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Research progress in the study of *Clinacanthus nutans* and treatment of liver diseases

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Author contributions

Qian-Jin Fu is responsible for reviewing the literature and writing the manuscript, while Sheng-Gang Sang is responsible for reviewing the manuscript and providing funding.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

C. nutans, *Clinacanthus nutans*; LUP, Lupeol; mRNA, message RNA; BSS, β-Sitosterol; IVX, Isovitexin; AIM2, Absent in melanoma; ALI, Acute Liver Injury; cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; HBsAg, Hepatitis B surface Antigen; NAFLD, Nonalcoholic fatty liver disease.

Citation

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Abstract

Background: Based on the long history of medicinal value and modern pharmacological research, a systematic collation of the *Clinacanthus nutans* has been carried out with a view to providing useful references for the clinical application of the *Clinacanthus nutans* in hepatoprotection and the research and development of new drugs. **Methods:** The research progress of a large number of domestic and international studies on the therapeutic mechanism of the antiliver diseases of *Clinacanthus nutans* and its active ingredients is summarized. The aim is to explore the greater value of the herb. **Results:** The major chemical constituents with pharmacological activities of the *Clinacanthus nutans* were summarized, and a feasible mechanism of action was compiled and analyzed. **Conclusion:** Many studies have confirmed the great medicinal value of *Clinacanthus nutans* and its active monomer components, especially in the prevention and treatment of liver diseases, which is worthy of more in-depth research, development and utilization. Chinese medicine is the treasure of China, therefore, it should be used in more clinical applications.

Keywords: *Clinacanthus nutans*; active ingredients; mechanisms of action; hepatoprotection

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Background

Clinacanthus nutans (abbreviated as *C. nutans*), meaning "life-saving grass", is a traditional medicinal plant in Southeast Asia and Guangzhou, Guangxi, Fujian, ect. It is a tall herb of the family Acanthaceae [1]. In the world, especially in the tropical and subtropical regions, many Acanthaceae plants are often widely used as folk medicine, most of the leaves into medicine, rich in terpenes, steroids, flavonoids, alkaloids and other active ingredients. Traditionally, *Clinacanthus nutans* has been used for the treatment of bacterial and viral infections, burns and scalds, insect and snake bites, rashes, gout and diabetes mellitus. Several studies have demonstrated its biological activities, including antidiabetic, antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral, and immunomodulatory activities, which are promising for medicinal development [2]. With the deepening of related research, more and more chemical components have been detected, and there are many new advances in the study of pharmacological effects, which are classified and summarized as follows.

Active ingredients

In recent years, the research on the bioactive components of *Clinacanthus nutans* has been increasing due to its therapeutic efficacy and low side effects. Among the compounds isolated and purified from *C. nutans*, more than 130 have been publicly reported, and their main chemical constituents are listed in Table 1 below [3–4]. They mainly include terpenoids, steroids, flavonoids, alkaloids, sulfur-containing compounds, and glycosides, etc. Some of these chemical components have good biological activities. According to the bioinformatics analysis of the herbal medicine-component-target network, the main active components of Lupeol, beta-sitosterol and isovitexin are important nodes in the network, suggesting that its pharmacological properties and therapeutic potentials have a broad development prospect.

Table 1 The summarization of the main chemical composition of *Clinacanthus nutans*

