 weeks	Impaired glucose tolerance and insulin resistance↓; PARα/PPARγ coactivator-1α pathway↑	Zhang, Deng et al. (2018)
	Diabetic db/db mice	25 and 50 mg	12 weeks	Blood glucose and serum lipid↓	Wu et al. (2020)
	HFD-fed Sprague-Dawley rats	20 mg/kg	8 weeks	Improving expression of key enzymes related to the glycerophospholipid, linoleic acid, and alpha-linolenic acid metabolism pathways↑	Cui et al. (2020)

	HFD-fed rats	10 mg/kg	8 weeks	*Firmicutes*/*Bacteroidetes*↓; LPS-producing genus *Desulfovibrio*; Relative abundance of SCFA-producing bacteria↑	Wang et al. (2020)
	HFD-fed C57BL/6 J mice	7.5 and 15 mg/kg	6 weeks	*Akkermansia muciniphila*↑	Yu et al. (2021)
Lotus leaf ethanol extract and nuciferine	3T3-L1 cells	25 or 50 μM	8 days	SREBP1, PPARγ, C/EBPα, aP2, and FAS; Akt-mTORC1 Signaling↓	Yoo et al. (2019)
Lotus leaf total alkaloids	HFD-fed rat	20, 40, and 80mg/kg/d	40 days	Blood lipids levels (TG, TC, LDL-C, and HDL-C) ↓	Fan et al. (2013)
Coclaurine, N-norarmepavine, armepavine, asimilobine, *O*-nornuciferine, *N*-nornuciferine, nuciferine	HEK293-D2 cells	100 μM	—	D1 or/and D2 receptors ↓	Zhou et al. (2021)
Hepatoprotective, renoprotective and lung-protective activity					
Nuciferine	HFD-fed rat	20 mg/kg	8 weeks	NAFLD activity score and MDA↓; Liver SOD and GSH-Px ↑	Cui et al. (2020)
	HFD-fed hamsters	10 and 15 mg/kg·	8 weeks	Scored for hepatic steatosis; necroinflammation hepatic mRNA expression involved in lipid	Guo et al. (2013)

			metabolism↓	
Uricase inhibitor potassium oxonate induced hyperuricemia mice	10, 20 and 40 mg/kg	6 day	Renal TLR4/MyD88/NF-κB signaling↓; NLRP3 inflammasome activation↓	Wang et al. (2015)
Folic acid-induced acute kidney injury	7, 14 and 28 mg/kg	6 weeks	TLR4/PI3K/NF-κB signaling and NLRP3 inflammasome activation↓	Li et al. (2021)
Caki-1 cells	0, 5, 10, 15, 20, and 25μM	24 h	Bcl-2 and p65↓	Kim et al. (2019)
Fructose-fed Rats	7, 14, and 28 mg/kg	6 weeks	TLR4/PI3K/NF-κB signaling and NLRP3 inflammasome activation↓	Wang et al. (2016)
Fructose stimulated HK-2 cells	2.5, 5, 10, 20, and 40 μM	24 h		
LPS-stimulated mice with acute lung injury	10 and 20 mg/kg	6, 12, and 18 h	Lung Wet/Dry Ratio and MPO activity↓; TNF-α, IL-6, and IL-1β mRNA levels↓; TLR4 and NF-κB Pathway↓	Wu et al. (2017)

	LPS-induced RAW264.7 cells	10 and 20 μg/mL	24 h	TNF-α, IL-6, and IL-1β mRNA levels↓;TLR4 qnd NF-κB Pathway↓	
Neferine	CCl_4-induced hepatic fibrosis in mice	5, 10, and 20 mg/kg	2 weeks	Hydroxyproline content of liver↓; Plasma ALT/AST levels↓; Expression of TGF-β↓	Chen et al. (2015)
	LPS-ATP induced HUVECs cells	2 μM	2 h	ROS/NLRP3/Caspase-1 signaling pathway↓	Tang et al. (2019)
	Amiodarone-induced pulmonary Fibrosis in Kunming mice	20 mg/kg	21 days	Anti-inflammation properties, SP-D inhibition and restoring CD4+CD25+ Tregs↑; Th2 response↓	Niu et al. (2013)
	Bleomycin-induced RAW264.7 macrophages	1, 3 and 10 μmol/L	24 h	NF-κB and TGF-β1↓	Zhao et al. (2010)
	Bleomycin-induced Kunming mice	20 mg/kg	21 days	Oxidant stress (SOD, MDA and MPO) and inflammation (TNF-α, IL-6 and endothelin-1) ↓	
Antitumor and anti-cancer activity					
Nuciferine	Human breast cancer cells lines, MDA-MB-231 and MCF-7	20, 40, and 60 μM		Migration and invasion↓; Apoptosis or necrosis↑ Bax/Bcl-2 ratio, activation of caspase-3, and subsequent cleavage of PARP↑	Kang et al. (2017)
	B16 melanoma 4A5 cells	10-30 μM	72 h	Tyrosinase and TRP-2 mRNA ↓	Nakamura et al. (2013)

	Nicotine stimulated A549 and H1299	10, 25 and 50 μmol/L	24 h	Bcl/Bax raito↓; Wnt (wingless)/β-catenin signaling↓	Liu et al. (2015)
	GBM cell lines (U87MG and U251)	0-150 μM	48 h	SOX2-AKT/STAT3-Slug signaling pathway↓	Li, Chen et al. (2019)
	BALB/c nude mice	15 mg/kg	2 weeks		
N-methylasimilobine	B16 melanoma 4A5 cells	3–30 μM	72 h	TRP-1 and TRP-2 mRNA↓	Nakamura et al. (2013)
Isoliensinine	MDA-MB-231 cells	0, 20 and 40 μM	48 h	ROS generation and p38 MAPK/JNK activation↑	Zhang et al. (2015)
	HepG2, Huh-7, and H22 hepatocellular carcinoma	3 and 10 μg/ml	24 h	p65 dephosphorylation at Ser536 and NF-κB activity↓	Shu et al. (2015)
	Huh-7 cells were injected into the dorsal flanks of nude mice	3 and 10mg/kg	3 weeks		
	Human HepG2 and Huh-7 HCC cells, HL-7702 untransformed hepatocytes and murine H22 ascitic	10 μg/mL	24 h	Dephosphorylation of NF-κB p65 subunit at Ser536 *via* a PP2A-dependent mechanism↑	Shu et al. (2016)

	hepatoma cells				
	Tumor-bearing nude mice	3 and 10 mg/kg	10 days		
	BGC823 and SGC7901 cells	0, 40, 60, and 80 μM	48 h	ROS generation↑; PI3K/AKT pathway↓	Yang, Yu et al. (2019)
	LNCaP cells	100 μM	24 h	PI3K/AKT signaling pathway↓	Liu, Wu et al. (2021)
Liensinine	LNCaP cells	100 μM	24 h	PI3K/AKT signaling pathway↓	Liu et al. (2021)
	Human breast cancer cells lines, MDA-MB-231 and MCF-7	20, 40, and 60 μM	24 h	Migration and invasion↓; Apoptosis or necrosis↑; Bax/Bcl-2 ratio↑, Activation of caspase-3, and subsequent cleavage of PARP↑	Kang et al. (2017)
Neferine	HepG2 cells	6 and 10 μM	48h	TNF-α, p38 and ERK1/2 MAP kinases↑; Caspase-9, caspase-3 and PARP↑; Bcl2 and P-Akt↓	Poornima, Quency, and Padma (2013)
	Human lung cancer cell line A549	10 and 15 μM		PI3K/Akt/mTOR signaling↓; ROS hyper generation↓	Poornima, Weng, and Padma (2013)

