The pharmacology, pharmacokinetics, and toxicity of spinosin: A mini review

Xiaolan Kuang1†, Ganshu She2†, Ting Ma1, Wanna Cai3, Jingjing Zhao3, Bo Liu1* and Fangfang Xu1*

1Guangdong Provincial Key Laboratory of Clinical Research on Traditional Chinese Medicine Syndrome, Guangzhou Key Laboratory of Chirality Research on Active Components of Traditional Chinese Medicine, The Second Clinical College of Guangzhou University of Chinese Medicine, Guangzhou, China, 2Department of Pharmacy, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China, 3Department of pharmacy, GuangDong Women and Children Hospital, Guangzhou, China

Spinosin, a natural flavone-C-glycoside that is mainly isolated from the seeds of Ziziphus jujuba Mill. var. spinosa. It exerts the effects to ameliorate the neurological disorders, such as hypnosis effects, improvement of cognitive function, sedation effects, and anxiolytic effects, as well as anti-melanogenic effect, cardioprotective effects, and anti-cancer activity. However, the insufficient basic research, unclear mechanisms, and poor bioavailability may limit the prospects of spinosin in clinical utilization. In this review, we comprehensively summarized the latest information on the pharmacology, pharmacokinetics, toxicity, and NMR characteristic of spinosin, to evaluate its potential therapeutic for clinical application, hoping to provide some rational perspective for the innovative agent development and usage of spinosin in future.

KEYWORDS spinosin, pharmacology, pharmacokinetics, toxicity, Ziziphus jujuba Mill. var. spinosa

Introduction

Spinosin, (PubChem CID: 24771055, CAS No.: 72,063-39-9, MW: 608.5 g/mol), with the molecular formula of C28H32O15, is a natural flavone-C-glycoside that mainly exists in dried and ripe seeds of Ziziphus jujuba Mill. var. spinosa (ZJS) (Figure 1) (Wu et al., 2011). Recently, spinosin has also been isolated from Cayaponia tayuya (Yell.) Cogn (Aquila et al., 2009), Passiflora edulis Sims (Zucolotto et al., 2009), Leonurus japonicus Houtt. (Liu et al., 2018), and so on.

In traditional Chinese medicine (TCM), ZJS is sweet and sour in flavor, neutral in nature, and belongs to the liver, gallbladder, and heart meridians (Chinese pharmacopoeia, 2020). ZJS possesses the functions of nourishing the heart and liver, calming the mind and nerves, and condensing sweat and producing fluid that has been widely used for hypnosis, palpitations, dreams, sweating, and thirst (Chinese pharmacopoeia, 2020). It also has been widely used as an herb in the preparations of Chinese material medica to treat insomnia and anxiety for its main
sedative and hypnotic effects. ZJS contains several groups of bioactive components including flavonoids, saponins, alkaloids, and fatty acids (Jiao et al., 2017), and saponins and flavonoids are the main effective components of sedation and hypnosis. Jujuboside A, jujuboside B, and spinosin are major components of total saponins and total flavones, respectively (Zhu et al., 2015). Spinosin is a C-glycoside flavonoid isolated from the ethanol extract of ZJS’s dried seeds (Huang et al., 2014), as one of the main bioactive components of ZJS, numerous explorations of the pharmacological effects of it have been reported, such as hypnosis effects (Wang et al., 2008, 2010, 2012; Wang et al., 2016a), improvement of cognitive function (Jung et al., 2014; Ko et al., 2015; Xu et al., 2020), anxiolytic effect (Wang and Yan, 2022), inhibition of melanin synthesis (Moon et al., 2019), and antioxidant effects (Zhang et al., 2020a).

Pharmacology research is crucial for the efficient and rational development of drugs, such as elucidating their mechanism, developing new usage of existing drugs, improving their efficacy, and reducing the toxicity of the medicine. The pharmacokinetic (PK) behavior of drugs plays an essential role in their pharmacological actions. The alterations of pharmacokinetic parameters may affect the drug’s therapeutic efficacy (Ma et al., 2012). Detailed toxicological data are the basis for risk assessment. The use of accurate and reliable toxicological data is the first step in hazard identification (Qu and Song, 2021). To better utilize the medicinal resource of spinosin, we discussed the research on spinosin in various research fields in the recent years covering its pharmacology, pharmacokinetics, and toxicology in this review.

**Pharmacology**

**Hypnosis effects**

The seeds of ZJS are used as a traditional herbal drug for the treatment of insomnia (Tsai et al., 2019). The potentiating sleep effect of spinosin was investigated in pentobarbital-treated (45 mg/kg, i.p.) mice. The results showed that pretreatment with spinosin (10 and 15 mg/kg) enhanced hypnotic effects, while using spinosin alone did not work. Further studies found that co-administration of spinosin (5 mg kg, p.o.) and 5-HTP (2.5 mg kg, i.p.) significantly reduced sleep latency and lengthened the sleep duration time (Wang et al., 2008). The aforementioned research suggested the regulation effect of the 5-HT system in pentobarbital-induced sleep.

