Saikosaponins: a review of pharmacological effects

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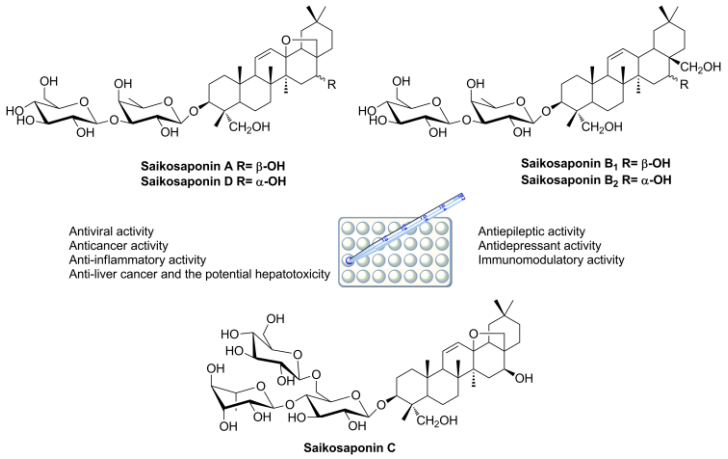
Abstract: Over the past decades, a number of phytochemicals have been reported to possess potent pharmacological effects. Saikosaponins represent a group of oleanane derivatives, usually as glucosides, which are common found in medicinal plants *Bupleurum* spp., which have been used as traditional Chinese medicine for more than 1,000 years in China. Emerging evidence suggests that saikosaponins have many pharmacological effects, including sedation, anticonvulsants, antipyretic, antiviral, immunity, anti-inflammation, antitumor, protecting liver and kidney and so on. The present review provides a comprehensive summary and analysis of the pharmacological properties of saikosaponins, supporting the potential uses of saikosaponins as a medicinal agent.



Keywords: *Bupleurum*; saikosaponins; pharmacological effects

1. Introduction

The root of *Bupleurum chinensis* DC., a well-known medicinal plant in China, was originally documented in the “Shennong's Herbal”, which is the oldest Chinese materia medica monographs. Radix Bupleuri is perennial herbaceous plants of Umbelliferae, and its action is regulating the function relation of internal organs to relieve fever, disperse the stagnation of liver-qi and uplift yang-qi to raise sinking in the traditional Chinese medicine (TCM) theory. Therefore, the TCM clinic is used for fever due to exogenous pathogenic factors with alternating episodes of chills and fever. And it is effective in eliminating the pathogenic factors located in the half-superficial and half-interior, hence it is an indispensable medicine for treating shaoyang disease, which is manifested as feeling of fullness and

oppression in the chest and hypochondrium, bitter taste in the mouth and dry throat, and relieving constraint for improving symptoms of emotionalin stability such as depression, anxiety and phobia. However, in recent 10 years, 43 kinds of saponins have been separated and identificated, and according to their differences of chemical structures bupleurum saponins can be divided into -a, -b, -c , -d, -m, -n, -p and -t, while saikosaponin d (SSd) is believed to be the most active among them, and saikosaponin a (SSa) is the second most active [1]. For the most part, saponins are extracted from bupleurum root, while saponins derived from the ground part are rare [2]. Saikosaponins (SS) are major biological active ingredients in *R. bupleuri*. Saikosaponins represent a group of oleanane derivatives, usually as glucosides, that are found in a number of plant families. Saikosaponins isolated from medicinal plants such as *Bupleurum* spp., *Heteromorpha* spp. and *Scrophularia scorodonia* have been reported to possess various biological activities, namely antihepatitis, antinephritis, antihepatoma, anti-inflammation and antibacterial effects, as well as being able to modulate immune function. Saikosaponins (a, b, c, and d) have been reported to have sedative and analgesic, anti-inflammatory, antibacterial, protect liver, kidney, anticancer, antivirus and other pharmacological effects. With regard to the latest use of saikosaponins (A, B, C and D) (Figure 1), we have summarized in Tables 1, 2, 3 and 4. In addition, the pharmacodynamic effect and mechanism of saikosaponins have the new research progress in the world and all literatures available are reviewed.

2. Pharmacology

2.1Central neverous systerm

2.1.1 Antiepileptic activity

Epilepsy is one of the most common neurological disorders, yet its treatment remains unsatisfactory. SSa, a triterpene saponin derived from *B. chinensis* DC., has been demonstrated to have significant antiepileptic activity in a variety of epilepsy models *in vivo*. Research found that SSa effectively terminated spontaneous recurrent epileptiform discharges (SREDs) in the HNC model of AE and continuous epileptiform high-frequency bursts (SE) in the hippocampal neuronal culture (HNC) model of SE, in a concentration-dependent manner with an IC_{50} of 0.42 mM and 0.62 mM, respectively [3].

By observing the electroencephalogram (EEG) of epileptic rats, the therapeutic effects of saikosaponins on epilepsy can be evaluated. Sixty 8-week-old healthy SD rats were randomized into normal control group, epileptic model group, lamotrigine group, and 3 saikosaponin groups of small, moderate and high doses, with 10 rats in each group. Penicillin was used to induce epilepsy in the latter 5 groups, and the EEG and onset of epileptic seizures were observed in each group. Result showed that saikosaponins can obviously alleviate the severity of epileptic seizure in epileptic rats [4]. Neonatal rat hippocampal astrocytes could be treated with SSa. SSa resulted in significant inhibition of cell proliferation, cell division and GFAP expression in the Glu-activated astrocytes. Glu-induced activation of rat hippocampal astrocytes can be inhibited by SSa, whose antiepileptic effect is probably mediated by inhibition of hippocampal astrocyte activation [5].