Categories	No.	Chemical composition	Parts of medicine	Biological activity
Terpenoids	1	Lupeol	Whole grass	Anti-cancer, vascular protection,etc
	2	Betulin	Rhizome	
	3	4,5-Dihydroblumenol A	Part above ground	
	4	(3E,6R,7E)-3-Hydroxy-4,7-megastigmadien-9-one	Part above ground	
	5	Grasshopper ketone	Branches and leaves	
	6	Loliolide	Branches and leaves	
	7	Isololiolide	Branches and leaves	
	8	(3S,5R,6S,7E)-5,6-Epoxy-3-hydroxy-7-megastigmen-9-One)	Branches and leaves	
	9	Phytol	Branches and leaves	
	10	(−)-α-Tocospirone	Branches and leaves	
Steroids	11	Stigmasterol	Part above ground	Lipid-lowering, anti-atherosclerosis, etc
	12	Stigmasterol-β-D-glucoside	Part above ground	
	13	Stigmasterol-3-O-β-D-glucopyranoside	leaf	
	14	β-Daucosterol	Rhizome	
	15	p-Sitosterol	Stem and leaf	
	16	β-Sitosterol-3-O-β-glucopyranoside	stems	
	17	β-Sitosterol-3-O-β-glucoside	stems	
	18	β-Sitostenone	Whole grass	
	19	β-Sitosterol palmitate	Whole grass	
Flavonoids	20	Shaftoside	Whole grass	Immunoregulation, antioxidation, etc
	21	Vitexin	Whole grass	
	22	Isovitexin	Whole grass	
	23	Orientin	Whole grass	
	24	Isoorientin	Whole grass	
	25	Apigenin	Whole grass	

Table 1 The summarization of the main chemical composition of *Clinacanthus nutans* (*continued*)

Categories	No.	Chemical composition	Parts of medicine	Biological activity
	26	Saponarin	leaf	
Flavonoids	27	5,7-Dihydroxy-8,2-dimethoxy-flavone-7-0-β-D-glucopyranoside	Part above ground	Immunoregulation, antioxidation, etc
	28	Isomollupentin-7-0-β-glucopyranoside	Part above ground	
	29	Api-genin6,8-di-C-α-L-arabinopyranoside	Part above ground	
	30	9β-Hydroxy-6-methoxy-sesamin	Part above ground	
	31	Epiyangambin	Part above ground	
Coumarins	32	Syringaresinol	Part above ground	Anti-cancer
	33	Seartemin	Part above ground	
	34	Sesangolin	Part above ground	
	35	Indazole	Part above ground	
	36	Aurantiamide	Part above ground	
Alkaloids	37	Aurantiamide acetate	Part above ground	Anti-cancer
	38	Piperine	Part above ground	
	39	1-[1-oxo-5-(3,4-methylenedioxyphenyl)-2E-heptatneyl] -piperidine	Part above ground	
	40	Clinamides A	Whole grass	
	41	Clinamides B	Whole grass	
	42	Clinamides C	Whole grass	
	43	Clinamides D	Whole grass	
	44	Clinamides E	Whole grass	
	45	2-cis-Entadamide A	Whole grass	
Sulfur containing compounds	46	Entadamide A	Whole grass	Anti-oxidation
	47	Entadamide C	Whole grass	
	48	Trans-3-methylsulfinyl-2-propenol	Whole grass	
	49	Clinacoside A	leaf	
	50	Clinacoside B	leaf	
	51	Clinacoside C	leaf	
	52	Cycloclinacoside A1	leaf	
	53	Cycloclinacoside A2	leaf	
	54	(2S)-1-0-linolenoyl-3-O-b-D-galactopyranosylglycerol	leaf	
	55	Methyl β-D-glucopyranoside	Branches and leaves	
Other glycosides	56	Methyl α-D-galactopyranoside	Branches and leaves	Anti-cancer
	57	3-Amino4,5-dihydroxyfuran-2(3H)-one	Part above ground	
	58	Polysaccharide-peptide complex	leaf	