The 5-HT1A receptor, a subtype of 5-HT receptors, plays an important role in the modulation of sleep and wakefulness. A deeper study revealed that spinosin (15 mg/kg, i.g.) lengthened the REM sleep time and increased the slow-wave sleep (SWS) mode in rats. A
5-HT_{1A} antagonist, the p-MPPI, reduced sleep latency and increased total sleep time and NREM sleep time. Conversely, a 5-HT_{1A} receptor agonist, the 8-OH-DPAT, reduced the NREM sleep, REM sleep, and SWS time in pentobarbital-treated rats. Spinosin could reverse the 8-OH-DPAT-induced reductions in the aforementioned sleep periods, suggesting spinosin may serve as an antagonist on the postsynaptic 5-HT_{1A} receptors (Wang et al., 2010). Further studies showed spinosin could potentiate pentobarbital-induced loss of righting reflex (LORR) in mice, and verified the antagonist of spinosin on presynaptic 5-HT_{1A} autoreceptor (Wang et al., 2012). Another research indicated that spinosin (20 mg/kg, i.p.) can increase the non-rapid eye movement (NREM) time and shorten the sleep latency time in the active phase of mice by antagonizing the 5-HT_{1A} receptor (Wang et al., 2016a).

Those results indicated that spinosin is an inhibitor on both somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} heteroreceptors, and this ingredient may be used as a potential drug for the treatment of hypnosis.

**Improvement of cognitive function**

Alzheimer’s disease (AD) is a neurodegenerative disease with the characteristics of memory deterioration, cognitive function reduction, and behavioral impairments (Chu et al., 2012). The hallmark of AD is hippocampal synaptic dysfunction, which is becoming a potential target for the AD therapy.

Spinosin exerted the neuroprotective effects on cholinergic blockade-induced memory impairment in mice by extending the latency time in the passive avoidance task, lengthening the swimming time, and increasing the expression levels of phosphorylated extracellular signal-regulated kinases and cAMP response element-binding proteins in the hippocampus (Jung et al., 2014). Further research revealed that spinosin increased the number of immature neurons in the dentate gyrus region of the hippocampus and the neuronal cell propagation, and stimulated the neurons’ differentiation by the ERK-CREB-BDNF signaling pathway. The results indicated that spinosin can be used for the treatment of neurological cognitive dysfunction or psychiatric disorders (Lee et al., 2016).

The amyloid-β_{1-42} (Aβ_{1-42})--induced mouse model was used to evaluate the activities and mechanisms of spinosin in the treatment of AD. Spinosin improves the memory impairment induced by amyloid Aβ_{1-42} oligomer in mice verified by the passive avoidance task and the Y-maze task, decreasing the GFAP or OX-42 in the hippocampus, reducing the number of activated microglia and astrocytes, and enhancing the choline acetyltransferase (ChAT) expression (Ko et al., 2015). Spinosin attenuated the long-term potentiation (LTP), which is the indicator that reflects learning and memory, by the improved plasmin level in hippocampi of 5XFAD mice induced by Aβ (Cai et al., 2020).

Numerous studies have revealed that neuroinflammation play a critical role in the occurrence and development of AD. Anti-inflammation has been considered to be one of the significant ways to improve or even treat AD. Spinosin has been reported to alleviate cognitive impairment by improving the neurotrophic factor (BDNF) and Bcl-2, decreasing the level of MDA, and inhibiting the inflammatory factor IL-6 in the brain (Xu et al., 2019). Another research showed that spinosin inhibited the expression of COX-2 and Bax protein caused by Aβ_{25-35} and improved the proportion of LTP. The conclusion suggested the repairment of spinosin on the learning and memory impairment induced by Aβ_{25-35} was mainly by inhibiting the inflammatory response (Du, 2021).

The oxidative stress is often accompanied by the occurrence of AD (Cervellati et al., 2016). Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), hydroxyl radical (OH•), and superoxide anions (O₂¬), is the major source of oxidative stress, which contributes to proteins, lipids, and DNA oxidation in brain tissues. p38MAPK is sensitive to stressful stimuli such as ROS and is related to the development process of AD. Spinosin showed the inhibitory effect on the intracellular ROS production induced by H₂O₂ in N2a cells. The in-depth mechanism research indicated spinosin inhibited Aβ_{1-42} production, decreased Tau phosphorylation, and improved synaptic structural plasticity induced by H₂O₂ through inhibiting the p38MAPK activation (Xu et al., 2020).

In addition, spinosin inhibited the production and accumulation of Aβ_{1-42} through influencing the amyloid precursor protein (APP) parade, by activating the antioxidative Nrf2/HO-1 pathway (Zhang et al., 2020a).