Acute epileptic seizure was induced by pentylenetetrazole (PTZ) in rats, and the seizure incubation period and the number of rats with tetanic convulsion were recorded to study the antiepileptic effect of SSa. After treatment with SSa, the incubation period of PTZ-induced seizure was significantly prolonged ($P<0.01$), and the rate of tetanic convulsion was significantly reduced ($P<0.05$). And SSa can inhibit epileptic seizure induced by PTZ [6,7]. Forty-eight healthy Sprague-Dawley rats were randomly divided into 6 equal groups, and except those in the blank control group, the rats in the other groups were all given different treatments to induce kindling by intraperitoneal injection of PTZ on a daily basis for 4 consecutive weeks. Seizure frequency in the 3 saikosaponins groups decreased 2 weeks later, which was especially obvious in the high-dose group ($P<0.05$). The kindling rate was significantly lower in high-dose saikosaponins group than in the other treatment groups after 4 weeks of the treatment ($P<0.05$), with also less intense seizure onset ($P<0.01$) and differences in the wave form of EEG [8].

2.1.2 Antidepressant activity

Pretreatment of PC12 cells with total saikosaponins (TSS) (3.125, 6.25, 12.5, 25 μg/mL) partly reversed corticosterone-induced neurotoxicity in a dose dependent manner. TSS (25 μg/mL) reversed the increase of dead cells in the hoechst stain, the accumulation in LDH leakage and the number of TUNEL positive cells induced by corticosterone to PC12 cells. Moreover, the cytoprotection of TSS was proved to be associated with the homeostasis of in

tracellular Ca^{2+}, the stabilization of ER stress via the down-regulation of GRP78, GADD-153, XBP-1, and the restoration of mitochondrial function, which included mPTP, MMP and caspase-3 activity. The result indicated that the antidepressant-like effect of TSS *in vivo* may be associated with the cytoprotection of neuron, and the neuroprotective mechanisms were co-related with inhibiting the ER stress and the mitochondrial apoptotic pathways [7].

2.2 Neuropathic pain

Neuropathic pain was induced by chronic constriction injury (CCI) of the sciatic nerve in rats. After CCI, rats were administered SSa (6.25, 12.50 and 25.00 mg/kg intraperitoneal, once daily) for 14 days. Mechanical withdrawal threshold and thermal withdrawal latency were assessed before surgery and on days 1, 3, 7, and 14 after CCI. Our results showed that CCI significantly decreased mechanical withdrawal threshold and thermal withdrawal latency on days 1, 3, 7 and 14, as compared with sham groups, however, SSa reversed this effects. In addition, SSa can inhibit the levels of TNF-α, IL-1β, IL-2 in rat spinal cord by CCI-induced. Western blot analysis demonstrated that saikosaponin a reduced the elevated expression of p-p38 mitogen-activated protein kinase (MAPK) and NF-κB in the spinal cord induced by CCI. These results suggest that saikosaponin a could effectively attenuate neuropathic pain in CCI rats by inhibiting the activation of p38 MAPK and NF-κB signaling pathways in spinal cord [9].

2.3 Anticancer activity

The effects of saikosaponins (a, b1, b2, c, d) on the induction of differentiation in rat C6 glioma cells were studied. SSa and d were shown to inhibit cell proliferation and alter cell morphology, and the results suggest that SSa can induce the differentiation of C6 glioma cells into astrocytes and/or oligodendrocytes, but SSd can only induce the differentiation of C6 glioma cells into astrocytes [10]. And SSd could inhibit the activated T lymphocytes via suppression of NF-κB, NF-AT and AP-1 signaling. Here, SSd significantly potentiated TNF-ß-mediated cell death in HeLa and HepG2 cancer cells via suppression of TNF-ß induced NF-κB activation and its target genes expression involving cancer cell proliferation, invasion, angiogenesis and survival. Also, SSd revealed a significant potency of abolishing TNF-ß induced cancer cell invasion and angiogenesis in HUVECs while inducing apoptosis via enhancing the loss of mitochondrial membrane potential in HeLa cells. These findings

indicate that SSd has a significant potential to be developed as a combined adjuvant remedy with TNF-ß for cancer patients [11]. SSd inhibits the cell growth of human lung cancer cell line A549 and provides a molecular understanding of this effect. It was showed that SSd inhibited the proliferation of A549 by inducing apoptosis and blocking cell cycle progression in the G1 phase [12]. To observe the effect of SSd on reversing the malignant phenotype of HepG2 cells and to investigate its mechanism in order to prove that SSd is a new choice to prevent and treat HCC. HepG2 cells were cultured and treated by different concentration of SSd demonstrating that SSd could reverse the malignant phenotype of HepG2 cells, and hinting SSd can be a new choice to prevent and treat HCC [13]. *In vitro*, MTT assay showed that SSd treatment inhibited cell proliferation in three human anaplastic thyroid cancer cell lines ARO, 8305C and SW1736. And SSd promoted cell apoptosis and induced G1-phase cell cycle arrest as shown by flow cytometric analysis. The research study suggested that SSd might be a new potent chemopreventive drug candidate for human undifferentiated thyroid carcinoma through induction of apoptosis and cell cycle arrest [14]. Used transmission electron microscopy (TEM) to observe ultrastructural changes of cells found the combination of SSd and radiotherapy had a time-dependent synergistic effect. Radiation caused ultrastructural damage to cells, and the damage was enhanced in combination with SSd. Radiation decreased the GSH content and increased the MDA content in cells, and this effect was suppressed after the intervention of SSd. SSd can inhibit the growth of SMMC-7721 hepatoma cell lines *in vitro*. Additionally, it significantly enhances the effects of radiation on inhibiting the growth of SMMC-7721 hepatoma cell lines, and up-regulates the antioxidant level after the radiotherapy [15]. Research showed that treatment with SSd inhibited DU145 cell proliferation in a concentration-dependent manner. Flow cytometric analysis showed that SSd inhibited the proliferation of DU145 cells by induction of apoptosis and cell cycle arrest at G0/G1 phase. Further mechanistic experiments demonstrated that SSd arrested the cell cycle at G0/G1 phase via upregulation of p53 and p21 and induced apoptosis by modulating B-cell lymphoma 2 family proteins, dissipation of the mitochondrial membrane potential, release of cytochrome c into the cytosol and activation of caspase-3.

