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Hepatoprotective Diterpenoids Isolated from *Andrographis paniculata*

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ABSTRACT

Andrographis paniculata (Burm.f.) Nees (Acanthaceae), a plant widely used as traditional herbal medicine in many countries, has drawn attention of the researchers in recent years. Its major constituents are diterpenoids and flavonoids. This article reviews the anti-hepatotoxic effects of *A. paniculata* extract and derivative compounds, such as andrographolide, the major active compound, most studied for its bioactivities. