

Pharmacological Action of Bupleurum

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Abstract: Bupleurum is one of the oldest and most used Chinese herbal medicines with a long medicinal history and high medicinal value. Modern pharmacological research shows that the radix Bupleurum has some pharmaceutical effects like antipyretic, analgesic, fall hematic fat, anti-cancer, antidepressant, anti-inflammatory, and protection of the heart, liver, and kidney. They play key roles mainly by regulating apoptosis signaling pathways, inflammation, signaling pathways, neuroendocrine system, oxidative stress signaling pathways, fibrosis, signaling pathways. This paper mainly discusses the main medical effects of Bupleurum, including its effects on healing fever, strengthening the immune system, inhibits tumor cell proliferation, inducing tumor cell apoptosis, Saikosaponin D inhibiting tumor cell invasion and metastasis and regulating tumor cells' autophagy.

1. Introduction

Bupleurum is widely distributed in northeast China, north China, northwest China, east China, Hubei, and Sichuan province. Dry Bupleurem tastes bitter and is slightly cold. It belongs to the liver and gallbladder meridian and has the effects like relieving superficies syndrome; antipyretic, soothing the liver, relieving depression, and rising Yang lift depression. It mainly treats fever, chills, heat exchanges, chest and hypochondriac distension pain, irregular menstruation, deficiency of vital energy, uterine prolapse, etc. According to the main pathophysiological changes of the main clinical symptoms of Bupleurum, its "relieving superficies syndrome and reducing fever" effect is mainly related to the infection of the respiratory system. "Liver depression" is related to liver and gallbladder diseases and central nervous system dysfunction. The effect of "Yang lifting depression" is related to the relaxation of the smooth muscle of some organs, such as fever caused by various respiratory infections, liver and gallbladder diseases, gastric ptosis, uterine prolapse, etc. Therefore, the modern study of Bupleurum mainly revolves around these aspects.

2. Antipyretic effect of bupleurem

2.1 Heating mechanism

People getting fever is due to the pyrogen (including bacteria and endotoxin, viruses, fungi, helix microorganisms, such as immune complex, hormone, etc.) by endogenous pyrogen (such as interleukin 1, tumor necrosis factor) for the stimulation of the hypothalamus of the temperature regulating center, lifting the temperature regulation point level, and makes the temperature exceed the normal range.

2.2 Antipyretic effect of bupleurem

In vivo experiments and clinical studies found that the total effective rate of Saikosaponin (figure 1, figure 2) on cooling down fever was as high as 95%, especially for wind-heat exogenous fever. It has poor effects on fever caused by Yin deficiency, but no obvious effect on fever caused by malignant tumor and collagen system disease. [1] It is generally believed that the volatile oil of Bupleurum has a strong antipyretic effect and is the main component of antipyretic. Radix Bupleurum Saikosaponin and saponidins also have antipyretic effect. Xue Yan et al. studied the antipyretic effect of essential oil and

Saikosaponin of Bupleurum on rats heated by subcutaneous injection of yeast, and the results showed that all three had antipyretic effect.