2.4 Anti-liver cancer and the potential hepatotoxicity

Treatment with SSd decreased the cell proliferation of Hep G2 and Hep 3B cells in a

dose dependent manner. SSd blocked the progression of cell cycle at G1 phase by inducing p53 expression and further up-regulating p21/WAF1 expression. Furthermore, SSd also inhibited the cell survival signaling by enhancing the amount of IkBa in cytoplasm and reducing the level and activity of NF-κB in the nucleus, and subsequently attenuated the expression of Bcl-XL in Hep G2 and Hep 3B cells. SSd decreased the cell proliferation and induced apoptosis both in p53-postive Hep G2 and p53-negative Hep 3B cell. Male SD rats were divided into control, model and SSd groups, and model and SSd groups given intragastric 0.2% (w/v) N-diethylnitrosamine to induce HCC. SSd group received 0.03% (w/v) SSd in saline. Liver samples were analysed immunohistochemically for syndecan-2, MMP-2, MMP-13 and TIMP-2 at 16 weeks. The model group had more malignant nodules than the SSd group, and SSd inhibited HCC development, and down-regulated expressions of syndecan-2, MMP-2, MMP-13 and TIMP-2 in rat HCC liver tissue [16]. An experimental model with diethylinitrosamine (DEN)-treated Sprague Dawley rats was used in the present study. The liver nodule formation, tumorous invasion to surrounding organs and increased cellular atypia induced by DEN were markedly reduced by SSd in the SSd + DEN group compared with the DEN group. This results suggest that SSd prevents DEN-induced hepatocarcinogenesis in rats through inhibition of C/EBPβ and COX-2 [17].

The proliferation and migration of hepatic stellate cells (HSCs) profoundly impact the pathogenesis of liver inflammation and fibrogenesis. SSa and SSd are the major active components of triterpene saponins in *Bupleurum falcatum*. Experimental results indicate that, in addition to suppressing HSC-T6 proliferation, wound healing activity and cell migration in a time- and dose-dependent manner, SSa and SSd significantly induce apoptosis. Additionally, SSa and SSd decreased the expressions of extracellular matrix-regulated kinase 1/2 (ERK1/2), platelet-derived growth factor receptor 1 (PDGFR1), and subsequently transformed growth factor-β1 receptor (TGF-β1R), α-smooth muscle actin, TGF-β1 and connective tissue growth factor. They also decreased phosphorylation of p38 (p-p38) and ERK1/2 (p-ERK1/2) of HSC-T6. Furthermore, both SSa and SSd can block PDGF-BB and TGF-β1-induced cell proliferation and migration of HSC-T6 [18]. Hepatic fibrosis model rats that were induced by subcutaneous injection of CCl_4 were injected intraperitoneally with SSd at different doses (1.0, 1.5 and 2.0 mg/kg). The results demonstrated that SSd could attenuate the area and extent of

necrosis and reduce the scores of liver fibrosis [19,20]. A rat model of liver fibrosis was established using the dimethylnitrosamine. Liver tissue and serum were used to examine the effect of SSd on liver fibrosis. A hepatocyte culture was also used to investigate how SSd can protect hepatocytes from oxidative injury induced by carbon tetrachloride. The results showed that SSd significantly reduced collagen I deposition in the liver and alanine aminotransferase level in the serum. SSd was able to alleviate hepatocyte injury from oxidative stress. In conclusion, SSd could postpone the development of liver fibrosis by attenuating hepatocyte injury [21].

However, recent investigations and clinical practice have indicated that this medicinal herb has potential hepatotoxicity, questioning the clinical safety of using it [22]. Saikosaponins have been identified to be the primary bioactive components contained in *B. falcatum*, and many saikosaponins have been isolated from this plant, but SSd is believed to be the most active among them [23]. Therefore, SSd is highly possible to act as the major culprit responsible for hepatotoxicity. And its trongly strengthened the research that SSd inhibited cell proliferation and induced mitochondrial apoptosis in human hepatocyte LO2 cells. This pro-apoptotic effect could be attributed to SSd interruption of PDGF-bR/p38 cascade. And these data offered mechanistic insights into the potential hepatotoxicity of saikosaponins as well as relevant herbal prescriptions.

2.5 Immunomodulatory activity

Saikosaponin has a very significant role in the protection of radiation in mice and enhancement of the immune effects. Experiments showed that the extracts of South and North *Bupleurum* were significantly enhanced lymphocyte proliferation, interleukin-2 and tumor necrosis factor secretion levels .

