Advances in toxicological studies of Radix Phytolaccae

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Abbreviations
P. ameri-cana L., Phytolacca americana L; EsA, Esculentoside A; CNKI, China National Knowledge Infrastructure; EdB, Esculentoside B; EsC, Esculentoside C; LDDG, Lethal Dose 50; CK, Creatine Kinase; CK-MB, Creatine kinase-MB; PRO, Protein; URO, Urobilinogen; ALT, Alanine transaminase; AST, Aspartate Aminotransferase; ALB, Albumin; NGAL, Neutrophil Gelatinase-Associated Lipocalin; KIM-1, Kidney injury molecule 1; IL, Interleukin; MTT, Methyl Thiazolyl Tetrazolium; L-02, human normal liver cell L 02; IC50,Half maximal inhibitory concentration; KEGG, Kyoto Encyclopedia of Genes and Genomes; HIF-1, Hypoxia-inducible factor; MAPK, Mitogen-activated Protein Kinase; P38-AKT, Phosphatidylinositol 3-Kinase And Protein Kinase B; MAPK1, Mitogen-activated Protein Kinase 1; CASP-3, Caspase-3; p53, Tumor protein p53; TNF, Tumor necrosis factor; Bcl-2, B-cell lymphoma-2; NOD, Nod-like Receptor Signaling Pathway; VEGF, Vascular Endothelial Growth Factor; NF-κB, Nuclear factor-kappa B; JAK-STAT, Janus kinase-signal transducer and activator of transcription; HXC, Human Renal Tubular Epithelial Cell; LDH, Lactate dehydrogenase; SOD, SuperoxideDismutase; MDA, Malondialdehyde; IPSCs, induced pluripotent stem cells; FoxO, Forkhead box O; GC-MS, Gas Chromatography-mass Spectrometry; TGF, Transforming growth factor; ACE, Angiotensin Converting Enzyme; HE, Hematoxylin and Eosin staining; PSA, Prostate Specific Antigen; PGE2, Prostaglandin E2; PCR, Polymerase chain reaction; MUC2, Mucin 2; HPLC, High-Performance Liquid Chromatography; LC-MS, Liquid chromatography-mass spectrometer.

Citation

Abstract
Radix Phytolaccae is the dried root of Phytolacca acinosa Roxb or P. ameri-cana L, which is commonly used as a traditional Chinese medicine to treat diseases like cirrhotic ascites, hepatitis B, nephrotic syndrome, psoriasis , etc. However, there is no exact basis for its clinical application safety. In this paper, the toxic effects and mechanism of Saponin A (EsA), the main component of Radix Phytolaccae, were summarized by searching the results and reports of toxicology related to the plant from 1991 to 2023 on CNKI and pubmed, aiming to provide reference for the toxicological research and future research direction of Radix Phytolaccae, so that Radix Phytolaccae can be safely and effectively used in clinical practice.

Keywords: Radix Phytolaccae; Radix Phytolaccae Saponin A; toxicity; toxicity mechanism
Introduction

Phytolacca Radix is a commonly used Chinese medicine. There are 35 species of Phytolaccae as plant in the world. The dried roots of Phytolacca acinosa Roxb or P. ameri-cana L. are used as medicinal products. It has cold nature, bitter taste, which is toxic and belongs to lung, kidney and other meridians [1]. As a common Chinese medicine, Phytolacca Radix is widely used in clinical treatment of cirrhosis ascites, hepatitis B, nephrotic syndrome, psoriasis and other diseases [2-3]. Phytolaccae Radix contains a large number of saponin compounds [4], which have analgesic, antiinflammatory properties and anti-oxidative effects [5]. In this paper, systemic toxicity, target organ toxicity and mechanism of Saponin A (EsA), the main component of the plant were studied. We summarized recent studies by international scholar in order to provide reference for further research and utilization of P. plantarum.

Chemical composition

The types of compounds contained in Phytolaccae Radixo are mainly triterpene saponins, polysaccharides, flavonoids, phenolic acids, sterols, and some alkaloids and organic acids [6]. Among them, triterpenoid saponins, flavonoids, and phenolic acids are the main pharmacological active ingredients, and modern studies are also more extensive on the above components. Saponin A (EsA) is one of the main toxic components of Saponin, and Saponin B (EsB) and Saponin C (EsC) also have toxic effects.