Terpenoids-lupeol

Lupeol (LUP), one of the terpenoids, is currently recognized as a representative active ingredient in *Clinacanthus nutans*. Lupeol is a pentacyclic triterpene with the chemical structure of Lup-20(29)-en-3.beta.-ol, which is currently a hot spot in the research of active ingredients of Chinese herbal medicines, and has a wide range of biological activities, such as anti-inflammatory, antioxidant, anticancer, antidyslipidemic, antiproliferative and apoptotic. Normally, the inflammatory response is a defense reaction of the organism to external stimuli, but if the defense is excessive it will attack the organism and cause diseases. Numerous studies have shown that LUP has significant anti-inflammatory effects, and it can inhibit the inflammatory response and bone loss in an osteoporosis (op) rat model constructed by induction with retinoic acid [5]. LUP has been shown to be a potent inhibitor of the PI3K/AKT/NF-κB signaling pathway, which can inhibit cardiac inflammatory responses by inhibiting PI3K/AKT/NF-κB signaling, which in turn inhibits aortic constriction-induced cardiac hypertrophy in mice in vivo and phenylephrine-induced NRCM hypertrophy of rat cardiomyocytes in vitro [6]. Nowadays, the development of many diseases is closely related to the inflammatory response, so lupeol, which possesses good anti-inflammatory effects, has been found to be of great importance in the treatment of diseases. Lupeol has been shown to induce cytotoxic effects such as antiproliferative, apoptosis-inducing and cell cycle arrest in many human cancer cell lines. A large body of evidence also suggests that lupinol induces cytotoxic effects in human cancer cells through cell signaling pathways, including alteration of pro-apoptotic Bcl-2 related X protein (BAX) and anti-apoptotic B-cell lymphoma-2 (BCL2) proteins, pro-apoptotic schizogenin-activated protein kinase (MAPK) and protein kinase B (AKT) levels [7]. Numerous studies have demonstrated the potential of LUP to inhibit cell migration and cancer cell invasion, and its antitumor activity has been recognized in a number of different cancer cells, including leukemia, lymphoma, colon, ovarian, cervical, prostate, and breast cancers [8]. And a variety of mechanisms have been proposed for lupeol's antitumor activity, including inhibition of glycolysis, up-regulation of cell cycle inhibitors such as p21WAF1 and p27KIP1, and down-regulation of oncogene expression. It has been shown by vivo and vitro experiments that lupeol has an inhibitory effect on the proliferation of hepatocellular carcinoma cells, and its mechanism of action may be realized by increasing the p38 and MEKK1 mRNA and protein in the p38 MAPK signaling pathway, and decreasing the levels of ERK1/2, JNK, c-Jun, and c-MYC mRNA and protein, and inducing cell apoptosis [9]. In recent years, the incidence of cancer diseases is getting higher and higher, but most of the anticancer drugs have problems such as drug resistance, expensive and serious side effects, etc. Chinese medicine can alleviate the side effects of radiotherapy and play a certain role in cancer treatment. In the stage of precancerous lesions, the early intervention of TCM can also nip some malignant tumor cells in the bud.

Steroids-β-sitosterol

There is a natural active substance in steroids - phytosterols, known as the "key to life", the most common and abundant phytosterols include β-Sitosterol (β-Sitosterol, BSS), showing important physiological functions, which is widely distributed in the roots, stems, leaves, seeds and fruits of many kinds of oleaginous plants and traditional Chinese medicine plants. Studies have shown that β-sitosterol interferes with multiple cell signaling pathways including cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardioprotective, and antidiabetic effects, and was found to be non-significantly toxic during the pharmacological screening. Not only that, it also has a modulatory effect on the innate or acquired immune system. It has been reported that β-sitosterol can attenuate rheumatoid inflammation in mice by regulating macrophage polarization, inhibit angiogenesis in rheumatoid synovium through the vascular endothelial growth factor signaling pathway, and inhibit TNF-α-induced proliferation, migration, invasion, and secretion of inflammatory factors of MH7A cells through activation of the PPARα pathway [10–11]. In addition, it was found that BSS could significantly reduce the lipid peroxide content in rat liver tissues, and at the same time, it could up-regulate the activity of intracellular antioxidant enzymes, which could play a protective role against carbon tetrachloride-induced chronic liver disease mediated by oxidative stress [12]. BSS can significantly alleviate peptidoglycan-induced oxidative stress in keratinocytes and reduce intracellular reactive oxygen species levels, and its antioxidant effect is related to the elevated protein expression level of HO-1 [13]. In vivo experiments have demonstrated that β-sitosterol can also inhibit neuroinflammation, neural apoptosis, and oxidative stress through the IL-17-p53 signaling pathway mechanism of action, and improve the cognitive function of Alzheimer's disease (AD) mice [14]. Inflammation is at the root of many chronic diseases such as chronic hepatitis B, obesity, hypertension, atherosclerosis, cancer and Alzheimer's disease. β-sitosterol has been shown to contain a wealth of pharmacological activities, with anti-inflammatory effects being particularly notable, which provides numerous theoretical underpinnings for its use in the treatment of liver disease.