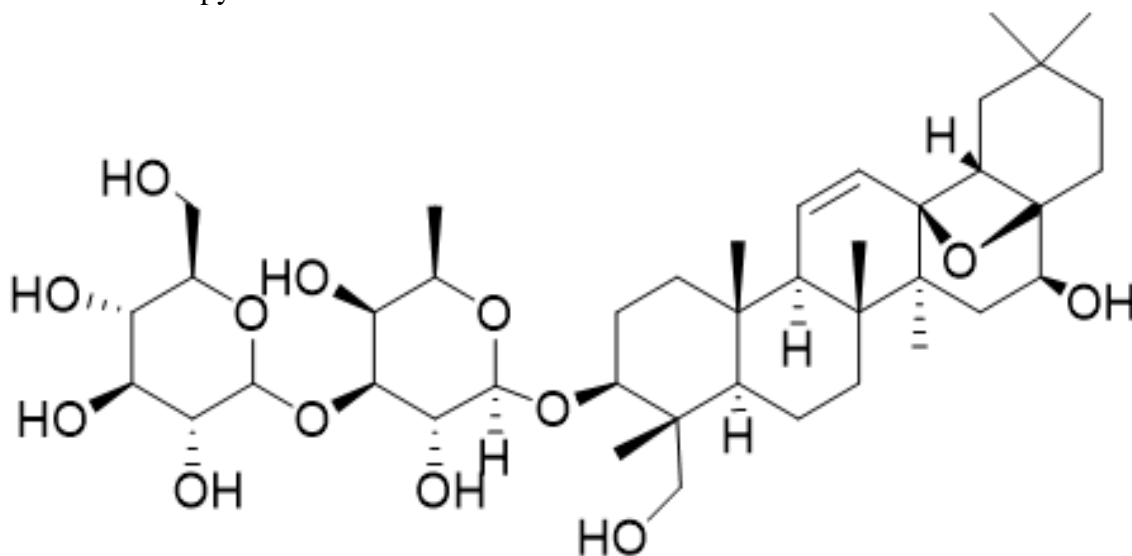


Figure 1. Saikosaponin D.

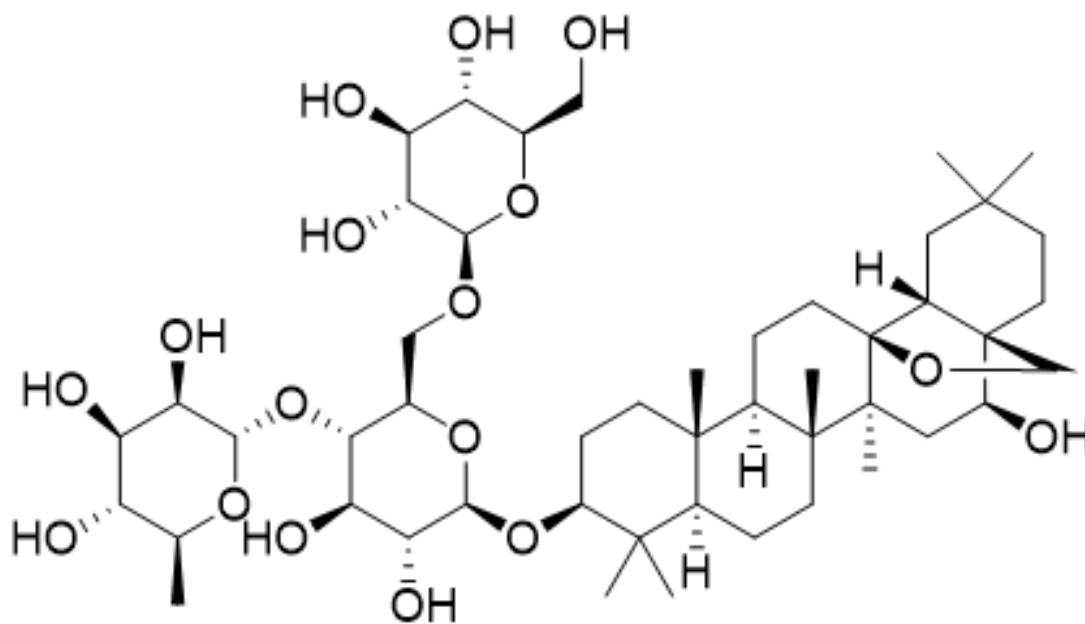


Figure 2. Saikosaponin C.