Toxicity tests study

Acute toxicity

When Phytolacca Radixo aqueous infusion, decoction and tincture were given to mice by gavage, their LD50 values were 26.0, 28.0 and 46.5 g/kg, respectively, while by intraperitoneal injection, their LD50 values were 1.05, 1.3 and 5.3 g/kg, respectively, and at high doses, most of the mice showed reduced activity, slower and weaker breathing and general convulsions within 3 h, and finally died of poisoning [7].

In addition, there have been clinical reports of acute poisoning from Phytolacca Radixo [8], where patients who mistakenly consumed Phytolacca Radixo had varying degrees of symptom of sympathetic excitation and gastrointestinal irritation, mainly manifested as dizziness and headache, nausea and vomiting, abdominal pain, general weakness, etc. Laboratory tests showed elevated leukocytes, cardiac enzymes (CK, CK-MB elevated), tachycardia, urinary routine (PRO, URO abnormal), liver function (ALT, AST elevated, ALB decreased) and other abnormalities [9].

Subacute toxicity

Phytolacca Radixo ater decoction (40, 20 g/kg) was given to rats for 35 consecutive days, and it was detected that the levels of kidney injury markers NGAL, KIM-1 and IL-18 began to increase on the 7th day, and significantly increased on the 14th day, degeneration and necrosis of renal tubular epithelial cells appeared on the 14th day, and protein tubular patterns were visible in the lumen of renal tubules on the 21st day [10]. The expression of renal injury factor KIM-1 was significantly enhanced in the proximal convoluted tubule epithelial cells regenerated after injury. Wenxue Sun gavaged to rats of low, medium and high doses of water extract from the Phytolacca Radix for 120 days continuously, and conducted urine tests weekly. The results showed that compared with the control group, the level of KIM-1 in Phytolacca Radix medium dose group increased significantly on the 28th, 91st, 105th and 112th day. In the high dose group, there was an increasing trend on the 7th day, and it was significantly increased from the 14th day to the 120th day [11], indicating the occurrence of kidney injury. Toxicokinetic study found that the exposure of EsA in rat plasma increased with the increase of dose and administration cycle, and the nephrotoxic effect of Phytolacca was stronger.

Study on toxicity and mechanism of toxicity in specific organs

Hepatotoxicity (Table 1)

Rui Ding et al. extracted the n-butanol part of the plant and fed it to mice for administration. After 4 weeks, the serum biochemical analysis found that the activity of ALT and AST in the serum increased, the arrangement of liver tissue cells was disordered, degeneration and necrosis occurred, and inflammatory cell infiltration occurred around the central vein [12]. The mice were injected with 10mg/kg of Saponin A through the tail vein for 7 consecutive days, and ALT and AST levels in the serum of the mice were significantly increased, and multiple focal necrosis of hepatocytes and hepatocyte regeneration were observed in the liver of the mice under light microscopy [13]. By using MTT assay to measure the effect of different concentrations of Phytolacca Radixo Saponin A culture solution from 150 to 400 μg/mL, we found that the viability of hepatocytes (L-02 cells) was significantly decreased in all groups, and the degree of decrease increased with the increase of concentration. The IC50 was 360.18 μg/mL. The number of hepatocytes decreased and apoptotic and necrotic cells increased in 300 μg/mL group. With the increase of dose, apoptotic and necrotic cells increased significantly.

Xuohan Guo et al., combined network toxicology and molecular docking methods to predict the target of liver injury induced by C. planataram, and conducted enrichment analysis of KEGG pathway for the core target [14]. The results showed that the signaling pathways closely related to liver injury mainly included HIF-1 signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, etc. Involved in the regulation of hepatocyte apoptosis, liver fibrosis and so on. Similar studies have found that EsA has 58 key targets in induced hepatotoxicity, such as ALB, MAPK1, CASP-3, etc. [15]. It was also found in acute toxicity experiments [16] that the mortality rate of zebrafish larvae was increased in a concentration dependent manner when exposed to the solution of Phytolacca Radixo. It was found that ALT and AST values in plasma of Zebrafish larvae significantly increased with the increase of Phytolacca Radixo concentration. Through enrichment analysis of KEGG pathway, it was found that arginine and proline metabolism were the main metabolic pathways related to liver toxicity. The PI3K-Akt signaling pathway is related to environmental information processing, and the p53 and TNF signaling pathways in cell process are related to apoptosis.