	Diethylnitrosamine-induced lung carcinogenesis in Wistar rats	10, 15, and 20 mg/kg	20 days	PI3K/Akt/NF-κB pathway↓	Sivalingam et al. (2019)
	Neferine induces autophagy of human ovarian cancer cells via p38 MAPK/ JNK activation	0, 5, and 10 μM	24 h	p38 MAPK/ JNK activation↑	Xu et al. (2016)
	Hepatocellular carcinoma cells	10 μM	48 h	Oxaliplatin-induced apoptosis↑; TGF-β1-promoted migration and invasion abilities↓	Deng et al. (2017)
	xenograft nude mice	20 mg/kg	3 weeks	Oxaliplatin sensitivity *via* EMT inhibition↑	
	LNCaP cells	100 μM	24 h	PI3K/AKT signaling pathway↓	Liu, Wu et al. (2021)
7-Hydroxydehydronuciferine	Mice translated into A375.S2 cells	20 mg/kg	28 days	Diameters and volume of xenograft tumor↓	
	A375.S2 cells	0, 10, 25, 50 and 100 μM	24 h	Autophagic-related proteins (beclin-1 (ATG-6), ATG-3, ATG-12, ATG-7, ATG-10, ATG-16 and ATG-5) ↑; Apaf-1, caspase-9, caspase-7, caspase-6, caspase-3, PARP and Bax↑	Wu et al. (2015)
Cardiovascular protective activity					
Alkaloids from *Nelumbinis Plumula*	Rat aortic VSMC A7R5	125, 250, and 500 ng/mL	24 h	RhoA/ROCK signaling pathway↓	Li et al. (2019)

Alkaloid rich fraction	Vascular smooth muscle cell	10, 50, and 100 μg/ml 1, 5, 10, and 20 μM	24 h	PDGF-BB induced MAPKs and NF-κB activation↓	Jun et al. (2016)
Neferine		0.1−5.0 μmol/L	24 h	Ang II-induced proliferation through HO-1↓	Li et al. (2010)
	RASMC line	5 μmol/L	24 h	Fkn expression and at the AngII-induced RASMC proliferation↓	Zheng et al. (2014)
	H9c2 cells	10 μM	24 h	IGF-1R/Nrf2 signaling↑	Bharathi Priya et al. (2018)
				Activation of NADPH oxidase↑; activation of ERK1/2 and p38↓; NF-κB activation↓	Priya et al. (2017)
	IL-1β-stimulated HUVECs	4, 10 and 30 μM	30 min	Vascular endothelial inflammation ↑; NF-κB signaling pathway↓	Zhong et al (2020)
	Isoproterenol induced albino rats	10 mg/kg	30 days	Lipid Peroxidation↑; Glutathione↓; GSH content and the activity of antioxidant enzymes (GPx, GST, SOD, and CAT) in heart	Lalitha et al. (2013)
Higenamine	Cardiomyocytes were isolated from Sprague-Dawley rats	50 μM	12 h	PI3K/Akt Pathway↑	Chen et al. (2013)
	Neonatal rat ventricular myocytes	60 μM	24 h	Activation of β2-AR/PI3K/AKT signaling pathway↑	Wu et al. (2016)

	Adult mouse ventricular myocytes	100 μM	24 h		
Antiviral activity					
Benzylisoquinoline alkaloids (e.g., coclaurine, norcoclaurine, nuciferine, liensinine. negferine, isoliensinine)	T cell line, H9 infected with HIV-1	100, 20, 4, and 0.8 μg/mL	4 days	P24 antigen↓	Kashiwada et al. (2005)
Neuromodulatory properties					
Total alkaloids extracted from lotus leaf (4.23% of 2-hydroxy-1-methoxyaporphine; 13.23% of *N*-nornuciferine and 27.76% of nuciferine)	Adult male ICR mice	10, 20, and 40 mg/kg	30 min	Binding to the GABAA receptor and activating the monoaminergic system↑	Yan et al. (2015)
Liensinine Isoliensinine	ICR mice	10, 25, and 50 mg/kg	30 min	5-HT_{1A} receptor↑; immobility time↓	Sugimoto et al. (2015)

Neferine	Diabetic db/db mice	25 and 50 mg/day	10 weeks	NLRP3 inflammasome pathway ↓; endoplasmic-reticulum stress↓	Wu et al. (2020)
	Male ICR mice	20 mg/kg	14 days	5-HT_{1A} receptor↑; immobility time↓	Sugimoto et al. (2010)
	Synaptosomes from Sprague Dawley rats	30 μM	10 min	Calcium influx and glutamate release ↓; Activation of Gi/o protein and the inhibition of adenylyl cyclase/cAMP/protein kinase A cascade↑; Activation of 5-HT_{1A} receptor↑	Yeh et al. (2020)
	Parkinson-induced C57BL/6 mice	20 mg/kg	14 days	Dopamine and tyrosine hydroxylase protein expression↑; Levels of proinflammatory cytokines TNF-α, IL-1β, IL-6, iNOS and Cox-2↓	Jing et al. (2021)
Aporphine-type alkaloids in lotus flowers	PC-12 cells	1ng/mL	72 h	TrkA, Vav3, and Rac-1 mRNA↑	Yano et al. (2020)
	C6 cells	0~100 μM	15 min or 1h		Ha et al. (2012)
Higenamine	Healthy male rats	10 mg/kg	24 h	PI3K/Akt/Nrf-2 signal pathways↑; HO-1 induction↑	
Intestinal activity					
Lotus leaf powder rich in nuciferine, nornuciferine,	Nile tilapia fingerlings	0.1, 0.2, and 0.4%	60 days	Intestinal villous heights, numbers of goblet cells, and intraepithelial lymphocytes↑	Abdel Rahman, Hassanin, and ElHady (2019)