**Sedative action**

The sedative activities of spinosin were evaluated by the climbing test and caffeine-induced hyperactivity model in mice. The results revealed that the number of mice that could not climb the ladder was increased and the frequency of crossing the hole was decreased after spinosin (500 and 1,000 mg/kg) was given (i.p.) (Shin et al., 1978; Shin et al., 1981). Spinosin was injected into mice (15 mg/kg, i.p.), and then 90 min later their brains were isolated and used for immunohistochemical analysis. The results revealed that spinosin markedly decreased c-Fos expression in the lateral hypothalamic area (LHA) and locus coeruleus (LC), suggesting that inactivation of the LHA and LC neuronal was the mechanism of spinosin on sedation (Zhang et al., 2020a).

**The anxiolytic effects**

The anxiety disorder is one of the most common psychiatric disorders that affect the health of the general population. The
TABLE 1 Pharmacology of spinosin.

| Pharmacological effect | Detail | Cell line/model | Dose | Application | Reference |
|------------------------|--------|-----------------|------|-------------|-----------|
| Hypnosis effects       | Increase sleep time and reduce sleep latency assessed with the loss-of-righting reflex | Male ICR mice | 0.1 ml/10 g | In vivo | Wang et al. (2008) |
|                        | Reduce sleep latency and increase total sleep time, slow-wave sleep (SWS) sleep time, and REM sleep time as inhibitor of postsynaptic 5-HT1A receptors | Male SD rats | 5, 10, 15 mg/kg | In vivo | Wang et al. (2010) |
|                        | Potentiate pentobarbital-induced loss of righting reflex (LORR) in mice | ICR male mice | 5, 15 mg/kg | In vivo | Wang et al. (2012) |
|                        | Increase non-rapid eye movement (NREM) time and shorten the sleep latency time in the active phase of mice | CS7BL/6j mice | 5, 10, 20 mg/kg | In vivo | Wang et al., 2016a |
| Improvement of cognitive function | Exert the neuroprotective effects on cholinergic blockade-induced memory impairment in mice by extending the latency time in the passive avoidance task, prolonging the swimming time, increasing the expression levels of phosphorylated extracellular signal-regulated kinases, and cAMP response element-binding proteins in the hippocampus | Male ICR mice | 2.5, 5, 10, or 20 mg/kg | In vivo | Jung et al. (2014) |
|                        | Improve the memory impairment induced by amyloid Aβ (1–42) oligomer in mice though the passive avoidance task and the Y-maze task, reduce the number of activated microglia and astrocytes, enhance the choline acetyltransferase expression | Male ICR mice | 5, 10, 20 mg/kg | In vivo | Ko et al. (2015) |
|                        | Increase the proliferation and survival of neuronal cells and the number of immature neurons in the hippocampal dentate gyrus region, stimulate the differentiation of newly generated cells into mature neurons by activating the ERK-CREB-BDNF signaling pathway | Male ICR mice | 1.25, 2.5, 5, or 10 mg/kg | In vivo | Lee et al. (2016) |
|                        | Alleviate the cognitive impairment by decreasing the level of MDA and Aβ (1–42) accumulation in hippocampus, improve the neurotrophic factor (BDNF) and B-cell lymphoma-2 (Bcl-2) in the brain, and inhibit the inflammatory response in the brain | Male-specific pathogen-free KM mice | 10, 100 μg/kg | In vivo | Xu et al. (2019) |
|                        | Attenuate amyloid β-induced long-term potentiation (LTP) impairment, and improve plasmin activity and protein level in the hippocampus of SXFAD mice | ICR mice | 3, 10, 30 μM | In vivo | Cai et al. (2020) |
|                        | Prevent H2O2-induced oxidative damage via inhibiting Aβ (1–42) production, decrease Tau phosphorylation, and improve synaptic structural plasticity though inhibition of p38MAPK. | N2a cell | 25 μM | In vitro | Xu et al. (2020) |
|                        | Reduce Aβ (1–42) production by activating the Nrf2/ HO-1 pathway in N2a/WT and N2a/APP695 cells | N2a/WT N2a/ APP695 cell | 6.25, 12.5, 25 μM | In vitro | Zhang et al., 2020c |
|                        | Repairment of spinosin on the learning and memory impairment induced by Aβ (1–42) by inhibiting the inflammatory response | Aβ15-35 | 30 μmol/L | In vitro | Du, (2021) |
| Sedation effects       | Increased the number of mice unclimbed the ladder and increased the frequency of the hole crossing | Male dd mice | 200, 500, and 1,000 mg/kg | In vivo | Shin et al. (1978), Shin et al. (1981) |
|                        | Decrease c-Fos expression in the lateral hypothalamic area (LHA) and locus coeruleus (LC) | pathogen-free adult male mice | 15 mg/kg | In vivo | Zhang et al., 2020a |
| The anxiolytic effects | Induce anxiolytic-like effects in the elevated plus maze, light/dark box test, and open field test but do not influence spontaneous activity | Male ICR mice | 1.25, 2.5, and 5 mg/kg | In vivo | Liu et al. (2015) |