Flavonoids-isovitexin

Isovitexin (IVX), also known as apigenin-6-C-glucoside, is a kind of natural flavonoids, which is a natural bioactive ingredient present in various medicinal plants, with the advantages of easy extraction, stable performance, high bioavailability, weak or even non-toxicity. As a functionally pleiotropic molecule, Isovitexin acts on a variety of intracellular targets and cell signaling processes, and has been shown to possess significant biological effects such as antioxidant activity, anti-inflammatory activity, anti-apoptotic activity, hypoglycemic activity, neuroprotective activity, and so on [15]. It has been shown that Isovitexin exhibits antioxidant capacity by scavenging superoxide anion radicals [16]. Nuclear factor red factor-2-related factor 2 (Nrf2) exerts antioxidant effects by controlling the expression of antioxidant enzymes, and as important regulators of oxidative stress, the HO-1/Nrf2 signaling pathway and NF-κB transcription factors may be potential targets for the action of Isovitexin. Deng et al. found the involvement of the flavonoid isomugulin in ameliorating the inflammatory response through KEGG pathway enrichment and in vitro experiments [17]. Isomonthocarpine is a potent COX-2 inhibitor, which can inhibit the production of pro-inflammatory factors such as PGE2, IL-1 and IL-6 in plasma at non-cytotoxic concentrations, showing strong anti-inflammatory activity [18]. In addition, Isovitexin can prevent and ameliorate various diseases by modulating inflammation-related signaling pathways. Isovitexin inhibits the production of TNF-α, IL-1 and IL-6 in renal tissue by inhibiting the activation of NF-κB [19]. Another study demonstrated that isovitexin can inhibit the NF-κB/COX-2 signaling pathway by promoting Nrf2 expression, which in turn inhibits IL-1β-mediated inflammatory activation and osteoarthritis [20]. In addition, Sun et al found that isovitexin inhibited inflammation through the TLR4/MyD88/NF-κB pathway to alleviate acute gouty arthritis in rats, which may be a potential alternative drug for acute gouty arthritis [21]. PKM2 can promote cellular glucose and energy metabolism, and play an important role in maintaining the metabolic program of cancer cells. Isovitexin can significantly inhibit the expression of PKM2, promote apoptosis of lung cancer cells, and then enhance the anti-tumor activity of chemotherapeutic drugs on lung cancer cells [22]. Another study demonstrated that isovitexin treatment induced endoplasmic reticulum stress in hepatocellular carcinoma cells and subsequently led to apoptosis and autophagy, which in turn inhibited the growth of hepatocellular carcinoma cells [23]. In addition, isovitexin inhibited sphere and colony formation, reduced the number of CD44+ cells, mediated the upregulation of miR-34a to induce apoptosis and inhibited the stem cell properties of hepatocellular carcinoma cells [24]. Isovitexin has been reported to have antioxidant activity comparable to that of α-tocopherol, a chemical that has been found to reduce TNF-α, prostaglandin E2 (PGE2), and COX-2 expression in LPS-activated RAW 264.7 macrophages [25]. In a model of

LPS-induced acute lung injury, isovitexin also attenuated histopathological changes, polymorphonuclear granulocyte infiltration and endothelial cell activation. It also inhibited MAPK phosphorylation, reduced NF-κB nuclear translocation, and upregulated Nrf2 and HO-1 expression in RAW 264.7 cells [26]. It also protects against LPS/D-Gal-induced liver injury by inhibiting oxidative stress and inflammatory responses and reducing cisplatin-induced renal injury [27]. Isovitexin is an active monomer with proven hepatoprotective effects that has been isolated by extraction, and as a potential hepatoprotective agent, isovitexin is very promising and may become a new natural drug for the treatment of liver diseases [28].

