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Comment

The Cape Gooseberry Constituent Physalin B Ameliorates Nonalcoholic Steatohepatitis and Attenuates Liver Fibrosis

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Abstract: Physalin B belongs to a family of Physalins that can be isolated from the genus *Physalis* (*Solanaceae*). In traditional Chinese Medicine, *Physalis angulata* L. is frequently used to treat a variety of illnesses such as dermatitis, trachitis, rheumatism, and hepatitis. Physalin B promotes cellular apoptosis and has antitumor, antimalarial, and antimycobacterial activities. Two recent studies evaluated the therapeutic activities of Physalin B in pre-clinical hepatic disease models. In this comment, a brief summary of the most important findings of these two studies is given and discussed.

Keywords: inflammation; fibrosis; liver disease; steroid; hepatic stellate cells; myofibroblasts

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1. Introduction

Currently, hundreds of pharmacological active, naturally occurring substances have been identified as showing fibropreventive, fibrostatic, or fibrolytic effects in pre-clinical rodent models [1]. In most cases, these compounds counteract intracellular reactive oxygen species (ROS) formation, prevent the infiltration of various immune cell populations into the diseased liver, or target pro-inflammatory and/or pro-fibrotic signaling pathways. Although possessing a variety of pharmacological activities, their limited oral bioavailability, quick degradation in the gastrointestinal tract, poor permeation through the intestinal membrane, or low plasma half-life render these compounds ineffective in humans [2]. Therefore, there are only a few natural compounds that have been tested in clinical trials, including silymarin, glycyrrhizin, curcumin, and resveratrol [2]. Furthermore, most animal studies evaluating potential anti-fibrotic activities are only descriptive in nature and do not provide mechanistic insights.

Physalin B can be isolated from *Physalis angulata* L. This exotic fruit plant belongs to the nightshade *(Solanaceae*) family and grows in subtropical and warm-weather region, such as Central and South America, Africa, India, and the Pacific Islands [3]. Based on its chemical constituents, different *Physalis species* have received high nutritional and pharmacological interest [3]. Beside many phenolic acids with anti-oxidant properties, these plants contain different physalins such as Physalin B. This ingredient has an unusual 13,14-*seco*-16,24-*cyclo*-steroidal ring structure (Figure 1). Together with phenolic acids and flavonoids, these substances have a large variety of pharmacological effects. In traditional Chinese Medicine (TCM), plants from the genus *Physalis* are used for the treatment of tumors, leishmaniasis, eczema, cough, urinary problems, and hepatitis [4]. However, the efficacy of Physalin B in preventing liver steatosis and fibrosis has not been systematically analyzed yet.

In two very recent studies, the laboratory of Ling-yi Kong (Jiangsu Key Laboratory of Bioactive Natural Product Research and State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University Nanjing, China) published the beneficial effects of Physalin B in models of experimental non-alcoholic steatohepatitis (NASH) [5] and hepatic fibrosis [6].

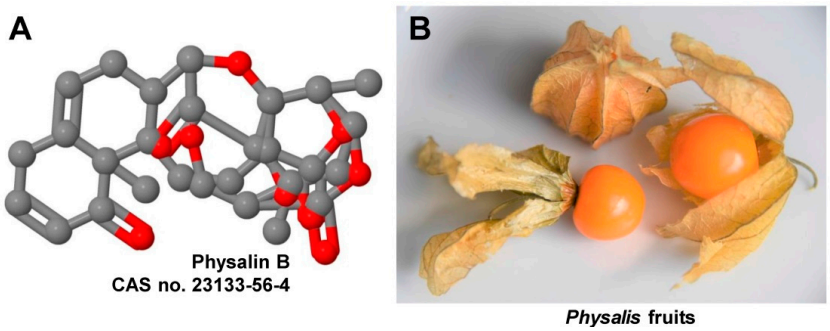

Figure 1. Physalin B: (**A**) The *seco*-steroid Physalin B has a complex highly oxygenated, cage-shaped structure. (**B**) The compound naturally occurs in *Physalis angulata* L. (*Solanaceae*), which produces exotic tasty fruits that expand inside a bladder-like calyx which looks like a small Chinese lantern.

In the first paper, the authors performed experiments in methionine-choline deficient diet (MCD)-induced NASH mice and in cultured human fetal hepatocyte line L02 [5]. The authors showed that orally administered Physalin B significantly ameliorated hepatic injury during MCD, as assessed by lowered transaminase activities, decreased hepatic lipid accumulation, and attenuated ROS formation. Furthermore, the compound increased the expression of the ubiquitin-binding protein p62 and the ratio of microtubule-associated protein light chain 3-II (LC3-II)/LC3-I in vitro and in vivo. In addition, the substance promoted the interaction of the Kelch-like ECH-associated protein 1 (KEAP1) and p62, while reducing the interaction between KEAP1 and NFE2-related transcription factor 2 (NRF2). This resulted in elevated activation and nuclear translocation of NRF2, increased expression of *Nrf2* target genes, and reduced oxidative stress. Moreover, the change in the LC3-II/LC3-I ratio reflected increased autophagic activity, suggesting that the observed beneficial effects of Physalin B on NASH is mediated by induction of anti-oxidative signaling and triggering of autophagic cell death.

In the second paper, the authors analyzed the effects of Physalin B on liver fibrosis in two different in vivo models, namely the carbon tetrachloride (CCl_4) and the bile duct ligation (BDL) models [6]. Mechanistic details in Physalin B activity were investigated in the human hepatic stellate cell (HSC) line LX-2 and in primary mouse HSCs. In both in vivo models, Physalin B attenuated hepatic fibrosis, lowered serum transaminases, and diminished expression of classical fibrosis markers, including collagen type I, tissue inhibitor of metalloproteinase 1 (TIMP1), α-smooth muscle actin (α-SMA), and transforming growth factor-β (TGF-β). In vitro, Physalin B decreased α-SMA expression and reduced TGF-β-induced activity of a luciferase-based collagen gene reporter. Mechanistically, the authors found that Physalin B repressed expression, acetylation, and nuclear translocation of the glioma-associated oncogene 1 (GLI1). As a consequence, the expression of the GLI1 target genes' Hedgehog Interacting Protein (HHIP), Cyclin D, Cyclin E, and c-*myc* were reduced. The authors further demonstrated that the reduction in GLI1 acetylation hindered complex formation between the Lamina-associated polypeptide 2α (LAP2α) and the histone deacetylase 1 (HDAC1), which normally promotes GLI1 deacetylation.

2. Discussion

These two studies suggest Physalin B as a potential new drug candidate for the treatment of NASH and hepatic fibrosis. The therapeutic activity of this compound is mediated by the induction of anti-oxidative signaling, autophagic cell death, or interaction with the GLI1/LAP2α/HDAC1 network that is crucial in the control of fibrogenic marker gene expression. Thus, Physalin B beneficially impacts a broad range of biological processes that contribute to the pathogenesis of hepatic lesions in experimental models of NASH and fibrosis.

Previous studies have shown that Physalin B induces apoptosis in the human HCT116 colon cancer cell line by inducing autophagosome formation and accumulation of LC3-II and p62, underpinning the finding that Physalin B triggers autophagic cell death [7].

Another recent study investigating anti-ulcerative colitis effects has demonstrated that Physalin B suppresses the activation of STAT3, β-arrestin 1, and NLRP3 inflammasome in dextran sulfate sodium-challenged Balb/c mice [8]. It further reduced the levels of TNF-α, IL-6, and IL-1β in lipopolysaccharide-stimulated RAW 264.7 cells [8]. In line, Physalin B acts as an immunosuppressive agent by targeting lymphocyte function and reducing nitric oxide production by macrophages [9,10].

A previous study comparing the biological activities of different physalin members showed that only Physalin B and Physalin F are suitable to block NF-κB activation in phorbol 12-myristate-13-acetate-induced HeLa cells, suggesting that the epoxy ring between carbons 5 and 6, which is lacking in Physalin D, is mandatory for the observed anti-inflammatory activity [11]. In sum, all these findings support the assumption that Physalin B might be a novel potential therapeutic for the treatment of inflammatory and fibrotic diseases.

As such, this compound can be added to the list of substances or mixtures of components with proven beneficial effects in the therapy of experimental fibrosis [1]. Diverse other compounds were previously shown to induce antioxidant and anti-inflammatory activities in rodent disease models, including glutathione, *N*-acetyl-L-cysteine, S-allylcysteine, bucillamine, lipoic acid, taurine, α-tocopherol, ascorbic acid, resveratrol, caffeic acid, genistein, luteolin, quercitin, apigenin, narigenin, and other polyphenolic compounds. However, most of these 'pipeline drugs' have not been exploited systematically in clinical trials yet. There are only a few natural compounds that have been tested in clinical trials as therapeutics for NASH or hepatic fibrosis, including silymarin, glycyrrhizin, curcumin, and resveratrol [2].

In the two studies discussed, Physalin B protected against liver injury without causing toxicity. Even mice that were treated with 300 mg/kg or 600 mg/kg Physalin B showed no histological changes in the heart, kidney, or liver [5]. Most importantly, the compound reduced serum levels of aspartate transaminase (AST), alanine transaminase (ALT), and hepatic triglyceride, as well as total cholesterol content during the pathogenesis of NASH [5]. The association of Physalin B with autophagic cell death [5] and the GLI1/LAP2α/HDAC1 network [6] underpins the notion that Physalin B is more than just a simple antioxidant.

Although these findings are highly encouraging, there are still some critical issues that need attention. In contrast the observed low toxicity, Physalins, and in particular Physalin B, have been shown by others to have a broad cytotoxic activity toward many human cell lines, thus questioning their drug safety [12]. Pharmacokinetics and stability studies have shown that the absorption of Physalin B is limited, and that the substance is quickly transformed by intestinal bacteria [13]. When intravenously administered at 5 mg/kg in rats, Physalin B showed a wide tissue distribution, with a higher penetration in the lung. This was >20-fold higher than that of the heart, liver, spleen, kidney, and brain, suggesting that the lung and not the liver is the main target organ of Physalin B [14]. In addition, the finding that Physalin B interferes with Hedgehog signaling and changes the balance between activator and repressor forms of GLI1 might provoke unwanted side effects. The hedgehog pathway and GLI1 are evolutionarily conserved and play key roles in development processes, wound healing, and in the maintenance of somatic stem cells and pluripotent cells, which are eminently important for tissue repair [15].

In future, it will be necessary to test the curative effectiveness of Physalin B in suitable models. In the discussed studies, the preventive therapeutic effects of Physalin B were only tested in three models of ongoing hepatic damage (i.e., MCD, CCl_4, and BDL). However, therapies are commonly initiated when hepatic damage or fibrosis has already progressed. Last but not least, recovery of normal liver tissue architecture requires liver regeneration and remodeling, as well as other well-orchestrated processes which depend on cell proliferation and differentiation. It is crucial to further demonstrate that the cape gooseberry constituent Physalin B does not interfere with the molecular and cellular mechanisms that drive these coordinated processes.

3. Conclusions

The two studies from Nanjing provide in vitro and in vivo evidence for the beneficial effects of Physalin B in the therapy of hepatic inflammation and fibrosis. The compound acts by inducing anti-oxidative signaling, autophagic cell death, and modulating the GLI1/LAP2α/HDAC1 network that is crucial in the pathogenesis of liver disease. It is now essential to test the curative potential of this drug and to design safety and efficacy studies in healthy adults. This will unmask potentially unwanted side effects and prove whether the proposed therapeutic efficacy of this *seco*-steroid can be integrated into new treatment options.