(Continued on following page)
Table 1 (Continued) Pharmacology of spinosin.

| Pharmacological effect | Detail                                                                 | Cell line/model     | Dose                   | Application | Reference |
|------------------------|------------------------------------------------------------------------|---------------------|------------------------|-------------|-----------|
| Anti-melanogenic effect| Suppress αMSH- or UVB-induced melanogenesis in B16F10 cells without cytotoxicity | B16F10 melanoma cells | 2, 5, 10, 20 μM         | In vitro    | Moon et al. (2019) |
| Cardioprotective effects| Weaken the myocardial tissue injury, reduce the serum levels of cTnI and LDH levels, and attenuate the apoptosis by increasing LC3B-II and reducing p62 in AMI rats | Male Wistar albino rat | 5 mg/kg               | In vivo     | Gu et al. (2019) |
| Anti-cancer activity    | ZJSP inhibited the proliferation, increased the apoptosis, and promoted the chemo-sensitivity of damaged organs (heart, liver, spleen, lung, kidney, and pancreas), and raise the CRC early marker (COX-II, EMR1, and Ki67) in CAC mice | CRC, HCT-116, HCT-8, HCT-8FU cells | ZJSP 0, 50, 100, 150, 200 μg/ml | In vitro    | Shan et al. (2020) |
|                        | ZJSP could reduce the CAC polyps, promote the recovery of damaged organs (heart, liver, spleen, lung, kidney, and pancreas), and raised the early CRC markers (COX-II, EMR1, and Ki67) in CAC mice | male C57BL/6J mice | ZJSP 100, 200 mg/Kg     | In vivo     | Shan et al. (2020) |

Skin-lightening effects

As tyrosinase is the key factor in melanin production, inhibiting the tyrosinase can be used for skin pigmentation. Spinosin showed tyrosinase inhibitory activity (IC50 = 47 μM) and anti-melanogenesis effect (10, 20 μM) in B16F10 cells induced by αMSH- or UVB. The protein docking analysis further demonstrated that spinosin repressed the tyrosinase activity through the hydrogen bonds (Moon et al., 2019). This evidence suggested the potential of spinosin developed as a candidate for skin-lightening cosmetics.

Cardioprotective effects

The acute myocardial infarction (AMI) rat model was used to investigate the cardioprotective effects of spinosin and its analog 6'-feruloyl-spinosin. As a result, pretreatment with spinosin lessened myocardial tissue injury, reduced the serum levels of cTnI and LDH, and promoted autophagy by increasing LC3B-II levels in AMI rats. The mechanism data suggested that spinosin worked by inhibiting the GSK3β and activating the autophagy and the activity of the PGC-1α/Nrf2/HO-1 pathway (Gu et al., 2019).

Anti-colorectal cancer effects

The polyphenol extraction from ZJS (ZJSP, 0, 50, 100, 150, and 200 μg/ml) exhibited anti-colorectal cancer (CRC) activity by inhibiting HCT-116 cell growth, increasing cell apoptosis in the HCT-8 and HCT-116 cells, and enhancing the sensitivity of HCT-8FU cells for 5-FU. The AOM/DSS-induced CAC mice were used to evaluate the CRC effect and the results showed the ZJSP (100 and 200 mg/kg) reduced the CAC polyps, promoted the recovery of damaged organs (heart, liver, spleen, lung, kidney, and pancreas), and raised the early CRC markers (COX-II, EMR1, and Ki67) in CAC mice. Further isolation and RP-HPLC-MS/MS results indicated spinosin was the anti-CRC constituent in ZJSP (Shan et al., 2020). This study indicated the potential usage of spinosin as a natural agent against CRC.

The details pharmacological activities of spinosin are depicted in Table 1 and Figure 1.

Pharmacokinetics

The pharmacokinetic properties are the premise of preclinical and clinical research of drugs. It provides drug toxicity and clinical application information to screen the candidate agents in the process of innovative agent development. Nowadays, HPLC (Li et al., 2014) and HPLC/MS/MS (Liu et al., 2013) were used to investigate the pharmacokinetic parameters of spinosin in vivo with rats (Bao et al., 2013) and dogs (Lee et al., 2020). The rat oral administration of ZJS extract (20 g/kg) containing spinosin revealed the pharmacokinetic parameters with Cmax at 224 ± 82 μg/L, Tmax at 5.5 ± 0.6 h, and T0.5 at 5.8 ± 0.9 h in rat plasma using the HPLC method (Li et al., 2003). The pharmacokinetic parameters of spinosin in ZJSP between the control group (NC) and insomnia model (IM) group were determined using
### Table 2: Pharmacokinetic information of spinosin.