Research found that SSd can inhibit T cell activation through the suppression of CD69 and CD71 expressions and IL-2 production, and the modulation of PKC pathway through PKCh, JNK, and NF-κB transcription factor. This may candidate for use in the treatment of inflammatory and autoimmune diseases including SLE. The effects of SSd on activated mouse T lymphocytes through the NF-κB, NF-AT and AP-1 signaling pathways, cytokine secretion, and IL-2 receptor expression have been investigated [24]. The results demonstrated that SSd not only suppressed OKT3/CD28-costimulated human T cell proliferation, it also

inhibited PMA, PMA/Ionomycin and Con A-induced mouse T cell activation *in vitro*. These results indicate that the NF-κB, NF-AT and AP-1 (c-Fos) signaling pathways are involved in the T cell inhibition evoked by SSd, so it can be a potential candidate for further study in treating T cell-mediated autoimmune conditions [1]. The effects of SSd on the signaling pathways of T cell activation were examined. The results showed that SSd potently suppressed both early (CD69) and late (CD71) expressions of mouse T cells stimulated with Con A or PMA. It interfered with PKCh translocation from cytosol to membrane fraction and inhibited the phosphorylations of IjBa and JNK in PMA-activated mouse T cells. Additionally, it inhibited PMA and ionomycin-stimulated IL-2 production in mouse T cells. These results indicate that the mechanism by which SSd inhibits T cell activation would involve the suppression of CD69 and CD71 expressions and IL-2 production, and the modulation of PKC pathway through PKCh, JNK, and NF-κB transcription factor. This may herald a novel approach for further studies of SSd as a candidate for use in the treatment of inflammatory and autoimmune diseases [24]. The aim of the present study was to investigate the effect of SSd on the differentiation, maturation and function of human monocyte-derived dendritic cells (DCs) isolated from condylomata acuminate patients. The results of the present study demonstrated that SSd reduced the differentiation of DCs, as evidenced by decreased expression levels of cluster of differentiation (CD)1a, CD80 and CD86 molecules and increased CD14 expression. Expression levels of the mannose receptor and CD32 were also significantly elevated, which was associated with enhanced fluorescein isothiocyanate-dextran endocytic activity. Furthermore, SSd treatment promoted DC maturation by increasing the expression levels of CD40, CD83, CD80 and CD86. In addition, the function of mature DCs, including the secretion of IL-12 and the stimulation of lymphocyte proliferation, was significantly increased following SSd administration. In conclusion, the present study indicated that SSd exhibited immunomodulatory effects and may be a novel potent chemopreventive drug candidate for the treatment of condylomata acuminate [25].

2.6 Anti-inflammatory activity

Bupleurum saponins exhibit multiple pharmacological activities including anti-inflammatory and anti-cancer effects. Moreover, SSd has been reported to attenuate toxin-induced hepatocyte injury and hepatic fibrosis in animal models through the inhibition

of several types of inflammatory mediators. Buddlejasaponin I and saikosaponins 1 and 2, biologically active compounds from *S. scorodonia* and *Bupleurum rigidum* respectively, exert potent *in vivo* antiinflammatory effects on mouse ear edema induced by phorbolmyristate acetate (PMA). The effects of these compounds on swelling and other inflammatory parameters are described. The effects of saikosaponins on cellular systems generating cyclooxygenase (COX) and lipoxygenase (LOX) metabolites were also evaluated *in vitro* [26]. SSa and SSd exhibited significant anti-inflammatory activity in two different murine models of acute inflammation, carrageenan-induced paw edema in rats and acetic acid-induced vascular permeability in mice. SSa and SSd showed potent anti-inflammatory activity through inhibitory effects on NF-κB activation and thereby on iNOS, COX-2 and pro-inflammatory cytokines [27]. Current study examines the effects of SSd on progression of mesangioproliferative glomerulonephritis induced by anti-Thy1 monoclonal antibody 1-22-3 (mAb 1-22-3) in uninephrectomized rats. SSd inhibits the progression of mesangioproliferative glomerulonephritis through reduction of the expression of TGF-beta 1 and the infiltration of macrophages and CD8+ T lymphocytes [28]. Chorioallantoic membrane (CAM) model was established successfully in chicken embryos. They were divided into SSd treated groups and the control group treated with PBS. Microscopic examinations showed that the number of microvessels and infiltrated inflammatory cells in the sponge and peripheral CAM mesenchyme in the SSd groups were less than those in the control group, especially on vessels of medium and small size ($P < 0.05$, $P < 0.01$, respectively), but was insignificant on great vessels ($P > 0.05$). SSd could inhibit the physiological angiogenesis of chicken embryoe, especially for the medium and small vessels, while there was no significant effect on great vessels ($P > 0.05$). Its mechanism of action may be related to its inhibition on leukocyte migration and activation [29]. Three saikosaponins were isolated from the MeOH extract of the roots of *B. falcatum* L.: saikosaponins B_3 (**1**); B_4 (**2**); and D (**3**). Of the three, compound **3** inhibited the interaction of selectins (E, L, and P) and THP-1 cells with IC_{50} values of 1.8, 3.0 and 4.3 μM, respectively. Also, the aglycone structure of compound **3** showed moderate inhibitory activity on L-selectin-mediated cell adhesion. From these results, researchers suspect that compound **3** isolated from *B. falcatum* roots would be a good candidate for therapeutic strategies to treat inflammation [30]. C57/BL6 mice were administered SSd