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References

1. Weiskirchen, R. Hepatoprotective and anti-fibrotic agents: It's time to take the next step. *Front. Pharmacol.* **2016**, *6*, 303. [CrossRef] [PubMed]
2. Lin, L.; Zhou, F.; Shen, S.; Zhang, T. Fighting liver fibrosis with naturally occurring sntioxidants. *Planta Med.* **2018**, *84*, 1318–1333. [CrossRef] [PubMed]
3. de Oliveira, A.M.; Malunga, L.N.; Perussello, C.A.; Beta, T.; Hoffmann Ribani, R. Phenolic acids from fruits of Physalis angulata L. in two stages of maturation. *S. Afr. J. Bot.* **2020**, *131*, 448–453. [CrossRef]
4. Wang, A.; Wang, S.; Zhou, F.; Li, P.; Wang, Y.; Gan, L.; Lin, L. Physalin B induces cell cycle arrest and triggers apoptosis in breast cancer cells through modulating p53-dependent apoptotic pathway. *Biomed. Pharmacother.* **2018**, *101*, 334–341. [CrossRef] [PubMed]
5. Zhang, M.H.; Li, J.; Zhu, X.Y.; Zhang, Y.Q.; Ye, S.T.; Leng, Y.R.; Yang, T.; Zhang, H.; Kong, L.Y. Physalin B ameliorates nonalcoholic steatohepatitis by stimulating autophagy and NRF2 activation mediated improvement in oxidative stress. *Free Radic. Biol. Med.* **2021**, *164*, 1–12. [CrossRef] [PubMed]
6. Zhu, X.; Ye, S.; Yu, D.; Zhang, Y.; Li, J.; Zhang, M.; Leng, Y.; Yang, T.; Luo, J.; Chen, X.; et al. Physalin B attenuates liver fibrosis via suppressing LAP2α-HDAC1 mediated deacetylation of GLI1 and hepatic stellate cell activation. *Br. J. Pharmacol.* **2021**. [CrossRef] [PubMed]
7. Ma, Y.M.; Han, W.; Li, J.; Hu, L.H.; Zhou, Y.B. Physalin B not only inhibits the ubiquitin-proteasome pathway but also induces incomplete autophagic response in human colon cancer cells in vitro. *Acta Pharmacol. Sin.* **2015**, *36*, 517–527. [CrossRef] [PubMed]
8. Zhang, Q.; Xu, N.; Hu, X.; Zheng, Y. Anti-colitic effects of Physalin B on dextran sodium sulfate-induced BALB/c mice by suppressing multiple inflammatory signaling pathways. *J. Ethnopharmacol.* **2020**, *259*, 112956. [CrossRef] [PubMed]
9. Soares, M.B.; Brustolim, D.; Santos, L.A.; Bellintani, M.C.; Paiva, F.P.; Ribeiro, Y.M.; Tomassini, T.C.; Ribeiro Dos Santos, R. Physalins B, F and G, *seco*-steroids purified from Physalis angulata L., inhibit lymphocyte function and allogeneic transplant rejection. *Int. Immunopharmacol.* **2006**, *6*, 408–414. [CrossRef] [PubMed]
10. Soares, M.B.; Bellintani, M.C.; Ribeiro, I.M.; Tomassini, T.C.; Ribeiro dos Santos, R. Inhibition of macrophage activation and lipopolysaccaride-induced death by *seco*-steroids purified from Physalis angulata L. *Eur. J. Pharmacol.* **2003**, *459*, 107–112. [CrossRef]
11. Jacobo-Herrera, N.J.; Bremner, P.; Marquez, N.; Gupta, M.P.; Gibbons, S.; Muñoz, E.; Heinrich, M. Physalins from Witheringia solanacea as modulators of the NF-κB cascade. *J. Nat. Prod.* **2006**, *69*, 328–331. [CrossRef] [PubMed]
12. Fang, L.; Chai, H.B.; Castillo, J.J.; Soejarto, D.D.; Farnsworth, N.R.; Cordell, G.A.; Pezzuto, J.M.; Kinghorn, A.D. Cytotoxic constituents of Brachistus stramoniifolius. *Phytother. Res.* **2003**, *17*, 520–523. [CrossRef] [PubMed]
13. Zheng, Y.; Lin, M.; Hu, X.; Zhai, Y.; Zhang, Q.; Lou, Y.; ShenTu, J.; Wu, L. Simultaneous pharmacokinetics and stability studies of physalins in rat plasma and intestinal bacteria culture media using liquid chromatography with mass spectrometry. *J. Sep. Sci.* **2018**, *41*, 1781–1790. [CrossRef] [PubMed]
14. Zheng, Y.; Chen, J.; Liu, L.; Liang, X.; Hong, D. In vivo pharmacokinetics of and tissue distribution study of physalin B after intravenous administration in rats by liquid chromatography with tandem mass spectrometry. *Biomed. Chromatogr.* **2016**, *30*, 1278–1284. [CrossRef] [PubMed]
15. Doheny, D.; Manore, S.G.; Wong, G.L.; Lo, H.W. Hedgehog signaling and truncated GLI1 in cancer. *Cells* **2020**, *9*, 2114. [CrossRef] [PubMed]