2.3 Testing antipyretic effect with animal experiments

Compared with the normal control group, the temperature of the model control group increased significantly ($P < 0.01$) [2], indicating that the model was successfully built. Compared with the model control group, the aspirin group had the most significant antipyretic effect 1 and 2 hours after the first administration ($P < 0.01$), and 3 and 4 hours after the first administration, the antipyretic effect was significant ($P < 0.05$). Compared with the model control group, the antipyretic effect of *p*. Bupleurum volatile oil group was extremely significant in each period after the first administration ($P < 0.01$). Compared with the model control group, the antipyretic effect of the Bupleurum group was significant 1 and 2 hours after the first administration ($P < 0.05$), and extremely significant 3 and 4 hours after the first administration ($P < 0.01$); Compared with the model control group, the antipyretic effect of Bupleurum decoction group was significant at each time after the first administration ($P < 0.05$). See table 1.

Table 1. Antipyretic effect of Bupleurum components on dry yeast induced temperature in rats ($\bar{x}\pm s$) °C.

Group	Amount	Initial temperature	fever temperature after 4 h	Body temperature changes after administration			
				1 h	2 h	3 h	4 h
Normal control	10	37.40±0.30	37.50±0.20	0.15±0.17	0.24±0.23	0.32±0.21	0.44±0.14
Model control	10	37.50±0.20	39.30±0.40**	- 0.27±0.32 **	- 0.45±0.40 **	- 0.65±0.52 **	- 0.77±0.35 **
Aspiring	10	37.30±0.20	39.10±0.30	- 2.08±0.31 **	- 1.95±0.44 **	- 1.76±0.55 *	- 1.67±0.57 *
Essential oil	10	37.40±0.40	39.40±0.40	- 2.03±0.48 **	- 2.28±0.51 **	- 2.40±0.43 **	- 2.48±0.37 **
Saikosaponin	10	37.50±0.30	38.90±0.30	- 1.77±0.33 *	- 1.81±0.44 *	- 1.95±0.28 **	- 2.07±0.38 **
Decoction	10	37.20±0.40	39.60±0.50	- 1.14±0.21 *	- 1.25±0.40 *	- 1.34±0.29 *	- 1.41±0.26 *

(Compared with normal control group, ** indicated $P < 0.01$; Compared with the model control group, * represents $P < 0.05$, ** represents $P < 0.01$.)

3. Influence of Bupleurum on immune function

The most important pharmacological action of plant polysaccharides is immune regulation. Plant polysaccharides can regulate immunity in many ways, for instance, by affecting ion channels and cell signal transduction, directly enhancing organ immunity and promoting cytokine release. First of all, plant polysaccharides can enhance or activate macrophages and cellular immune reaction activities, and enhance the immune response of the body to tumor cells. Plant polysaccharides can also activate lymphocytes and play an immunomodulatory role.

3.1 Discovery of BCPS reactions to antibodies

In ZHANG Xing-quan, CHEN Hong-shan's Immuno-pharmacological effects of Bupleurum polysaccharide. BCPS further confirmed and found a variety of immune-promoting effects. BCPS significantly increased the splenic coefficient but had little effect on the thymus coefficient. It increased the titer of serum neutralizing antibody specific to influenza virus several times but did not affect the amount of lysin secreting by splenic fine cells. It is indicated that BCPS has a different reaction to the formation of IgM and IgG antibodies, which needs further investigation. BCPS significantly increased macrophage endocytosis and macrophage function, which is consistent with domestic and foreign reports. BCPS can make the delayed hypersensitivity reaction of mice inhibited by influenza virus show a certain degree of recovery increase the activity of natural killer cells in spleen, and improve the conversion rate of splenic gonorrhea activated by ConA.

4. Antitumor effect of Bupleurum

4.1 Saikosaponin D blocks the cell cycle and inhibits tumor cell proliferation

Cell proliferation is an important character of cell life activities, and infinite proliferation is one of the important characteristics of tumor cells in different from normal cells. Therefore, restraining this infinite proliferation is the key to the treatment of malignant tumors. The cell cycle is closely related

to cell proliferation. Among them, Cyclin, cyclin-dependent kinase (CDK), proto-oncogene C-Myc, tumor suppressor gene p53, and proliferating cell nuclear antigen (PCNA) are of great importance in cell cycle regulation. Cell test showed that Saikosaponin D could effectively inhibit the proliferation of various tumor cells, and the inhibition showed a certain dose or time dependence.