intraperitoneally once daily for 5 days, followed by APAP challenge. Biochemical and pathological analysis revealed that mice treated with SSd were protected against APAP-induced hepatotoxicity. SSd markedly suppressed phosphorylation of nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) and reversed the APAP-induced increases in the target genes of NF-κB, such as pro-inflammatory cytokine Il6 and Ccl2, and those of STAT3, such as Socs3, Fga, Fgb and Fgg. SSd also enhanced the expression of the anti-inflammatory cytokine Il10 mRNA. Collectively, these results demonstrate that SSd protects mice from APAP-induced hepatotoxicity mainly through down-regulating NF-κB and STAT3-mediated inflammatory signaling. This study unveils one of the possible mechanisms of hepatoprotection caused by *B. falcatum* and/or SSd. Bacterial lipopolysaccharide (LPS) is an important mediator of inflammation and a potent inducer of endothelial cell damage and apoptosis. LPS triggered caspase-3 activation, which was found to be important in LPS-induced HUVEC apoptosis. Inhibition of caspase-3 also inhibited LPS-induced degradation of focal adhesion kinase (FAK), indicating that caspase-3 is important in LPS-mediated FAK degradation as well as in apoptosis in HUVECs. SSc significantly inhibited LPS-induced apoptotic cell death in HUVECs through the selective suppression of caspase-3. SSc was also shown to rescue LPS-induced FAK degradation and other cell adhesion signals. Furthermore, the protective effects of SSc against LPS-induced apoptosis were abolished upon pretreatment with a FAK inhibitor, highlighting the importance of FAK in SSc activity. Taken together, these results show that SSc efficiently inhibited LPS-induced apoptotic cell death via inhibition of caspase-3 activation and caspase-3-mediated-FAK degradation. Therefore, SSc represents a promising therapeutic candidate for the treatment of vascular endothelial cell injury and cellular dysfunction [31].

2.7 Antiviral activity

Saikosaponins represent a group of oleanane derivatives, usually as glucosides, that are found in a number of plant families, and saikosaponins (a, b2, c and d) have been shown to be active against human immunodeficiency virus (HIV), measles, influenza virus, herpes simplex virus 14 and varicellazoster virus [32]. In an attempt to find new anti-HCoV compounds, researcher conducted a series of experiments to investigate the antiviral activity and mode of action of saikosaponins against HCoV-229E *in vitro*. In the present study,

saikosaponin b_2 (SSb_2) was found to: (i) inhibit HCoV-229E viral infection at concentrations of 25 mmol/L or less; (ii) inhibit viral attachment to cells in a dose-dependent manner; (iii) block viral penetration into cells; and (iv) interfere with the early stage of viral replication, such as virus absorption and penetration [32-39]. In the time-of-addition studies, SSb_2, at 6 mmol/L, significantly inhibited human coronavirus 229E infection following its addition at various time pre-infection (-4 to -1 h), coinfection (0 h) and post-infection (1–4 h). Furthermore, SSb_2 also showed an inhibitory effect on viral attachment and penetration [32].

Infectious HCV culture systems were used to examine the effect of saikosaponins on the complete virus life cycle (entry, RNA replication/translation, and particle production). SSb_2 inhibited infection by several genotypic strains and prevented binding of serum-derived HCV onto hepatoma cells. Finally, treatment with the compound blocked HCV infection of primary human hepatocytes. SSb_2 may be of value for development as an antagonist of HCV entry and could be explored as prophylactic treatment during the course of liver transplantation [40].

3. Discussion

Bupleurum saponins are the main effective components of *R. Bupleuri*, which is reported to have various biological and pharmacological properties. Their important biological activities have attracted broad interests from both natural products chemists and pharmacologist over the last half century. At present, the deep research on therapeutic effects of single *Bupleurum* saponins, especially in the aspects of metabolic regulation, which has become a focus research, such as, receptor of ion channels and the effect of many links of signal transduction pathway, and so on. However, the research of *Bupleurum* saponins in composition and dosage on the compatibility was still insufficient, and it needs to be strengthened in future. In addition, the development of the SS medicinal effective still has a lot of work to do, such as, the technology of separation and extraction is improved, in order to get more active ingredients. The focus of future work should be on the discovery of more active *Bupleurum* saponins, and the clinical application of these compounds.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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References

[1] V.K. Wong, H. Zhou, S.S. Cheung, T. Li, and L. Liu, *J. Cell Biochem*. **107**, 303 (2009).

[2] L. Chen, F. Zhang, D. Kong, X. Zhu, W. Chen, A. Wang, and S. Zheng, *Chem. Biol. Interact*. **206**, 76 (2013).

[3] Y.H. Yu, W. Xie, Y. Bao, H.M. Li, S.J. Hu, and J.L. Xing, *PloS One* **7**, e50694 (2012).

[4] Y.S. Huang, W. Xie, and B.T. Chen, *Acad. J. First Med. Coll. PLA* **24**, 1379 (2004).

[5] Y. He, Z.F. Hu, P. Li, C. Xiao, Y.W. Chen, K.M. Li, J.Z. Guo, L. Pan, and J.P. Xiong, *J. Chin. Mater. Med.* **33**, 915 (2008).

[6] C.Z. Li, W. Xie, Y. Bao, and Y. Zhou, *J. South. Med. Coll.* **27**, 839 (2007).

[7] Z.Y. Li, Z. Guo, Y.M. Liu, X.M. Liu, Q. Chang, Y.H. Liao, and R.L. Pan, *J. Ethnopharmacol*. **148**, 794 (2013).

[8] W. Xie, Y. Bao, L.J. Yu, G.N. Hou, and H.X. Tan, *J. South. Med. Coll.* **26**, 177 (2006).

[9] X. Zhou, H. Cheng, D. Xu, Q. Yin, L. Cheng, L. Wang, S. Song, and M. Zhang, *Neurochem. Res.* **39**, 2136 (2014).

[10] Y.J. Tsai, I.L. Chen, L.Y. Horng, and R.T. Wu, *Phytother. Res.* **16**, 117 (2002).

[11] V.K. Wong, M.M. Zhang, H. Zhou, K.Y. Lam, P.L. Chan, C.K. Law, P.Y. Yue, and L. Liu, *Evid.-Based Complement. Alt. Med*. **2013**, 745295 (2013).

[12] Y.L. Hsu, P.L. Kuo, and C.C. Lin, *Life Sci*. **75**, 1231 (2004).

[13] B. H. Zhu, R. Pu, G.P. Zhang, M.Y. Li, L.T. Wang, and J.K. Yuan, *Chin. J. Liver Dis.* **19**, 764 (2011).