Roemerine (Peak area:70.74%)					
Nuciferine	HFD-fed Sprague-Dawley rats	10 mg/kg	8 weeks	Ratio of *Firmicutes/Bacteroidetes*↓; Relative abundance of the LPS-producing genus Desulfovibrio and bacteria↓	Wang,Yao et al. (2020)
	HFD-fed C57BL/6 J mice	7.5, 15, and 30 mg/kg	6 weeks	*Akkermansia muciniphila*↑	Yu et al. (2021)
Lotus leaf extract (nuciferine: 0.71 mg/g; total flavonoids: 2.49 mg/g)	Broiler chicks	1.0, 2.5, and 5 g/kg	21 days	Abundance of *Clostridiaceae* and *Bacteroidales* S24-7↑; *Peptostreptococcaceae*↓	Cheng, Zhang et al. (2021)

ABTS: 2,2-azino-bis-(3-ethylbenzodihydrothiazoline-6-sulfonic acid) diammonium salt; ACC: acetyl CoA carboxylase; aP2: adipocyte fatty acid binding protein; AKT: protein-serine- threonine kinase; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; Apaf-1: fapoptotic protease activating factor-1; ATGL: Adipose triglyceride lipase; Bcl-2: B-cell lymphoma-2; CAT:catalase; CCl_4: Carbon tetrachloride; C/EBPα,β:CCAAT/enhancer binding protein α and β; Cox-2, cyclooxygenase-2; DNCB: 2,4-dinitrochlorobenzene; DPPH: DPPH:2,2-Diphenyl-1-picrylhydrazyl; EMT: epithelial-mesenchymal transition; FAS: fatty acid synthase; GSH:glutathione; GSH-PX: glutathione peroxidase; GST: glutathione-S-transferase; HDL-C: high-density lipoprotein cholesterol; HFD: high fat diets; HO-1: heme oxygenase-1; HUVECs: human umbilical vein endothelial cells; HSL: hormone-sensitive lipase; IL:interleukin; iNOS: inducible NOS; JNK: c-Jun N-terminal kinase; LDL-C: low-density lipoprotein cholesterol; LPS: lipopolysaccharide; MDA: malondialdehyde; MAPK, mitogen-activated protein kinase; MPO: myeloperoxidase; MyD88: myeloid differentiation factor 88; NF-κB: nuclear factor kappa-B; NLRP3: pyrin domain containing 3; Nrf 2: nuclear respiratory factor 2; PARP: poly(ADP-ribose) polymerase; PDGF-BB: platelet-derived growth factor; PGE2: prostaglandin E2; PI3K: phosphatidylinositol 3 kinase; PPARγ: peroxisome proliferator-activated receptor γ; PP2A: protein phosphatase 2A; Rac-1: ras-related C3 botulinum toxin substrate 1; SOD: superoxide dismutase; SREBP-1: sterol-regulatory element binding protein-11; TBARS: thiobarbituric acid reactive substance; TC: total cholesterol; TG: triglyceride; TNF: tumor necrosis factor; TLR4: toll-like receptor 4; TrkA: nerve growth factor receptor; TRP: tyrosinase-related protein-2; VSMC: vascular smooth muscle cell

Figure Legends

Fig. 1. Diverse phenotypes of lotus germplasms (Asian) (Deng et al. 2016; Lin, Zhang et al. 2019). Reprinted (adapted) with permission from the Chemical Society and under open access from the MDPI publisher. (A) flower lotus showing various colours and forms of the bloom; (B) seed lotus showing different sizes and shapes of seeds and seedpods; (C) leaves showing different sizes, shapes and colours at different developmental stages; (D) rhizome lotus showing various branching, elongation and expansion patterns of the roots.

Fig. 2. VOSviewer co-occurrence network visualization (A) and Density visualization of most frequent all keywords (minimum of 5 occurrences) of “lotus & alkaloids” research (Web of Science Core Collection) (B).

Fig. 3. The potential biosynthesis route for benzylisoquinoline alkaloids in lotus. This figure was adapted from (Deng et al. 2018; Menéndez-Perdomo and Facchini 2019). CNDM: (S)-coclaurine N-methyltransferase; CYP80A/G: cytochrome P450 monooxygenase 80A/G; CYP719A: cytochrome P450 monooxygenase 719A; NCS: norcoclaurine synthase; NDM: N-demethylase; TYDC: tyrosine/DOPA decarboxylase; ODM: O-demethylase; 7OMT: 7-O-methyltransferase; 4′OMT: 4-O-methyltransferase

Fig. 4. The main alkaloids with their structurally and chemical formula in different parts of lotus. This figure was adapted from (Menéndez-Perdomo et al. 2018). LL: lotus leaf; LE: lotus embryo; LF: lotus flower; LS: lotus seed; LR: lotus rhizome

Fig. 5. The possible anti-cancer mechanisms of lotus alkaloids. 7-HDF: 7-Hydroxydehydronuciferine; AKT: protein kinase B; Bcl-2: B-cell lymphoma-2; ERK**:** extracellular signal-regulated kinase; GSH: glutathione; LPO: lipid peroxidation; MAPK: mitogen-activated protein kinase; NF-κB: nuclear factor-κB; MMP: matrix metalloproteinase; JNK: c-Jun NH 2-terminal kinase; ROS: reactive oxygen species; SOX2: SRY (sex-determining region Y)-box 2; STAT3; signal transducer and activator of transcription-3; Wnt/β-catenin: beta-catenin dependent Wnt signaling

Fig. 6. The possible mechanisms of lotus alkaloids on cardiovascular diseases. AKT: protein kinase B; β2-AR: β2-adrenergic receptor; EGFR: epidermal growth factor receptor; GPCR: G-protein-coupled receptor; HO-1: heme Oxygenase-1; IKB: immunome knowledge base; IL: interleukin; NQO1: NAD(P) H:quinone oxidoreductase 1; JNK: c-Jun NH 2-terminal kinase; Keap1: Kelch-like ECH-associated protein 1; mTOR: mammalian target of rapamycin; NF-kB: nuclear factor kB; Nrf2: nuclear factor E2-related factor 2; PI3K: phosphoinositide 3-kinase; p-RLC: phosphorylated regulatory light chains; RhoGEF12: 145 multidomain guanine nucleotide exchange factor 12;

ROCK: Rho-associated, coiled-coil containing kinases; TGF: transforming growth factor; IGF-1R: type I insulin-like growth factor receptor; TRAF-6: Tumor necrosis factor receptor-associated factor 6; TLR: toll-like receptor

Fig. 7. Proposed mechanisms underlying the neuroprotective effect of lotus alkaloids. AKT: protein kinase B; BNDF: brain neurotrophic derived factor; BIP: heavy chain binding protein; DA: dopamine; eIF2α: α subunit of eukaryotic translation initiation factor 2; $GABA_A$: binding to the γ-amino butyric acid A; $GABA_AR$: binding to the γ-amino butyric acid receptor; HMGB1: High mobility group box 1; JNK: c-Jun NH 2-terminal kinase; LC3B-II: microtubule-associated protein-2 light chain-3; NLRP3:pyrin domain-containing protein 3; NO: nitric oxide; Nrf2: E2-related factor 2; PI3K: phosphoinositide 3-kinase; TrkB: tyrosine kinase receptor B; 5-HT: serotonin, 5-HIAA: 5-hydroxyindoleacetic acid;

Fig. 8. The potential applications of lotus alkaloids in food and healthcare, cosmetic and pharmaceutical industries.