Mechanisms of action

According to a wide range of literature and modern pharmacological studies, *Clinacanthus nutans* has a variety of pharmacological activities, the efficacy of the *Clinacanthus nutans* has been fully confirmed and affirmed, and future research will also focus on the investigation of the mechanism of action. The signaling pathways in which the *Clinacanthus nutans* acts mainly include

PI3K/AKT/NF-κB signaling pathways

Studies have shown that the PI3K/AKT/NF-κB signaling pathway is mostly focused on inflammatory diseases and is closely related to inflammatory response and bone homeostasis. Lu et al. found that inhibition of the PI3K/AKT/NF-κB signaling pathway ameliorated cartilage inflammation and other injuries in osteoarthritic mice [29]. The NF-κB signaling pathway is a classical pro-inflammatory pathway, and PI3K/AKT signaling is the upstream element regulating the activation of the NF-κB pathway. Akt is a key downstream effector of PI3K, an intracellular phosphatidylinositol kinase [30–31]. NF-κB is inhibited through inhibition of the PI3K/AKT pathway, which subsequently reduces the release of inflammatory factors and attenuates the inflammatory response. Activation of the PI3K/AKT/NF-κB signaling pathway exists after viral infection, and the PI3K/AKT signaling pathway is involved in the activation of inflammatory factors, such as NF-κB and AP-1 [32]. Inhibition of the PI3K/AKT signaling pathway effectively suppressed COVID-19-associated fibroproliferation, cytokine storm, platelet activation, and inflammation, and ameliorated lung tissue injury in septic acute respiratory distress mice [33]. NF-κB is a downstream factor of PI3K/AKT and is activated by PI3K/AKT through phosphorylation of IκB kinase. NF-κB is a key transcription factor in the production of other inflammatory mediators during human respiratory syncytial virus (HRSV) infection, including RANTES, IL-6, interleukin-11 (IL-11), and intercellular adhesion factor-1 (ICAM-1), thus, NF-κB activation is a central determinant of the inflammatory response triggered by HRSV [34]. During viral infection, NF-κB can increase reactive oxygen species (ROS) production, leading to apoptosis in various tissues [35]. It has also been noted that activation of NF-κB increases the release of IL-1β, IL-6 and TNF-α, which are cytokines associated with apoptosis and inflammation [36–39].

AIM2-caspase-1 signaling pathways

Absent in melanoma 2 (AIM2) was first identified in human melanoma cell lines and was named "absent in melanoma 2" because of its lack of expression in melanoma cell lines and its ability to reverse the phenotype of the tumor. AIM2 is an innate immunoreceptor that specifically senses mutated or misplaced DNA molecules in the cytoplasm. Also, AIM2 is a key receptor for pathogens to sense exogenous DNA accumulated in the cytoplasm [40]. Therefore, AIM2 is also known as a cytoplasmic receptor. During infection, pathogens can induce the release of a variety of PAMPs and DAMPs, and thus host-mediated perception of infection occurs [41]. As a result, AIM2 inflammasome is commonly activated after host infection with pathogens such as bacteria, viruses, fungi and parasites, which in turn induces cytokine maturation, release and cellular pyroptosis, playing a key role in the development of autoinflammation and diseases such as cancer. In a study in a mouse stroke model, histone deacetylase 3 (HDAC3) inhibitors attenuated the inflammatory response by inhibiting the activation of AIM2 inflammatory vesicles in microglia to prevent ischemic brain injury [42]. AIM2 inflammatory vesicles can induce pro-caspase-1 self-shearing to activated caspase-1, which in turn activates IL-1β and IL-18, activating a series of downstream inflammatory responses and promoting the process of myocardial fibrosis. Meanwhile, AIM2 can trigger cardiomyocyte pyroptosis, a specific mode of programmed cardiomyocyte death. AIM2 acts as a cytoplasmic receptor that recognizes aberrant cytoplasmic dsDNA in bacterial, viral, or host cells, leading to activation of the cysteinyl aspartate specific proteinase-1 (Caspase-1) pathway, which promotes the maturation and release of a variety of inflammatory factors,such as Interleukin-1β (IL-1β), which further triggers the inflammatory response, and thus it plays an important role in inflammation caused by the body's intrinsic immune response. Lvy sebrink et al. activated AIM2 by giving mice intravenous injections of synthetic dsDNA reagents and found that it was able to induce the release of large amounts of pro-inflammatory cytokines in mice, which impaired reendothelialization of carotid arteries and led to atherosclerotic plaque formation [43]. In contrast, vascular reendothelialization was not impaired in AIM2 knockout mice. Caspase-1, a member of the caspase family, is an essential "component" for the activation of inflammatory vesicles. Caspase-1 is recognized as both an IL-1β converting enzyme and a key player in the mature secretion of IL-18 [44]. AIM2 expression was found to be increased in HBV-infected liver tissues, and hepatic AIM2 expression levels were positively correlated with Caspase-1, IL-18 expression and liver fibrosis in chronic hepatitis B patients [45].