[14] R.Y. Liu, and J.P. Li, *Eur. Rev. Med. Pharmacol. Sci*. **18**, 2435 (2014).

[15] B.F. Wang, S. Lin, M.H. Bai, L.Q. Song, W.L. Min, M. Wang, P. Yang, H.B. Ma, and X.J. Wang, *Med. Sci. Monit.* **20**, 1340 (2014).

[16] X.L. Jia, S.S. Dang, Y.N. Cheng, X. Zhang, M. Li, Y.P. Li, and S.Y. Li, *J. Tradit. Chin. Med*. **32**, 415 (2012).

[17] X.L. Lu, S.X. He, M.D. Ren, Y.L. Wang, Y.X. Zhang, and E.Q. Liu, *Mol. Med. Rep.* **5**, 637 (2012).

[18] S. Mallick, B.C. Pal, D. Kumar, N. Chatterjee, S. Das, and K.D. Saha, *J. Asian Nat. Prod. Res.* **15**, 1197 (2013).

[19] S.J. Wu, K.W. Tam, Y.H. Tsai, C.C. Chang, and J.C. Chao, *Am. J. Chin. Med*. **38**, 99 (2010).

[20] S.S. Dang, B.F. Wang, Y.A. Cheng, P. Song, Z.G. Liu, and Z.F. Li, *World J. Gastroenterol.* **13**, 557 (2007).

[21] J. Fan, X. Li, P. Li, N. Li, T. Wang, H. Shen, Y. Siow, P.Choy, and Y. Gong, *Biochem. Cell Biol.* **85**, 189 (2007).
[22] S.D. Klein, S. Becker, and U. Wolf, *Forsch. Komplementmed.* **19**, 242 (2012).
[23] L.L. Tan, X. Cai, Z.H. Hu, and X.L. Ni, *J. Integr. Plant Biol.* **50**, 951 (2008).
[24] C.Y. Leung, L. Liu, R.N. Wong, Y.Y. Zeng, M. Li, and H. Zhou, *Biochem. Biophys. Res. Commun.* **338**, 1920 (2005).
[25] Z.L. Ying, X.J. Li, H. Dang, F. Wang, and X.Y. Xu, *Exp. Ther. Med.* **7**, 1354 (2014).
[26] C.N. Lu, Z.G. Yuan, X.L. Zhang, R. Yan, Y.Q. Zhao, M. Liao, and J.X. Chen, *Int. Immunopharmacol.* **14**, 121 (2012).
[27] P. Li, Y. Gong, N. Zu, Y. Li, B. Wang, and F. Shimizu, *Nephron. Exp. Nephrol.* **101**, e111 (2005).
[28] B.F.Wang, Y.A. Cheng, S.S. Dang, *Chin. J. Integr. Tradit. West. Med.* **29**, 425 (2009).
[29] M.J. Jang, Y.S. Kim, E.Y. Bae, T.S. Oh, H.J. Choi, J.H. Lee, H.M. Oh, and S.W. Lee, *Molecules* **19**, 20340 (2014).
[30] A. Liu, N. Tanaka, L. Sun, B. Guo, J.H. Kim, K.W. Krausz, Z. Fang, C. Jiang, J. Yang, and F.J. Gonzalez, *Chem. Biol. Interact.* **223**, 80 (2014).
[31] T.H. Lee, J. Chang, and B.M. Kim, *Biochem. Biophys. Res. Commun*. **445**, 615 (2014).
[32] P.W. Cheng, L.T. Ng, L.C. Chiang, and C.C. Lin, *Clin. Exp. Pharmacol. Physiol.* **33**, 612 (2006).
[33] J. Balzarini, D. Schols, J. Neyts, D.E. Van, W. Peumans, and E.D. Clercq, *Antimicrob. Agents. Chemother.* **35**, 410 (1991).
[34] S. Shigeta, K. Konno, M. Baba, T. Yokota, and C.E. De, *J. Infect. Dis.* **163**, 270 (1991).
[35] D. Cosman, N. Fanger, and L. Borges, *Immunol. Rev.* **168**, 177 (1999).
[36] D.L. Evers, C.F. Chao, X. Wang, Z. Zhang, S.M. Huong, and E.S. Huang, *Antiviral. Res.* **68**, 124 (2005).
[37] R. Snoeck, G. Andrei, J. Neyts, D. Schols, M. Cools, J. Balzarini, and E.D. Clercq, *Antiviral Res*. **21**, 197 (1993).
[38] K. Numazaki, and H. Asanuma, *Vivo* **13**, 239 (1999).
[39] R. Snoeck, L. Lagneaux, A. Delforge, D. Bron, V.D. Auwera P., P. Stryckmans, J. Balzarini, and E.D. Clercq, *Eur. J. Clin. Microbiol. Infect. Dis.* **9**, 615 (1990).
[40] L.T. Lin, C.Y. Chung, W.C. Hsu , S.P. Chang, T.C. Hung, J. Shields, R.S. Russell, C.C. Lin , C.F. Li, M.H. Yen, D.L. Tyrrell, C.C. Lin, and C.D. Richardson, *J. Hepatol*. **62**, 541 (2015).
[41] J. Zhu, C. Luo, P. Wang, Q. He, J. Zhou, and H. Peng, *Exp. Ther. Med.* **5**,1345 (2013).
[42] M. Xu, and S.Y. Rhee, *Trends Plant Sci*. **19**, 619 (2014).
[43] K.H. Park, J. Park, D. Koh, and Y. Lim, *Phytother. Res*. **16**, 359 (2002).
[44] S.S. Yoon, J.W. Seo, S.H. Ann, H.Y. Kim, H.S. Kim, H.Y. Cho, J. Yun, E.Y. Chung, J.S. Koo, and C.H. Yang, *Neurosci. Lett*. **555**, 198 (2013).
[45] W. Xie, Y.H. Yu, Y.P. Du, Y.Y. Zhao, C.Z. Li, L. Yu, J.H. Duan, and J.L. Xing, *Evid.-Based Complement. Alt. Med.* **2013**, 413092 (2013).
[46] S.J. Wu, Y.H. Lin, C.C. Chu, Y.H. Tsai, and J.C. Chao, *J. Med. Food* **11**, 224 (2008).
[47] W. Xie, J. Lin, Z.W. Zhang, Y. Zhou, and Y. Bao, *J. South. Med. Coll.* **28**, 1798 (2008)..
[48] M.J. Hsu, J.S. Cheng, and H.C. Huang, *Br. J. Pharmacol.* **131**, 1285 (2000).
[49] B.F. Wang, Z.J. Dai, X.J. Wang, M.H. Bai, S. Lin, H.B. Ma, Y.L. Wang, L.Q. Song, X.L. Ma, Y.Zan, W.L. Min, and Y.A. Cheng, *BMC Complement. Alt. Med.* **13**, 1 (2013).
[50] X. Jia, S. Dang, Y. Cheng, X. Zhang, M. Li, Y. Li, and S. Li, *J. Tradit. Chin. Med.* **32**, 415 (2012).
[51] C. Hahnvajanawong, S. Ketnimit, K. Pattanapanyasat, N. Anantachoke, B. Sripa, K. Pinmai, W.