Fig. 1

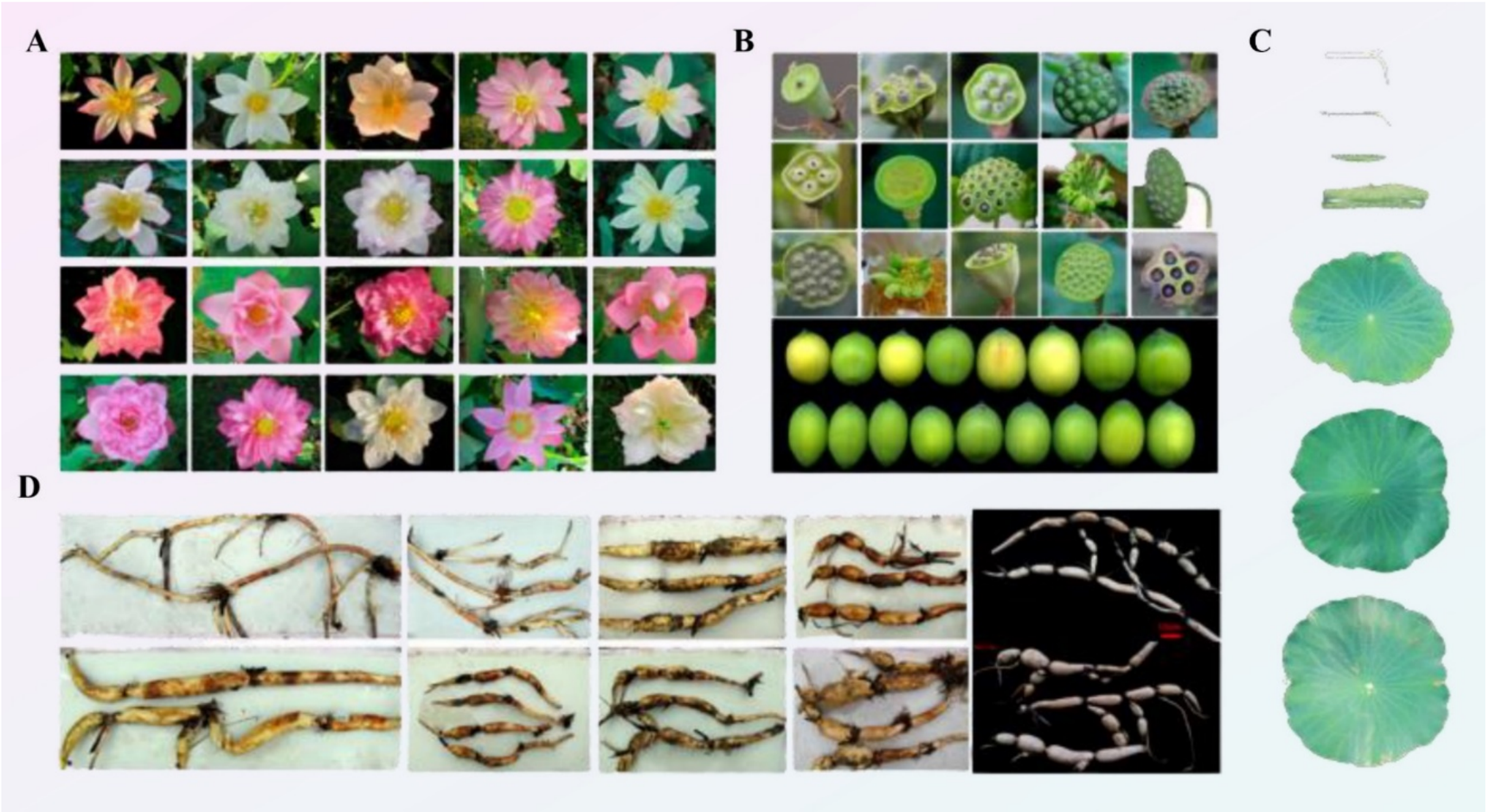
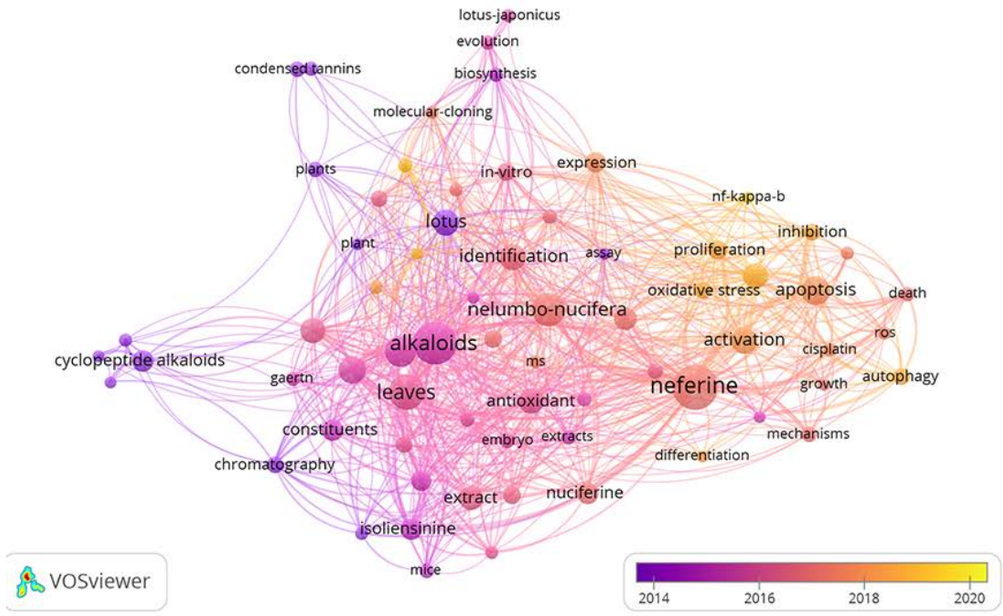