1) Liu Zhihua et al. [3] showed that the proliferation of HeLa cells was significantly inhibited by Saikosaponin D over 10 μ mol/L for 48 h in a dose-dependent manner. Saikosaponin D decreased Cyclin D1 and Cyclin E, and then affected the cell cycle, and blocked HeLa cells in G1 phase.

2) Yang Chunyan et al. [4] detected the effect of Saikosaponin D on the proliferation of human osteosarcoma 143B cells by MTT method and observed that low concentration of Saikosaponin D could significantly inhibit the growth of osteosarcoma 143B cells, with strong activity and dose-dependence.

3) Qinxiang Wu et al. [5] studied the effects of Saikosaponin D on the proliferation of human hepatoma HepG2 cell lines and the formation of hepatoma in nude mice. Compared with the control group, the expression of Ki67 in HepG2 cells in Saikosaponin D group was significantly decreased, and the expression of Cleaved caspase-3 was significantly increased. The tumor volume of Saikosaponin D group was significantly smaller than that of the control group ($P < 0.05$). Saikosaponin D could improve the survival rate of experimental mice, suggesting that Saikosaponin D could inhibit the proliferation of human hepatoma HepG2 cell lines and the formation of hepatoma in nude mice.

4) Yang Kunrong et al. [6] showed through MTT test that Saikosaponin D could significantly inhibit the proliferation of colorectal tumor cells SW480, but had no significant effect on the proliferation of normal colorectal cells FHC. Moreover, Saikosaponin D down-regulated the expression of G2-M cycle regulators CCNA1, CNA2, CCNB1 and CCNB2 at mRNA level. At the protein level, CCNA2, CCNB1 and P34 / CDC2 were down-regulated, p-H3S10 was up-regulated, and p21WAF1/CIP1 was significantly up-regulated. The results showed that Saikosaponin D inhibited the proliferation of COLORECTAL tumor cells SW480 by up-regulating p21WAF1/CIP1 expression and inducing G2-M cycle arrest. Dang Feng et al. investigated the molecular mechanism of Saikosaponin D inhibiting the proliferation of human colorectal cancer cells SW480, and detected the effect of Saikosaponin D on the adenosine-activated protein kinase (AMPK) signaling pathway of SW480 cells, and observed that after treatment with Saikosaponin D, the expression of P-AMPK α and P-ACC were both increased in SW480 cells. The up-regulation of P-AMPK α and P-ACC was inhibited by signal knockdown of AMPK α , and the proliferation of SW480 cells was inhibited by Saikosaponin D knockdown of AMPK α .

These results indicated that Saikosaponin D inhibited the proliferation of human colorectal cancer cells SW480 by activating the AMPK signaling pathway.

4.2 Induction of tumor cell apoptosis.

Apoptosis is an orderly process of cell death regulated by a series of related genes to maintain the stability of the internal environment, which plays an important role in the occurrence and development of tumors [7]. There are many kinds of apoptosis genes in tumor cells, including Caspase family, Bcl-2 family, p53 family and C-Myc family, etc [8]. After these genes are activated, they synthesize related substances required for apoptosis to induce apoptosis by starting apoptosis-related procedures. Through the use of drugs to change tumor cells or their living environment, inducing tumor cell apoptosis in vivo, thereby controlling tumor cell proliferation is the main mechanism of most anti-tumor drugs. Saikosaponin D can regulate the apoptosis of tumor cells by regulating the expression of apoptosis-related genes in various ways. The results of Li Yan et al [7] showed that the apoptosis index of human neuroblastoma SH-SY5Y cells pretreated with 8 ~ 10 μ mol/L Saikosaponin D increased significantly after TUNEL staining, and positive cells could be seen after acridine Orange (AO)/ethidium bromide (EB) staining. Saikosaponin D could induce the apoptosis of SH-SY5Y cells. The mechanism may be related to affecting mitochondrial membrane potential. Zhuang Yifu et al. showed that 25 and 50 μ mol/L Saikosaponin D inhibited the expression of P-I κ B α and P-P65, down-regulated the expression of P-ERK and P-P38, and then inhibited the activation of NF- κ B/MAPK signal transduction pathway. It promoted the expression of Bax and Cleaved caspase-3, reduced the