Seubwai, and V. Reutrakul, *Biol. Pharm. Bull.* **35**, 1914 (2012).
[52] I. Dobashi, F. Tozawa, N. Horiba, Y. Sakai, K. Sakai, and T. Suda, *Neurosci. Lett.* **197**, 235 (1995).
[53] B.F. Wang, X.J. Wang, H.F. Kang, M.H. Bai, H.T. Guan, Z.W. Wang, Y. Zan, L.Q. Song, W.L. Min, S. Lin, and Y.A. Cheng, *Cell Physiol. Biochem.* **33**, 37 (2014).
[54] Y. Hao, X. Piao, and X. Piao, *Int. Immunopharmacol.* **13**, 257 (2012).
[55] S. Bu, J. Xu, and J. Sun, *Chin. J. Integr. Tradit. West. Med.* **20**, 350 (2000).
[56] J.X. Zheng, K.Q. Lu, D.G. Xia, G.R. Tian, and Z.J. Huang, *Nat. Med. J. China* **90**, 808 (2010).
[57] B.Z. Zhang, X.T. Guo, J.W. Chen, Y. Zhao, X. Cong, Z.L. Jiang, R.F. Cao, K. Cui, S.S. Gao, and W.R.Tian, *Am. J. Chin. Med.* **42**, 1261 (2014).
[58] Z. Zong, K. Fujikawa Yamamoto, T. Ota, X. Guan, M. Murakami, A. Li, N. Yamaguchi, M. Tanino, and S. Odashima, *Cell Struct. Funct.* **23,** 265 (1998).

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Table 1. The pharmacologic activity of SSa.

Tissue or cell type	Observation	Activity	Mechanism of action	Refs:
Rat C6 Glimo Cells	*In vitro*	Atiproliferation and alerting morphology of cell	Induce the differentiation of C6 glioma cellsinto astrocytes	[10]
RAW264.7cells	*In vitro* and *In vivo*	Anti-inflammatory activity	Inhibitory effects on NF-κB activation	[26]
RAW 264.7 cells	*In vitro*	Anti-inflammatory activity response	Inhibiting the MAPK and NF-κB pathways in LPS-stimulated RAW 264.7 cells	[41]
Liver (LX-2cells)	*In vitro*	Treatment of liver disease	Elevated BMP-4 expression	[42]
Rat mast cell	*In vitro*	Inhibitory activity against allergic asthma	Antagonism of the histamine action and inhibition of allergic mediators	[43]
Neuronal system (Hippocampal Neuronal)	*In vitro*	Anticonvulsant	Inhibitions of NMDA receptor current and INaP	[3]
Neuronal system	*In vivo*	Attenuate cocaine-reinforced behavior	Activation of GABAB receptors	[44]
Neuronal system	*In vivo*	Anticonvulsant	Inhibits epileptiform discharges induced by 4AP	[45]
Liver	*In vivo*	Protects against CCL_4-induced liver injury	Attenuating hepatic lipids and lipid peroxidation	[46]
Hippocampal astrocytes	*In vivo*	Hippocampal astrocytes can be inhibited	Inhibition of hippocampal astrocyte activation	[47]

Table 2. The pharmacologic activity of SSb.