Fig. 2

A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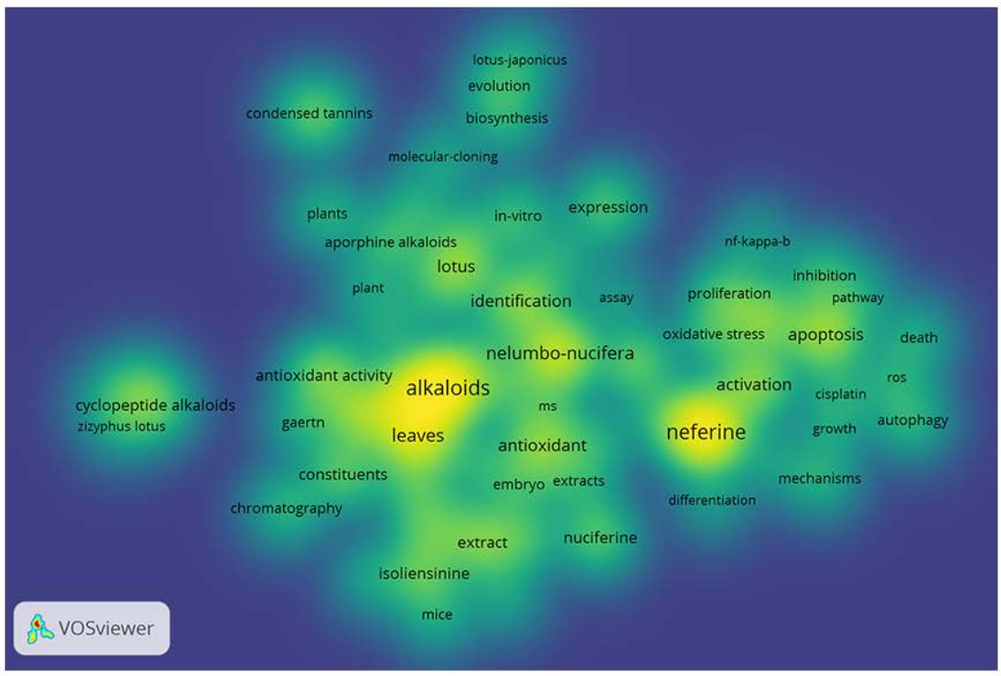
B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cisplatin
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gaertn
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growth
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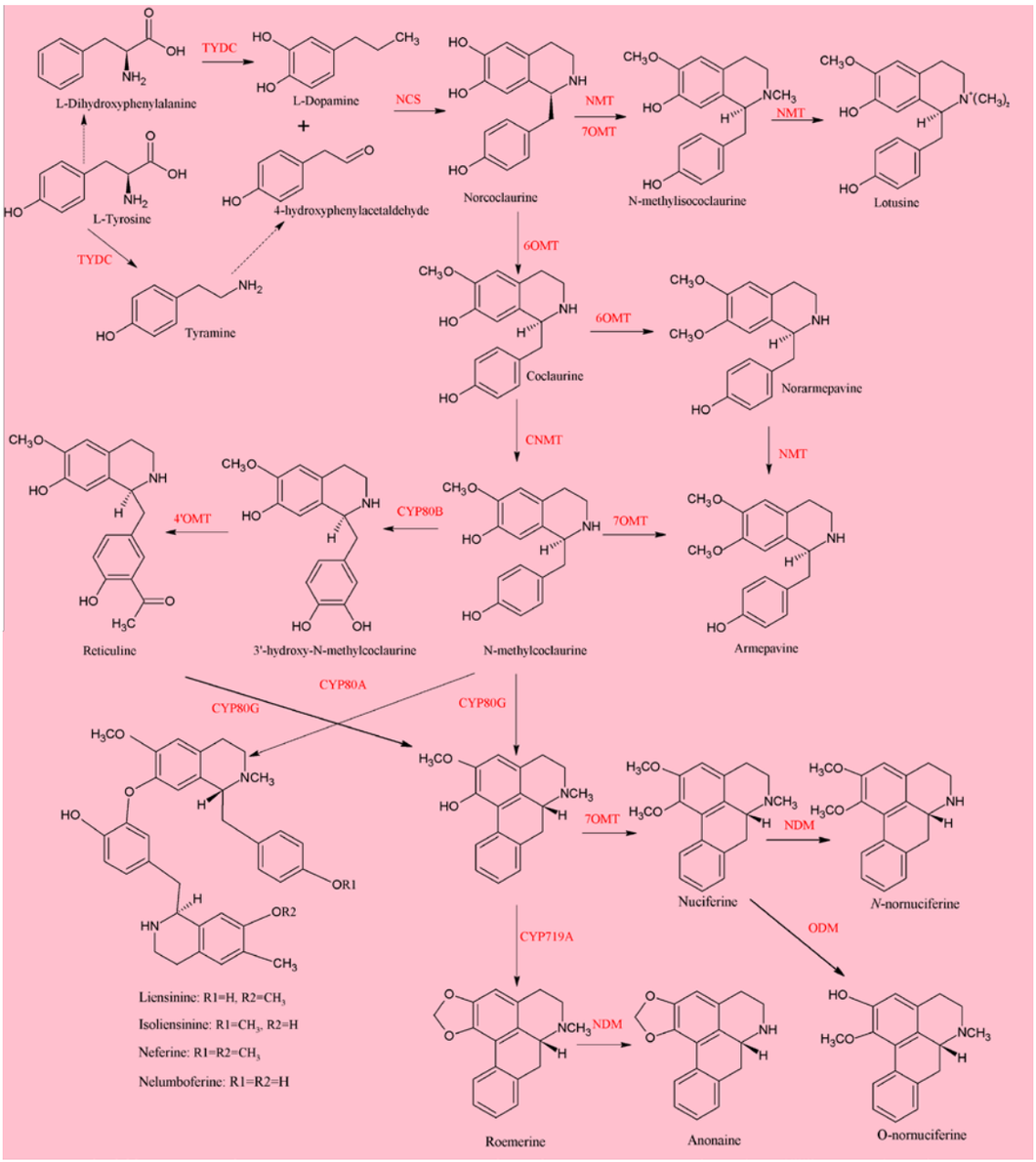
Fig. 3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L-Dopamine
NCS
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NMT
7OMT
N-methylisococlaurine
NMT
Lotusine
L-Tyrosine
4-hydroxyphenylacetaldehyde
TYDC
Tyramine
6OMT
Coclaurine
6OMT
Norarmepavine
CNMT
NMT
4'OMT
CYP80B
7OMT
Reticuline
3'-hydroxy-N-methylcoclaurine
N-methylcoclaurine
Armepavine
CYP80A
CYP80G
CYP80G
7OMT
NDM
Nuciferine
N-nornuciferine
ODM
CYP719A
NDM
Roemerine
Anonaine
O-nornuciferine
Liensinine: R1=H, R2=CH3
Isoliensinine: R1=CH3, R2=H
Neferine: R1=R2=CH3
Nelumboferine: R1=R2=H