Research on anti-liver disease of Clinacanthus nutans

Liver disease is an general term for all diseases occurring in the liver and is a huge threat to global public health and a major cause of disease and death worldwide [46]. They include viral hepatitis, alcoholic liver disease, drug-induced liver injury, autoimmune liver disease, cirrhosis, and hepatocellular carcinoma, etc., and generally have five developmental courses: a, liver injury; b, hepatitis; c, liver fibrosis; d, cirrhosis; e, hepatocellular carcinoma. At present, although the liver-protecting drugs used in clinical practice have remarkable efficacy, they have gradually shown many problems in their application, mainly due to high toxic side effects. Modern research has found that, as a traditional Chinese medicine with a long history of medicinal use, *Clinacanthus nutans* has the advantages of safety and high efficiency, and it is effective in the prevention and treatment of related liver diseases and their developmental process.

Anti-acute liver injury

Acute Liver Injury (ALI) is a disease in which liver cells are damaged or necrotic in a short period of time due to a variety of reasons such as microbial infections, toxic chemicals, drug abuse, excessive alcohol consumption, and other causes, resulting in abnormalities in liver function and even liver failure. It is the basis of chronic liver disease and the main initiating factor of cirrhosis and liver cancer. Therefore, timely prevention and treatment of ALI is of great significance for the prevention and treatment of chronic liver disease, cirrhosis, liver cancer and other liver diseases. At present, with the modernization of traditional Chinese medicine, researchers have found that traditional Chinese medicine plays a better clinical efficacy in the prevention and treatment of ALI, therefore, the prevention and treatment of ALI with safe traditional Chinese medicine has gradually become a common goal pursued by researchers. Wang Yao et al. used the fresh juice and aqueous extract of *Clinacanthus nutans* to conduct hepatoprotective experiments on carbon tetrachloride (CCl_4)-induced acute liver injury (ALI) mice, the results showed that *Clinacanthus nutans* extracts were able to dose-dependently reduce the ALT and AST level of model mice, and exerted a certain protective effect on the mice with ALI, and hypothesized that its mechanism might be related to the antioxidant effect [47].

Anti-hepatitis

Hepatitis is a collective term for inflammation of the liver. It is usually an inflammatory condition in which liver cells are damaged and the function of the liver is impaired by a variety of pathogenic factors-such as viruses, bacteria, parasites, chemical toxins, drugs, alcohol, and autoimmune factors. Currently, the most common hepatitis causative factors in China are hepatitis viruses, including hepatitis A, B, C, D, E, and G viruses, of which hepatitis B is the most common. Zhang R et al. demonstrated through animal experiments that *Clinacanthus nutans* could significantly reduce the serum HBsAg level of model mice, which showed the potential to alleviate the exhaustion of immune cells and to carry out immunomodulation [48]. In addition, *S. vulgaris* significantly reduced serum levels of TNF-α and IL-1β in model mice and exhibited excellent anti-inflammatory effects, suggesting that the efficacy of *Clinacanthus nutans* against the progression of HBV is partially attributable to its ability to inhibit the secretion of inflammatory cytokines. In addition, the study also indicated that *Clinacanthus nutans* significantly suppressed cccDNA levels in the nuclei of mouse hepatocytes and demonstrated excellent antiviral properties and long-term efficacy. *Clinacanthus nutans* has shown the ability to regulate key bacteria—*Alistipes*, by remodeling the intestinal flora of mice and limiting the metabolic levels of hippuric acid and bile acids in order to play a specific role in the prevention and treatment of HBV. In a model of acute ulcerative colitis induced by 4% dextrose sodium sulfate, the active ingredient of worrygrass-peonidin reduced the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α [49]. This phytochemical also reduced LPS-induced liver injury in mice by inhibiting the NF-κB pathway [50]. Isovitexin also reduced chronic stress and high-fat diet-induced hepatic fat deposition and liver inflammation in mice. This can be achieved by inhibiting the TLR4-NF-κB signaling pathway [51].