Tissue or cell type	Observation	Activity	Mechanism of action	Refs
Rat C6 Glimo Cells	*In vitro*	Anti-proliferation and alerting morphology of cell	Induce the differentiation of C6 glioma cells into astrocytes	[10]
RAW264.7 cells	*In vitro* and *In vivo*	Anti-inflammatory activity	Inhibitory effects on NF-κB activation	[26]
Liver (hepatocyte)	*In vivo*	Inhibiting hepatic fibrosis	Down-regulation of liver TNF-α, IL-6 and NF-κB, p65 expression and the increased I-κBα activity in liver.	[20]
Lymphocytes	*In vitro*	Induction of apoptosis	Increases in c-myc and p53 mRNA levels	[48]
Liver (hepatocellular carcinoma cells)	*In vitro*	Increases the radio sensitivity of smmc-7721 hepatocellular carcinoma	Adjusting the g0/g1 and g2/m checkpoints of the cell cycle	[49]
T cell	*In vitro*	Inhibition of T cell activation	Modulation of PKCh, JNK, and NF-κB transcription factor	[24]
Rat HCC liver tissue. (hepatocellular carcinoma(CC))	*In vivo*	Inhibition of HCC development	Down regulated expression of syndecan-2, MMP-2, MMP-13 and TIMP-2.	[50]
Liver (human hepatoma cell)	*In vitro*	Induction of apoptosis and cell cycle arrest	Decreased the cell proliferation and inducted apoptosis both in p53-postive, Hep G2 and p53-negative Hep 3B cells.	[51]
Rat hypothalamus	*In vivo*	Stimulates both CRF gene expression and CRF release	Not investigated	[52]
Liver	*In vivo*	Increasion radio sensitivity of hepatoma cells	Induced the upregulation of p53 and Bax as well as the down regulation of Bcl-2 by attenuating HIF-1α expression	[53]
Rat basophilic leukemia-2H3 cell	*In vitro*	Anti-allergic activity	Inhibiting rat basophilic leukemia-2H3 cell degranulation by suppressing these critical incidents in the signal transduction pathway	[54]
T cell	*In vivo*	Treating T cell-mediated autoimmune	Involved NF-kB, NF-AT and AP-1 (c-Fos)	[1]

		conditions	signaling pathways in the T cell inhibition	
T lymphocytes	*In vivo*	Anticancer	Inhibit the activated T lymphocytes via suppression of NF-κB, NF-AT and AP-1 signaling	[11]
Human leukemia cells	*In vivo*	Anticancer	Up-regulating GR mRNA expression and inhibiting cell growth	[55]
Chicken embryoe	*In vivo*	Inhibition of the physiological angiogenesis of chicken embryoe	Inhibition on leukocyte migration and activation.	[29]
Pulmonary fibrosis	*In vivo*	Anti-fibrosis	Anti-lipid peroxidation effect	[56]

Table 3.The pharmacologic activity of SSc

Tissue or cell type	Observation	Activity	Mechanism of action	Refs:
HUVECs	*In vitro*	Inhibiting lipopolysaccharide-induced apoptosis	Suppressing caspase-3 activation and subsequent degradation of focal adhesion kinase in human umbilical vein endothelial cells	[57]
Human umbilical vein endothelial cells.	*In vitro*	Treatment of vascular endothelial cell injury and cellular dysfunction.	Inhibiting LPS-induced apoptotic cell death via inhibition of caspase-3 activation and caspase-3-mediated-FAK degradation	[31]
Lung (human non-small cell lung cancer A549 cells)	*In vitro*	Inhibiting the proliferation	Inducing apoptosis and blocking cell cycle progression in the G1 phase.	[31]

Table 4.The pharmacologic activity of SSd

Tissue or cell type	Observation	Activity	Mechanism of action	Refs:
Melanoma cells	*In vitro*	Inducing differentiation in cultured B16 melanoma cells	Down-regulation of the PKC activity	[58]
Hepatoma cells	*In vitro*	Treatment with the compound blocked HCV infection of primary human hepatocytes.	Inhibiting infection caused by several genotypic strains and preventing the binding of serum-derived HCV onto hepatoma cells	[40]

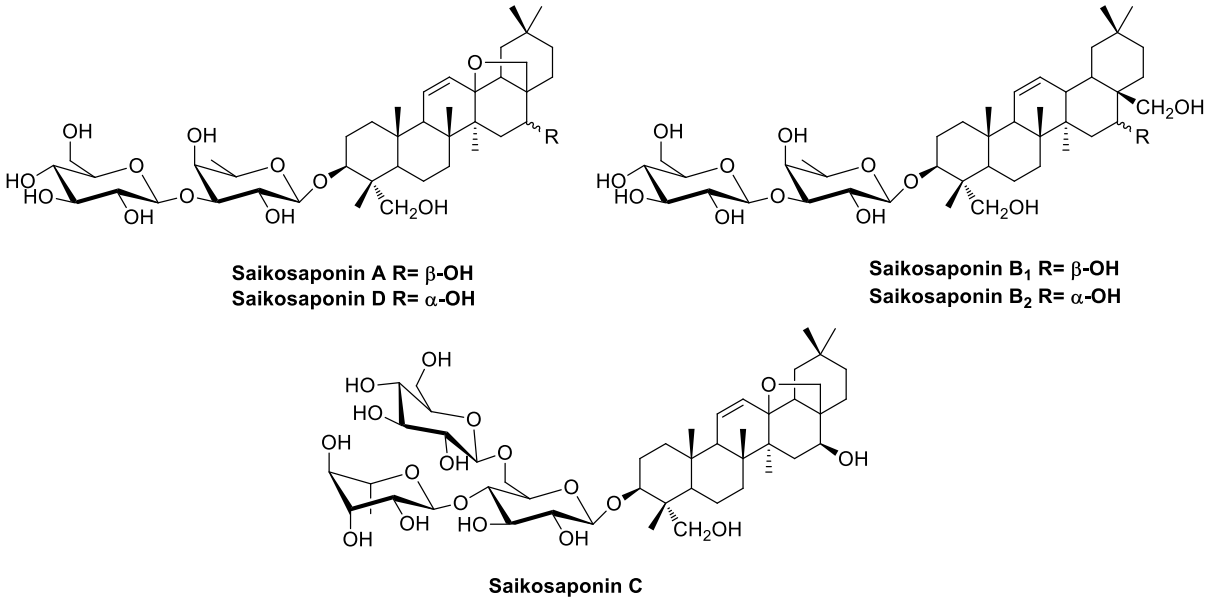

Figure 1. Structures of saikosaponins (A, B, C and D)