Fig. 4

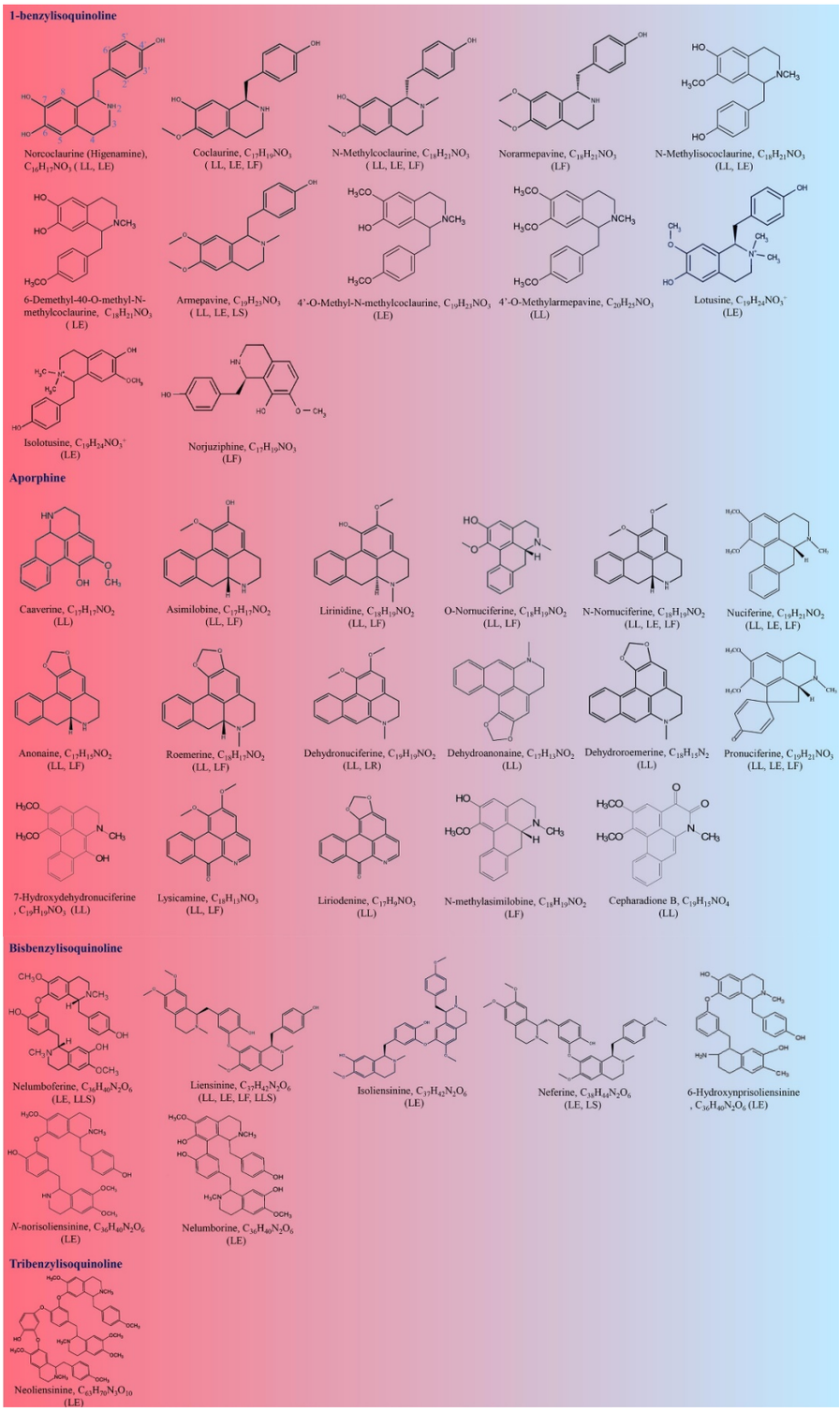
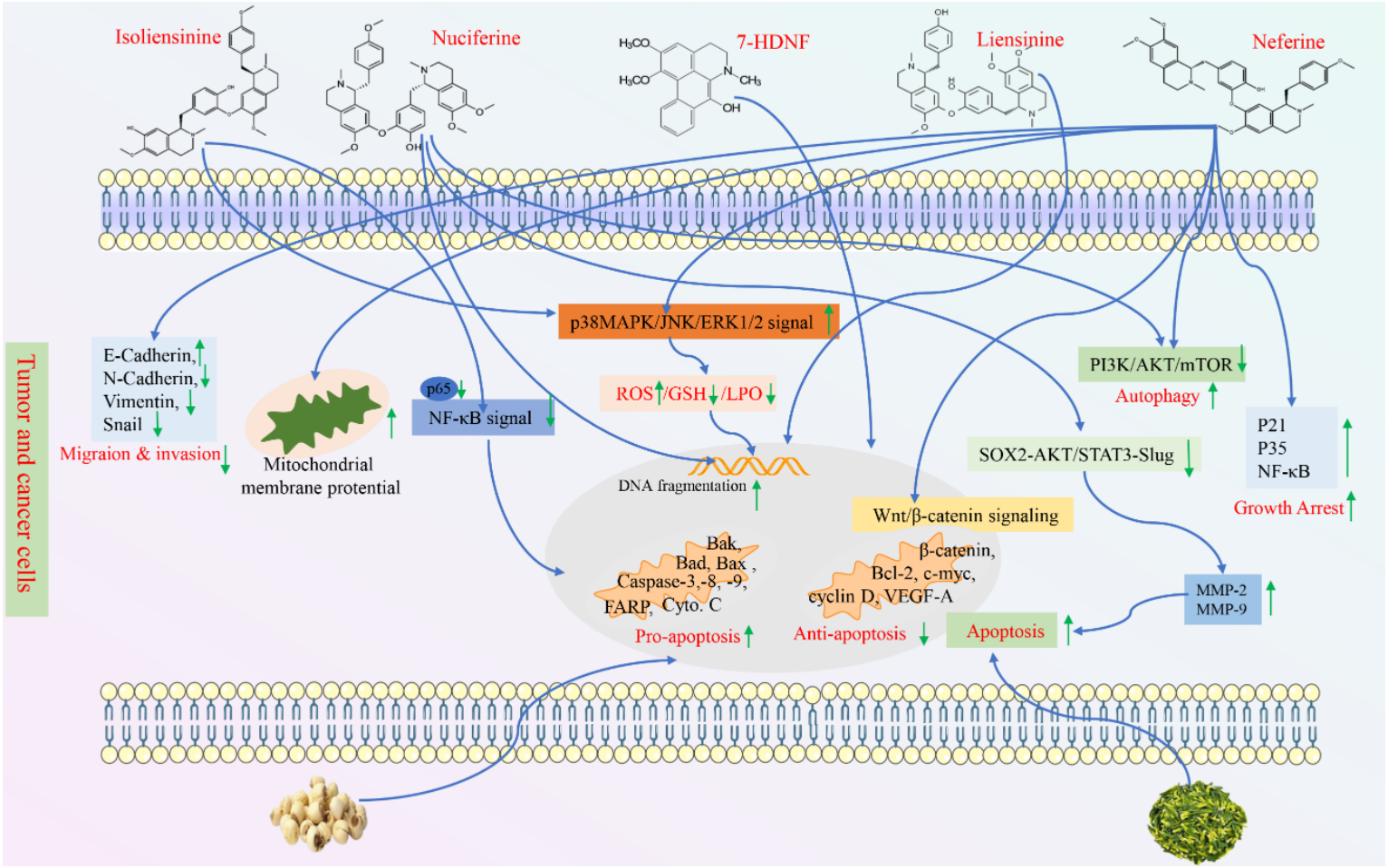