bcl-2 /Bax ratio, and promoted the apoptosis of osteoma SAOS-2 cells. Western blotting method by Zhao Wei et al. showed that Saikosaponin D could reduce the expression of Calnexin and Calreticulin in McF-7 cells and affect the Calnexin/Calreticulin circulation in McF-7 cells. Increased accumulation of misfolded proteins can cause ER stress (ERS) in McF-7 cells. Meanwhile, Saikosaponin D may increase the expression of GRP78 and CHOP proteins in McF-7 cells, break the ERS balance originally adapted to tumor cells, and activate ERK signaling pathway. It was confirmed that Saikosaponin D could induce the apoptosis of McF-7 cells from the molecular layer. Liu Zhihua et al. showed that 10 μ mol/L Saikosaponin D significantly induced apoptosis of cervical cancer Hela cells by increasing the expression of Bax gene and decreasing the expression of the Bcl-2 gene.

4.3 Saikosaponin D's inhibition to tumor cell invasion and metastasis

Tumor invasion and metastasis is a multi-step, multi-gene regulated process, which is realized through the interaction of various growth factors, cytokines, enzyme systems, signaling pathways and epithelial-mesenchymal (EMT). Invasion and metastasis of tumors are one of the main factors of tumor recurrence, so inhibition of invasion and metastasis of tumor cells is the key to the treatment of malignant tumors. Transwell migration test was used to detect the migration ability of GASTRIC cancer SGC-7901 cells [9]. The results showed that the number of migration cells in each dose of Saikosaponin D group was reduced in a dose-effect relationship, indicating that Saikosaponin D could inhibit the migration ability of SGC-7901 cells. At the molecular level, studies showed that medium and high doses of Saikosaponin D significantly down-regulated the expression of Norrin and Livin in SGC7901 cells, and the mRNA expression was consistent with the protein result, suggesting that Saikosaponin D may reduce the expression of anti-apoptotic factors by reducing the expression of Norrin and Livin. The expression ratio of apoptotic/anti-apoptotic cytokines was broken, resulting in a significantly increased apoptosis rate of SGC-7901 cells, and decreased proliferation and migration capacity of SGC-7901 cells. At the molecular level, studies showed that medium and high doses of Saikosaponin D significantly down-regulated the expression of Norrin and Livin in SGC7901 cells, and the mRNA expression was consistent with the protein result, suggesting that Saikosaponin D may reduce the expression of anti-apoptotic factors by reducing the expression of Norrin and Livin. The expression ratio of apoptotic/anti-apoptotic cytokines was broken, resulting in significantly increased apoptosis rates of SGC-7901 cells, and decreased proliferation and migration capacity of SGC-7901 cells. EMT is a biological process in which epithelial phenotype is transformed into mesenchymal cell phenotype. Under physiological conditions, EMT plays an important role in embryogenesis, organ development and tissue healing. EMT can also be activated under pathological conditions, especially during tumor invasion and metastasis. Ping'an Zhang et al. tested the effects of Saikosaponin D on the migration and invasion ability of human osteosarcoma cell mG-63 by scratch test, Transwell migration test and Transwell invasion test. The results showed that Saikosaponin D with no significant influence on the proliferation of MG-63 cells could significantly inhibit cell migration and invasion ($P < 0.001$). To further investigate the potential molecular mechanism of Saikosaponin D inhibiting cell migration and invasion of MG-63 cells, total protein was extracted after the cells were treated with different concentrations of Saikosaponin D. Western blot analysis of related proteins showed that Saikosaponin D significantly up-regulated the expression of epithelial marker E-cadherin and down-regulated the expression of interstitial marker N-cadherin and Vimentin, inducing EMT changes. The results indicated that Saikosaponin D inhibited the migration and invasion of human osteosarcoma cells mG-63 by reversing EMT. Dang FENG et al. [10] also showed that Saikosaponin D could inhibit the migration and invasion of human colorectal cancer cell SW480 by inhibiting EMT and cell dryness. In the scrape test and Transwell migration test, Saikosaponin D could significantly inhibit the migration ability of SW480. In Transwell migration test, Saikosaponin D also significantly inhibited the invasion ability of SW480. After treatment with Saikosaponin D, the expression of e-cadherin increased, and the expression of N-cadherin and Vimentin decreased. Meanwhile, Saikosaponin D inhibited the formation of clonal spheres in SW480 cells ($P < 0.05$).