Nephrotoxicity

Cong Zhang et al. used MTT method to detect the effect of EsA on the viability of HKC cells and found that EsA had a significant inhibitory effect on the viability of HKC cells when the EsA dose was greater than 0.25mmol/L, and when the EsA dose was increased to 0.6 mmol/L, half of the lethal dose was reached [17]. At the same time, LDH content of HKC cell supernatant was also increased to varying degrees. Qian Zhou et al. also used MTT method to investigate the renal cell toxicity of EsA [18], and found that EsA had a significant inhibitory effect on the viability of renal cells, and increased LDH content in the supernatant of renal cells, decreased SOD content and increased MDA content in the cells. EsA also causes changes in ultrastructure, necrosis and apoptosis of renal cells, suggesting that the mechanism of renal cell toxicity of EsA is related to oxidative stress and apoptosis. In vitro experiments, EsA administration can reduce the activity of intracellular antioxidants and induce cellular oxidative stress. EsA also down-regulates the expression of Bcl-2/apoptosis regulatory protein in kidney tissue, thereby accelerating apoptosis.

It has been found that the use of iPSCs-derived kidney organoids can effectively study the mechanism of EsA nephrotoxicity. iPSCs-derived kidney organoids were treated with EsA doses of 0, 15, 30, and 60μM for 48h, respectively. At the same time, the mice were injected with 25mg kg-1 EsA solution, and it was found that the cell viability decreased dose-dependent after EsA treatment. The relevant mechanism is endothelial damage caused by EsA, which in turn damages mitochondria and causes metabolic disorders. In addition, STING can be activated by translocation of mtDNA into the cytoplasm, triggering inflammatory cascade reactions, and ultimately changing
Table 1 Mechanism of hepatorenal toxicity induced by Cyanoplecta

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renal function and aggravating acute kidney injury, thus leading to renal interstitial inflammation and fibrosis [19]. Based on network pharmacology studies, Xue Yang et al. found that the pathways related to kidney inflammatory injury caused by Phytolaccae Radix mainly included HIF-1, NOD receptor, VEGF, NF-κB, and toll-like receptor signaling pathways. TNF, FoxO and other signaling pathways are closely related to the generation of apoptosis. The metabolism of arginine and proline, as amino acid metabolic pathways, is associated with oxidative stress [20].

Tingting Xu et al. studied metabolomics technology and detected the changes of metabolites in the urine of rats after gavage by gas chromatography-mass spectrometry (GC-MS), and found that the contents of α-ketoglutaric acid, citric acid and malic acid in urine increased, while the contents of isoleucine, creatinine and glycine decreased. It is suggested that the mechanism of kidney injury induced by Phytolaccae Radix is related to the changes of tricarboxylic acid cycle and amino acid metabolism pathway [21]. The levels of inflammatory factors IL-1β, IL-6 and TNF-α and the expression of NF-κB in renal tissue of rats were increased by gavage of Phytolaccae Radix decoction for 49 days. Through genomics, it has been found that the mechanism of kidney injury may be due to the activation of MAPK and JAK-STAT signaling transduction pathways by cytokines such as IL-1β and TGF-β1/β2, leading to inflammatory response and apoptosis [22].

Genotoxicity
Yifei Wang et al. added different concentrations of Phytolaccae Radix liquid into the semen of humans and rabbits respectively, and compared the inhibitory activity of the two, finding that Phytolaccae Radix liquid had a lethal effect on sperm, and human sperm was more sensitive [23]. Li Xiaohong et al. used mouse bone marrow polychromatophil micronucleus test and mouse embryo cell transfer micronucleus test to study the genotoxic effect of Phytolaccae Radix. Four groups of mice were intragastrically treated with Phytolaccae Radix decoction for 5 consecutive days at doses of 1g/kg, 5g/kg, 10g/kg and 20g/kg, respectively. The results showed that the dosage of Phytolaccae Radix water decoction was potentially mutagenic in mice when it was 5g/kg, and the toxic effect was related to the dose [24]. Yi Wenlong et al. added 1.0mL of rat sperm suspension into 1.0mL EsA liquid with different concentrations (0.5, 1.0, 2.0, 4.0g/L-1), and found that EsA had a rapid spermicidal effect when the SSA concentration reached 2.0g/L, and with the increase of the concentration, The decrease of ACE activity in each group also increased [25]. The above results proved that Phytolaccae Radix water decoction had certain reproductive genotoxicity.