Anti-nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD), also known as metabolic associated fatty liver disease (MAFLD), is a syndrome of hepatic impairment characterized by intracellular fat accumulation and steatosis in hepatocytes, in addition to alcohol and other definitive liver-damaging factors. Therefore, NAFLD can be prevented and treated by modulating the relevant signaling pathways or other pathways that can improve the accumulation of fat in the hepatocytes. In addition, NAFLD is a reversible, chronic liver disease that can be cured by effective treatment, but if left unchecked, it can partially evolve into irreversible liver diseases such as hepatic fibrosis, cirrhosis, and hepatocellular carcinoma, which can jeopardize human health. The results of related studies showed that *Clinacanthus nutans* could significantly reduce the body mass of NAFLD mice, attenuate the degree of inflammation and steatosis in liver tissues, lower the serum ALT, AST, TC and TG levels, and down-regulate the gene and protein expression of TLR4, MyD88, NF-κB, TNF-α, and IL-6 [52]. It is suggested that *Clinacanthus nutans* may promote hepatic lipid metabolism by down-regulating the TLR4/MyD88/NF-κB signaling pathway and play a hepatoprotective and lipid-lowering role, thus alleviating the progression of NAFLD.

Anti-hepatocellular carcinoma

Hepatocellular Carcinoma is the fourth most common cause of cancer-related deaths and one of the most common malignant tumors in China. Clinical treatment of liver cancer is mainly based on chemotherapeutic drugs, but its toxic side effects are large. Plant-derived drugs have unique advantages in preventing the occurrence of liver cancer, alleviating the pain of patients, improving the quality of life, and prolonging the survival time. Among them, paclitaxel, vincristine, and gibberellin are the more successful antitumor drugs. Numerous studies have demonstrated the antitumor effects of the major components in *Clinacanthus nutans*, such as lupeol, betulinol, and isovitexin. Liu Xu et al. established a Heps mouse hepatocellular carcinoma model through in vitro experiments, and confirmed that the n-butanol extracts of *Clinacanthus nutans* (CN-N) had good anti-tumor effects, and a high dose of CN-N increased the survival of the loaded mice, and its tumor-suppressive effects may be related to the induction of apoptosis and inhibition of cell proliferation [53]. After ethanol precipitation by hot water extraction, a novel polysaccharide peptide complex (CNP-1-2) was extracted from *Clinacanthus nutans*, which exhibited growth inhibition of human gastric cancer cells [54]. One of the methanolic extracts of the *Clinacanthus nutans* identified as a triterpene has been shown to have antiproliferative activity against Hep-G2 hepatocellular carcinoma cells [55]. Clinamide D and entadamide C, isolated from the powdered bark of the plant by methanol extraction, also showed anticancer effects in two breast cancer cell lines [56].

Summary and outlook

The excavation of natural, non-toxic liver-protecting drugs is particularly important in the prevention and treatment of liver diseases. As a traditional Chinese medicine with a long history of application, the chemical composition and pharmacological activity of the herb are currently the subject of research. Many researches have confirmed that the herb and its active monomer components are of great medicinal value, especially in the prevention and treatment of liver diseases, which is worthy of in-depth research, development and utilization. In conclusion, it is worthwhile to pay attention to the research and development of hepatoprotective drugs in the later stage of the development of hepatoprotective drugs. However, more in-depth and systematic studies are needed in order to evaluate the clinical application value and advantages of *Clinacanthus nutans* in liver diseases, to provide references for the development and utilization of *Clinacanthus nutans* related hepatoprotective drugs and health products, and to provide reference for the rational utilization of *Clinacanthus nutans* resources. Chinese medicine is the treasure of China, therefore, it should be used in more clinical applications.

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