| Model          | Dose      | Sample                  | Administration method | Quantitative method | Detail                                                                 | Reference       |
|----------------|-----------|-------------------------|-----------------------|---------------------|------------------------------------------------------------------------|-----------------|
| Rat blood      | 20 g/kg   | ZJS extract             | Oral administration   | HPLC                | \(C_{\text{max}} = 224 \pm 82 \mu g/L\) \(T_{\text{max}} = 5.5 \pm 0.6 \text{ h}\) \(T_{1/2} = 5.8 \pm 0.9 \text{ h}\) \(AUC_{0-\infty} = 269.02 \text{ mg h/L}\) \(CL = 0.06 \text{ L/kg/h}\) \(MRT = 12.15 \text{ h}\) | Li et al. (2003) |
| Rat blood      | 180 mg/kg | ZJS extract             | Oral administration   | HPLC                | \(C_{\text{max}} = 86.23 \text{ mg/L}\) \(T_{\text{max}} = 5.95 \text{ h}\) \(T_{1/2} = 5.34 \text{ h}\) \(AUC_{0-\infty} = 30.419 \pm 3.58 \text{ mg h/L}\) | Bao et al. (2013)|
| Beagle dog     | 200 mg/tablet | ZJS extract         | Intragastric gavage  | LC-MS/MS             | \(C_{\text{max}} = 21.3 \text{ ng/ml}\) \(T_{\text{max}} = 90 \text{ min}\) \(T_{1/2} = 103 \text{ min}\) \(AUC_{0-\infty} = 3.410 \text{ ng min/ml}\) | Lee et al. (2020)|
| Rat blood      | 6.67 g/kg | Zaoren An-shen granule | Oral administration   | HPLC                | \(C_{\text{max}} = 3.452 \pm 0.06 \text{ mg/L}\) \(T_{\text{max}} = 7 \pm 0.17 \text{ h}\) \(T_{1/2} = 2.341 \pm 2.63 \text{ h}\) \(AUC_{0-\infty} = 0.589 \pm 0.08 \text{ L/kg/h}\) | Li et al. (2014)|
| SD rat         | 6.8 g/kg  | ZJS extract             | Intragastric gavage  | UHPLC-Q-Orbitrap-MS  | \(C_{\text{max}} = 40.08 \text{ mg/L}\) \(T_{\text{max}} = 0.23 \text{ h}\) \(T_{1/2} = 2.75 \text{ h}\) \(AUC_{0-\infty} = 44.65 \mu g/h\) | Du et al. (2020) |
| SD rat         | 5 mg/kg   | Spinosin                | Femoral vein injection| HPLC-MS             | Blood \(C_{\text{max}} = 5.59 \pm 2.65\) \(AUC_{0-\infty} = 205.70 \pm 80.79 \text{ mg min/L}\) \(T_{1/2} = 48.07 \pm 4.71\) \(V = 3.00 \times 10^{-2} \pm 5.10 \times 10^{-3}\) | Ma et al. (2012) |
|                |           |                         |                       |                     | Blood \(C_{\text{max}} = 695.40 \pm 162.90\) \(AUC_{0-\infty} = 7.77 \times 10^{2} \pm 2.13 \times 10^{2} \text{ mg min/L}\) \(T_{1/2} = 97.20 \pm 37.63\) \(CL = 1.00 \times 10^{-2} \pm 2.00 \times 10^{-3}\) \(V = 9.40 \times 10^{-2} \pm 1.80 \times 10^{-2} \text{ L/kg}\) |                |
|                |           |                         |                       |                     | Brain \(C_{\text{max}} = 5.10 \times 10^{-2} \pm 7.5 \times 10^{-3}\) \(AUC_{0-\infty} = 2.09 \pm 0.03 \text{ mg min/L}\) \(T_{1/2} = 42.18 \pm 13.71\) \(CL = 1.72 \pm 0.28\) \(V = 101.67 \pm 16.45 \text{ L/kg}\) |                |
| Rat            | 10, 20, 40 mg/L | Spinosin        | Oral administration   | HPLC                | Spinosin was absorbed in all segments gastrointestinal in the pattern of first-order kinetics with the passive diffusion absorption mechanism | Zhang et al. (2012) |
| SD rat         | 5 mg/kg   | Spinosin                | Intravenous administration | UPLC-MS/MS       | Spinosin could permeate the blood-brain barrier, and reached the various areas of the brain such as the corpus striatum, hippocampus, cerebrum, cerebellum, and olfactory region | Zhang et al. (2015) |
| Wistar rat     | 20 mg/kg  | Spinosin                | Intravenous administration | HPLC                | Spinosin was detected in the liver, brain, spleen, and kidney | Li et al. (2007) |
| Rat bile       | 9 g/kg    | Shensong Yangxin capsules | Oral administration   | UPLC-MS/MS       | Total bile excretion of the original drug in 24 h accounted for 1.096% | Liu et al. (2013) |
| SD rats        | 1.0 g/ml  |                         | Intragastric gavage  | UPLC-MS/MS       | \(C_{\text{max}} = 2.83 \text{ mg h/L}\) \(CL = 1.42 \text{ L/kg/h}\) | Li et al. (2021) |