Gastrointestinal Toxicity
Some studies have found that the saponins contained in Phytolaccae Radix have strong anti-inflammatory toxicity [26], and these components are also related to the inflammatory stimulation and...
diarrhea caused by Phytolaccae Radix [27].

Lin Chen et al, after a single large dose of Phytolaccae Radix administration in mice, mainly caused lesions in the stomach. In the group of 35.1 g kg\(^{-1}\), local gastric mucosal epithelial cells necrosis, exfoliation and small focal bleeding occurred [28]. Some studies have confirmed that EsA has hemolytic properties, which may be related to gastrointestinal tissue injury bleeding [29]. Mice were intragastric with raw Phytolaccae Radix decoction for 15 consecutive days, and the intestinal mucosa was dissected and stained with HE. It was observed that diffuse infiltration of lymphocytes was observed in the Phytolaccae Radix group, while the goblet cells in the intestinal mucosa were significantly reduced by PSA staining, suggesting that the intestinal mucosa had inflammation and serious injury [30].

The extract of Phytolaccae Radix will cause swelling of the body’s mucosa, accumulate protein exudate, and promote the release of inflammatory mediators PGE2 and NO [31], causing irritation and toxicity to the mucosa. Based on network pharmacology, 52 mapping targets were found to be associated with catharsis [32]. Xiaoming Qi et al. used immunohistochemistry and real-time fluorescent quantitative PCR to detect the expression of MUC2 and aquaporin 9 (AQP9) in the colonic mucosa of rats after the gavage ofalaccae Radix liquid. The results showed that both Phytolaccae Radix Saponin A and Phytolaccae Radix raw product could reduce the expression level of MUC2 in the colonic mucosa of rats, thereby damaging the colonic mucosa. It can also produce cathartic effect by increasing the expression of AQP9 in rat intestinal mucosa [33].

Summary and prospect

Phytolaccae has a wide range of clinical uses, including analgesic, antiinflammatory and anti-inflammatory effects, and can be used to treat various diseases such as nephrotic syndrome and cirrhosis ascites. Many of its pharmacodynamic substances are also toxicological components, so it is of great significance to study its toxicological effects and mechanism in clinical application.

In terms of toxicological effects, studies on hepatotoxicity have been carried out in depth. Phytolaccae officinalis can cause liver and kidney damage by affecting multiple signaling pathways, and most of them are dose-dependent. The genotoxicity of Phytolaccae officinalis is reflected in excessive treatment, and spermicide and mutagenic toxicity can occur only when the concentration reaches a certain level. Gastrointestinal irritation symptoms such as diarrhea are often clinically manifested as adverse reactions such as diarrhea [34]. The clinical use of Phytolaccae is mainly made of vinegar, and vinegar can significantly reduce its toxic effect [35]. Studies have found that after the preparation of Phytolaccae vinegar, except for Phytolaccae Saponin A (EsA), the content of other saponin components has decreased [36], which confirms the scientific nature of vinegar toxicity reduction. At the same time, vinegar can reduce the content of active ingredients, so more in-depth pharmacological and toxicological studies are needed to clarify the exact mechanism of action and explore the optimization of processing technology to achieve the purpose of increasing efficiency and reducing toxicity.

This paper summarized the toxicological studies on EsA, the main component of Phytolaccae, and clarified that the toxicity of Phytolaccae and other mechanisms of action can not only find new indications, but also effectively guide the clinical safe application of Phytolaccae. In addition, the reasonableness and mechanism of toxicity reduction of Phytolaccae processing can be further studied in combination with modern analytical techniques such as HPLC and LC-MSn, so as to make it more scientifically applied in clinic and improve the use value of toxic Chinese medicine.

References