4.4 Saikosaponin D regulates tumor cells' autophagy

Autophagy refers to the recycling of proteins to maintain normal cell homeostasis while maintaining cell energy through damaged organelles and turnover proteins. For tumor cells, moderate autophagy can provide energy for cell survival and growth by degrading intracellular macromolecules. Overactivated autophagy leads to excessive consumption of essential proteins and organelles, leading to autophagy death of non-Caspase-dependent cells and inducing tumor cell death. Yang Chunyan detected by Western blot that the expression of autophagy-related protein LC3 ii in HepG2 cells treated with Saikosaponin D increased and showed a dose positive correlation. The formation of autophagosome was observed under laser confocal microscope at 24 h after Saikosaponin D (25 μ mol/L group). Studies have shown that Saikosaponin D can up-regulate the expression of autophagy-related protein LC3 ii and induce autophagy and cell death in HepG2 cells. Wang Yanli et al. [11] showed that autophagosomes appeared in human hepatocellular carcinoma cells PLC, MHCC-97H, MMC-7721 and Huh7 after the intervention of Saikosaponin D for 48h, suggesting that the antitumor activity of Saikosaponin D may be closely related to the autophagy process, and after the action of Saikosaponin D, the expression levels of autophagy-related protein LC3 ii and autophagy specific gene Beclin1 in HCC cells were significantly up-regulated compared with untreated cells ($P < 0.05$). Researchers further explored the mechanism of Saikosaponin D inducing hepatocyte autophagy. Ribosomal protein S6 kinase 1 (S6K1) is the main downstream substrate of mTORC signaling pathway, and the mTORC pathway can be activated by phosphorylation of S6K1. Western blot analysis showed that after Saikosaponin D treatment, the expression level of phosphorylated S6K1 protein (P-S6K1) in cells was significantly decreased, indicating that Saikosaponin D could induce autophagy death of HCC cells through negative regulation of mTORC signaling pathway, thus exerting anti-tumor activity. Studies have shown that Saikosaponin D can induce autophagy in human colorectal cancer SW48 cells, which is manifested in increased LC3A/B ii and LC3A/B ii /I ratio of autophagy-associated egg white, decreased p62 of classical substrate protein of autophagy, and positive LC3 flip test. Moreover, the autophagy inhibitor 3-MA can inhibit the occurrence of autophagy induced by Saikosaponin D. After 3-MA inhibition of autophagy, the inhibitory effect of Saikosaponin D on the proliferation of SW480 cells was weakened. The results suggested that Saikosaponin D could inhibit the proliferation of SW480 cells by inducing autophagy.

5. Conclusion

In this paper, several animal experiments were summarized to illustrate the pesticide effect of Bupleurum, for instance, we used decoction of Bupleurum, Bupleurum volatile oil, Bupleurum saikosid to observe the antipyretic effect of febrile mice in these experimental groups and got the experimental results of their pesticide effects. We also showed the effects of Bupleurum polysaccharide on the human immune system, and its power of protecting the human body from being invaded by tumor cells. We truly hope that not only Bupleurem but also every kind of traditional Chinese medicine can be widely accepted by the world and put into use in larger scope to save more lives.

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