(Continued on following page)
The Caco-2 cell model was used to investigate the transport characteristics of spinosin, the results indicated that spinosin was transported through the intestinal mucosa via a passive diffusion at low concentration, while affected by P-glycoprotein (P-gp) at a high concentration with reduced absorption (Huang et al., 2016). Song et al. (2020) found the absorption mechanism of spinosin was energy-dependent monocarboxylate transporter (MCT)–mediated active transport, and the efflux process was mediated by P-gp and multidrug resistance protein (MRP), which may result in a decrease in bioavailability.

The UPLC-MS/MS was carried out to identify metabolites and evaluate the in vivo metabolic profile of spinosin. Three metabolites of I-phase were identified from blood and urine in depression model rats (Wang et al., 2016b). Eight I-phase metabolites of spinosin were detected in the human liver microsome incubation samples (Zhang et al., 2020b). Spinosin was degraded by rat intestinal bacteria in vitro and its metabolite was swertisin (Zhang et al., 2013).

The serum proteins, such as BSA and HSA, have the ability of binding with drugs, which play an important role in the drug metabolism. The results revealed that spinosin bound with BSA and HSA through Van der Waals force and hydrogen bond and then changed the Tyr and Trp residue microenvironments. The findings may explain the metabolism behaviors of spinosin in oxidation, intestinal hydrolysis, demethylation, and reduction by rat intestinal bacteria and its metabolite was swertisin.
The pharmacokinetic studies on spinosin are shown in Table 2 and metabolites of spinosin are shown in Figure 2.

**Toxicity**

The toxicity of spinosin was studied by intraperitoneally injecting into mice with graded doses ranging from 200 mg/kg to 10 g/kg and there was no mortality of mice even at the highest dosage, which suggested the safety of spinosin (Shin et al., 1978). Spinosin also did not exhibit cytotoxicity on HaCat, B16F10, and Hs27 cells at 20 μM (Moon et al., 2019). Spinosin had cytotoxicity in N2a/APP695 cells at 200 and 400 μM, but no cytotoxicity in N2a/WT cells at 400 μM (Zhang et al., 2020c). The cytochrome P450 participated in the drug metabolism process and mediated the drug–herb interactions, which have attracted much attention in recent years. The activation effects between CYP450 and drugs may increase the risk of drug application. One report indicated that spinosin exhibited inactive effects on CYP3A4 in human liver microsomes (Bai et al., 2020).

Spinosin, the main active C-glycoside constituent from ZJS, is highly consistent with pharmacological and toxicological properties of ZJS. The ZJS decoction was administered to mice at 15 g/kg by gavage, and no toxicity was observed in 48 h (Shen, 2011). The administration routes affected the drug’s toxicity. ZJS decoction and ethanolic extract of ZJS were injected intravenously into mice and the LD50 values were determined as 14.3 ± 20.0 and 27.5 ± 2.4 g/kg, respectively. The same samples were orally administered to mice at the dosage of 340 g/kg, but no case of mortality was observed (Wang et al., 2009). The chickens were orally administered with ZJS solution at the dosages ranging between 2.5 and 20 g/(kg d) and the maximum tolerable dosage of ZJS solution was calculated as more than 20 g/(kg d), while the LD50 was not determined. The experiments suggested that ZJS solution had no acute toxicity and no long-time toxicity (Li et al., 2010).

**NMR phenomenon**

The interesting NMR phenomenon of spinosin was observed in 2000 (Gong et al., 2000). Both 1H and 13C NMR data of spinosin exhibited the partial carbons and
protons signal splits appearance at room temperature (298 K), and doublet signals disappeared as the temperature rose to coalescence temperature (\( T_c \) 363 K). The results of the variable-temperature experiments suggested the presence of two rotational isomerisms at room temperature. Compared to the NMR data with compounds that have similar structures, only the constituents with 7-OCH\(_3\) in the flavone-6-C-glycoside skeleton exhibited the aforementioned NMR signal pattern (Gong et al., 2000). The variable-temperature 1H NMR experiments explained that the high energy barrier about the C-6-C-1‴ bonds prevents the interchange between rotamers at room temperature. The theoretical (MM2) calculations revealed the minimum energy of two conformations (energy difference ca 0.84 kJ/mol) and the separated energy barrier of ca 67 kJ/mol (Lewis et al., 2000). The findings were approved by many reports in analogs of spinosin, such as 6‴-feruloyl-spinosin, 6‴-acetyl-spinosin, and isovitexin-2‴-O-arabinoside (Song et al., 2020; Zhou and Yan, 2021). The aforementioned NMR signal features can be used to quickly distinguish the chemical skeleton and analyze the structure of substituent position. The 1D NMR spectra of spinosin at 298 and 387 K are shown in Figure S1A-B.