1-benzylisoquinoline
Norcoclaurine (Higenamine), $C_{16}H_{17}NO_3$ (LL, LE)
Coclaurine, $C_{17}H_{19}NO_3$ (LL, LE, LF)
N-Methylcoclaurine, $C_{18}H_{21}NO_3$ (LL, LE, LF)
Norarmepavine, $C_{18}H_{21}NO_3$ (LF)
N-Methylisococlaurine, $C_{18}H_{21}NO_3$ (LL, LE)
6-Demethyl-40-O-methyl-N-methylcoclaurine, $C_{18}H_{21}NO_3$ (LE)
Armepavine, $C_{19}H_{23}NO_3$ (LL, LE, LS)
4'-O-Methyl-N-methylcoclaurine, $C_{19}H_{23}NO_3$ (LE)
4'-O-Methylarmepavine, $C_{20}H_{25}NO_3$ (LL)
Lotusine, $C_{19}H_{24}NO_3^+$ (LE)
Isolotusine, $C_{19}H_{24}NO_3^+$ (LE)
Norjuziphine, $C_{17}H_{19}NO_3$ (LF)
Aporphine
Caaverine, $C_{17}H_{17}NO_2$ (LL)
Asimilobine, $C_{17}H_{17}NO_2$ (LL, LF)
Liriniding, $C_{18}H_{19}NO_2$ (LL, LF)
O-Nornuciferine, $C_{18}H_{19}NO_2$ (LL, LF)
N-Nornuciferine, $C_{18}H_{19}NO_2$ (LL, LE, LF)
Nuciferine, $C_{19}H_{21}NO_2$ (LL, LE, LF)
Anonaine, $C_{17}H_{15}NO_2$ (LL, LF)
Roemerine, $C_{18}H_{17}NO_2$ (LL, LF)
Dehydronuciferine, $C_{19}H_{19}NO_2$ (LL, LR)
Dehydroanonaine, $C_{17}H_{13}NO_2$ (LL)
Dehydroroemerine, $C_{18}H_{15}N_2$ (LL)
Pronuciferine, $C_{19}H_{21}NO_3$ (LL, LE, LF)
7-Hydroxydehydronuciferine, $C_{19}H_{19}NO_3$ (LL)
Lysicamine, $C_{18}H_{13}NO_3$ (LL, LF)
Liriodenine, $C_{17}H_9NO_3$ (LL)
N-methylasimilobine, $C_{18}H_{19}NO_2$ (LF)
Cepharadione B, $C_{19}H_{17}NO_4$ (LL)
Bisbenzylisoquinoline
Nelumboferine, $C_{36}H_{40}N_2O_6$ (LE, LLS)
Liensinine, $C_{37}H_{42}N_2O_6$ (LL, LE, LF, LLS)
Isoliensinine, $C_{37}H_{42}N_2O_6$ (LE)
Neferine, $C_{38}H_{44}N_2O_6$ (LE, LS)
6-Hydroxynprisoliensinine, $C_{36}H_{40}N_2O_6$ (LE)
N-norisoliensinine, $C_{36}H_{40}N_2O_6$ (LE)
Nelumborine, $C_{36}H_{40}N_2O_6$ (LE)
Tribenzylisoquinoline
Neoliensinine, $C_{63}H_{70}N_2O_{10}$ (LE)

Fig. 5


Isoliensinine
Nuciferine
7-HDNF
Liensinine
Neferine
Tumor and cancer cells
E-Cadherin,
N-Cadherin,
Vimentin,
Snail
Migraion & invasion
Mitochondrial membrane protential
p65
NF-κB signal
p38MAPK/JNK/ERK1/2 signal
ROS/GSH/LPO
DNA fragmentation
Bak,
Bad, Bax ,
Caspase-3,-8, -9,
FARP, Cyto. C
Pro-apoptosis
β-catenin,
Bcl-2, c-myc,
cyclin D, VEGF-A
Anti-apoptosis
Wnt/β-catenin signaling
PI3K/AKT/mTOR
Autophagy
SOX2-AKT/STAT3-Slug
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P35
NF-κB
Growth Arrest
MMP-2
MMP-9
Apoptosis

Fig. 6

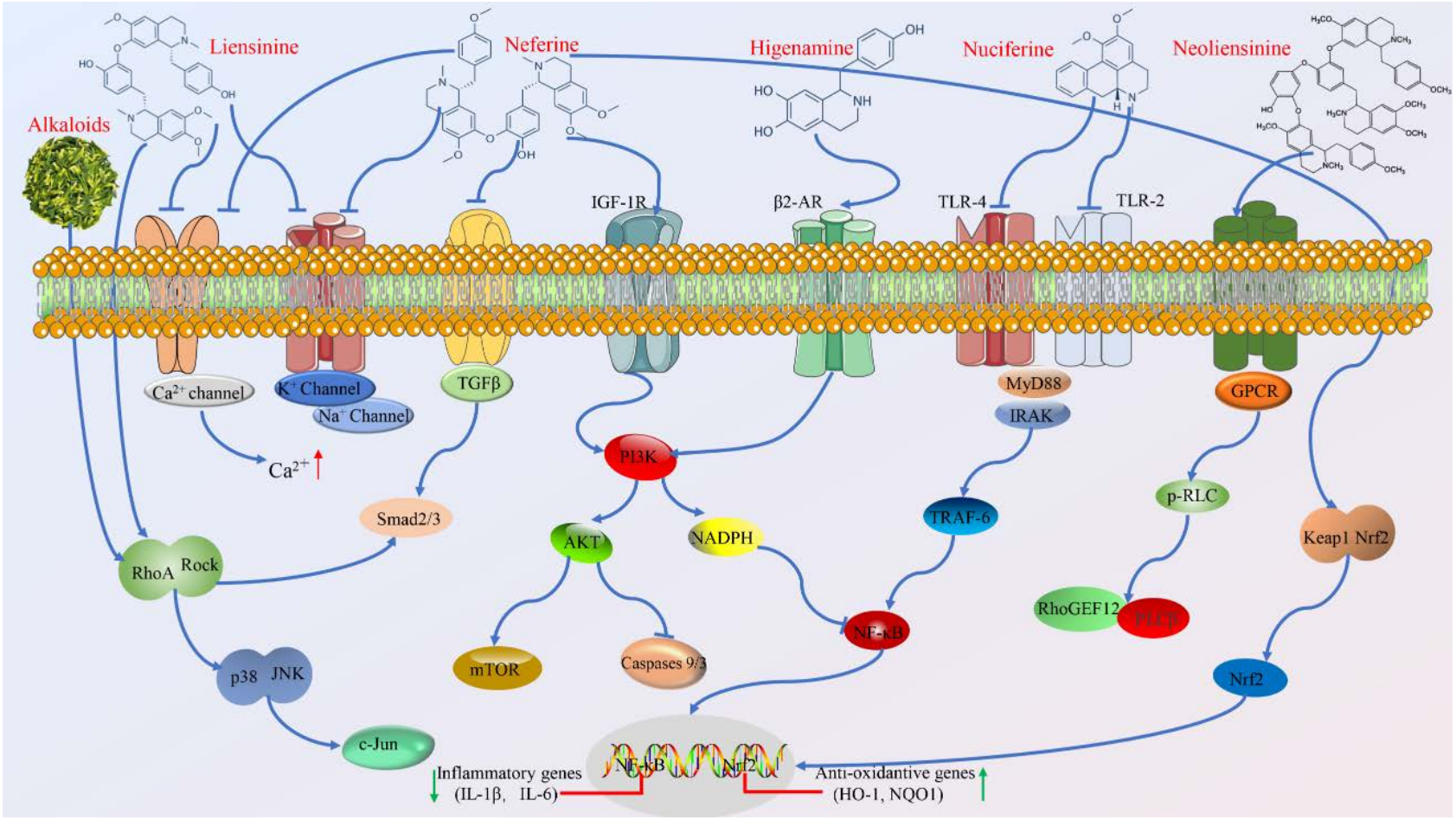
Liensinine
Neferine
Higenamine
Nuciferine
Neoliensinine
Alkaloids
IGF-1R
β2-AR
TLR-4
TLR-2
Ca2+ channel
K+ Channel
Na+ Channel
TGFβ
MyD88
IRAK
GPCR
Ca2+
PI3K
Smad2/3
AKT
NADPH
TRAF-6
p-RLC
Keap1 Nrf2
RhoA
Rock
RhoGEF12
PLCβ
NF-κB
p38
JNK
mTOR
Caspases 9/3
Nrf2
c-Jun
Inflammatory genes
(IL-1β, IL-6)
NF-κB
Nrf2
Anti-oxidantive genes
(HO-1, NQO1)