**Conclusion**

This article reviewed the bioactivities and the mechanisms of spinosin (Table 1), its pharmacokinetics parameters (Table 2) and security, as well as characteristic NMR performance. However, many issues need to be further illustrated in further studies. First, a few reports on pharmacological activities and the reported bioactivities of spinosin mainly focused on the phenotypic aspect, but there is lack of an in-depth specific interpretation on the mechanism research. Therefore, it is extremely meaningful to explore the molecular mechanism of its biological activities. Second, the little existing evidence suggested the safety of spinosin in vitro and in vivo, but it is difficult to fully evaluate its security due to lack of research evidence. Hence, it is necessary to systematically evaluate its safety and toxicity in vitro and in vivo for the clinical application. Third, mice are also widely used for pharmacokinetic studies but there have been no relevant reported pharmacokinetic studies on spinosin. So, the comprehensive pharmacokinetic research about spinosin and ZJS in rodent models should be conducted in future.

**Author contributions**

FX and XK analyzed the data and drafted the manuscript. BL and GS gave valuable advice on manuscript writing and revision. FX, WC, JZ, and TM collected and analyzed many references.

**Funding**

This work was supported by the National Natural Science Foundation of China (No. 82173700); Science and Technology Planning Project of Guangzhou (No. 202102021213); the special foundation of Guangzhou Key Laboratory (No. 20202010004); Special Funds for State Key Laboratory of Dampness Syndrome of Chinese Medicine (No. SZ2021ZZ33).

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.938395/full#supplementary-material

References

Aquila, S., Giner, R. M., Recio, M. C., Spegazzini, E. D., and Jose, L. R. (2009). Anti-inflammatory activity of flavonoids from Cayaponia tayuya roots. J. Ethnopharmacol. 121, 333–337. doi:10.1016/j.jep.2008.11.002

Bai, J., Li, L., Zhao, S. Y., Fan, X. Q., Zhang, J., Hu, M. W., et al. (2020). Heterotrophic activation of flavonoids on cytochrome P450 3A4: A case example of alleviating dromedrone-induced cytotoxicity. Toxicol. Lett. 319, 187–196. doi:10.1016/j.toxlet.2019.11.016

Bao, K. D., Zhao, J. H., Qi, L. W., Li, H., Yi, J. L., Wang, W., et al. (2013). Determination of spinosin and 6‴-feruloylspinosin in rat plasma after oral administration of flavonoid extract from zuziphi spinosae semen by SPE-HPLC-DAD. Chin. J. Mod. Appl. Pharm. 30, 707–711. doi:10.13746/cjmaap2013.07.010

Cai, M. D., Jung, I. H., Kwon, H. Y., Cho, E., Jeon, J., Yun, J., et al. (2020). Spinosin attenuates alzheimer’s disease-associated synaptic dysfunction via regulation of plasmin activity. Biomol. Ther. 28, 131–136. doi:10.4062/biomolther.2019.076
Wu, Y., He, F., Pan, Q., Shi, Y., Min, Z., and Liang, J. Y. (2011). C-glucosyl flavones from the seeds of Ziziphus jujuba var. spinosa. *Chem. Nat. Compd.* 47, 369–372. doi:10.1007/s10600-011-9936-y

Wu, B. A., Qu, C. H., Wang, Y. X., Zhao, J. F., and Du, H. Z. (2019). Comparison of the quenching effects of two main components of ziziphi spinosae semen on serum Albumin fluorescence. *J. Fluoresc.* 29, 1113–1123. doi:10.1007/s10895-019-02422-z

Xu, F., He, B. S., Xiao, F., Yan, T. X., Bi, K. S., Jia, Y., et al. (2019). Neuroprotective effects of spinosin on recovery of learning and memory in a mouse model of Alzheimer’s disease. *Biomol. Ther.* 27, 71–77. doi:10.4062/biomolther.2018.053

Xu, F. X., Zhang, X. Y., Wang, J. Y., Li, X., He, B. S., Xiao, F., et al. (2020). Spinosin protects N2a cells from H$_2$O$_2$-induced neurotoxicity through inactivation of p38MAPK. *J. Pharm. Pharmacol.* 72, 1607–1614. doi:10.1111/jphp.13334

Yang, J. Z., Cai, X. G., and Hao, H. J. (2019). Preparation, characterization and pharmacokinetic comparison of spinosin phospholipid complex and its solid lipid nanoparticles. *J. Chin. Med. Mat.* 42, 1855–1859. doi:10.13863/j.issn1001-4454.2019.08.030