Fig. 7

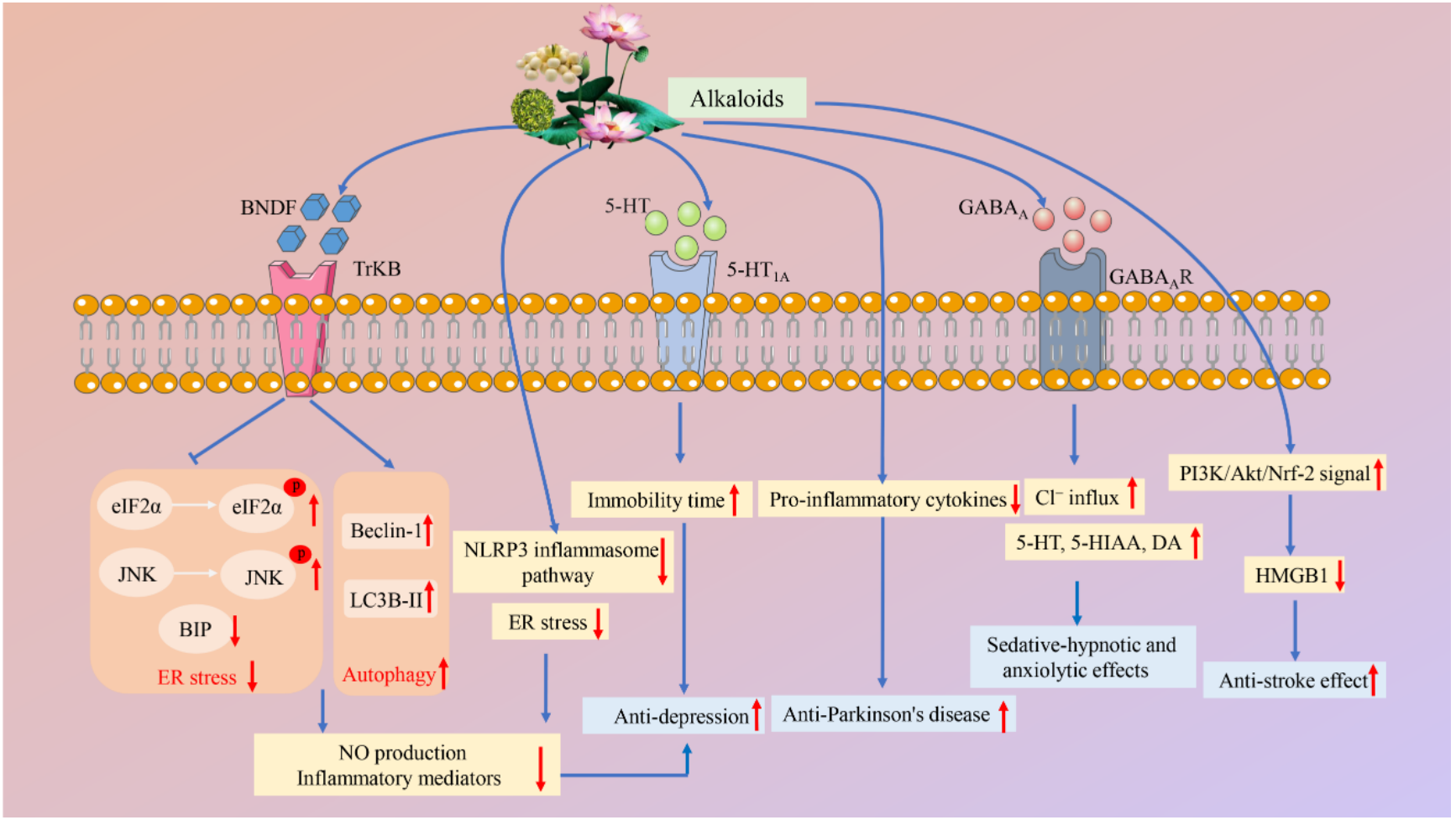
Alkaloids
BNDF
TrKB
5-HT
5-HT1A
GABAA
GABAAR
eIF2α
eIF2α
JNK
JNK
BIP
ER stress
Beclin-1
LC3B-II
Autophagy
NLRP3 inflammasome pathway
ER stress
Immobility time
Pro-inflammatory cytokines
Cl⁻ influx
5-HT, 5-HIAA, DA
Sedative-hypnotic and anxiolytic effects
PI3K/Akt/Nrf-2 signal
HMGB1
Anti-stroke effect
Anti-depression
Anti-Parkinson's disease
NO production
Inflammatory mediators

Fig. 8


Food and healthcare industry
Pharmaceutical industry
Cosmetic industry
美韵秀
脂降宁片
Zhijiangning Pian
心脑静片
Colgate