Zhang, T., Xie, J. B., Liu, Z. Y., Zhang, Y. C., and Cheng, D. W. (2012). Absorption kinetics of spinosin in rat gastrointestinal. *Chin. J. Exp. Tradit. Med. Formula.* 18, 134–139. doi:10.13422/j.cnki.syfjx.2012.03.052

Zhang, T., Xie, J. B., Zhang, Y. Q., and Cheng, D. W. (2013). High-performance liquid chromatography coupled with tandem mass spectrometry applied for metabolic study of spinosin by rat intestinal flora. *J. Liq. Chromatogr. Relat. Technol.* 36, 1391–1400. doi:10.1080/10826076.2012.691439

Zhang, Y. Q., Zhang, T., Wang, F. L., and Xie, J. B. (2015). Brain tissue distribution of spinosin in rats determined by a new high-performance liquid chromatography-electrospray ionization-mass/mass spectrometry method. *J. Chromatogr. Sci.* 53, 97–103. doi:10.1093/chromsci/bmu025

Zhang, T. S., Shang, S. Y., Wang, C. Y., and Zhang, Z. C. (2019). Preparation and *in vivo* pharmacokinetic behavior evaluation for two spinosin solid dispersions. *Chin. Tradit. Pat. Med.* 41, 2025–2029. doi:10.3969/j.issn.1001-1528.2019.09.001

Zhang, J. P., Liao, D. Q., Li, L., and Chu, L. (2020a). Reduced c-fos expression in orexin neurons of the lateral hypothalamic area and the locus coeruleus following injection of spinosin into mice. *Folia Morphol.* 79, 429–437. doi:10.5603/FM.a2019.0118

Zhang, Q. Y., Zhang, X., Liu, Y. Y., Wan, C. C., Sun, Y. P., and Zhang, L. T. (2020b). *In vitro* identification of spinosin metabolites in human liver microsomes using a simple and sensitive UHPLC-Q-TOF-MS/MS method. *Curr. Pharm. Anal.* 16, 40–46. doi:10.2174/1573412914666181003141210

Zhang, X. Y., Wang, J. Y., Gong, G. W., Ma, R. X., Xu, F. X., Yan, T. X., et al. (2020c). Spinosin inhibits αβ₂, α₂ production and aggregation via activating Nrf2/Keap1 pathway. *Biomol. Ther.* 28, 259–266. doi:10.4062/biomolther.2019.123

Zhou, G. H., and Yan, H. R. (2021). Variable-temperature 1H-NMR studies on three C-glycosylflavones exhibiting rotational isomerism. *Mod. Chem.* 9, 8–12. doi:10.11648/j.mc.20210901.12

Zhu, H. Y., Zhang, L. N., Tang, S., Lin, H. C., Wang, G. L., He, Z. M., et al. (2015). Determination of spinosin, jujubosides A and B in *Ziziphi Spinosae* Semen from three different origins by HPLC. *Chin. J. Pharm. Anal.* 35 (12), 2099–2104.

Zucolotto, S. M., Goulart, S., Montanher, A. B., Reginatto, F. H., Schenkel, E. P., and Froede, T. S. (2009). Bioassay-guided isolation of anti-inflammatory C-glucosylflavones from *Passiflora edulis*. *Planta Med.* 75, 1221–1226. doi:10.1055/s-0029-1185536
**Glossary**

5-HTP 5-hydroxytryptophan  
AD Alzheimer’s disease  
AMI acute myocardial infarction  
APP amyloid precursor protein  
Aβ1-42 amyloid-β1,42  
BDNF brain-derived neurotrophic factor  
BSA bovine serum albumin  
CAC colitis-associated cancer  
ChAT choline acetyltransferase  
CL clearance  
CRC colorectal cancer  
cTnI cardiac troponin I  
GABA\_A γ-aminobutyric acid A  
GSK3\_β glycogen synthase kinase-3β  
HPLC high-performance liquid chromatography  
HSA human serum albumin  
IM insomnia model  
LC locus coeruleus  
LC3B-II microtubule-associated protein one light chain 3B-II  
LDH lactate dehydrogenase  
LHA lateral hypothalamic area  
LORR loss of righting reflex  
LTP long-term potentiation  
MCT monocarboxylate transporter  
MDA malondialdehyde  
MRP multidrug resistance protein  
NC normal control  
NMR nuclear magnetic resonance  
NREM non-rapid eye movement  
PCPA p-chlorophenylalanine  
P-gp P-glycoprotein  
PK pharmacokinetic  
RDA Retro Diels–Alder reaction  
REM rapid eye movement  
ROS reactive oxygen species  
SWS slow-wave sleep  
TCM traditional Chinese medicine  
ZJS Ziziphus jujuba Mill. var. spinosa  
ZJSP polyphenols of Ziziphus jujuba Mill. var. spinosa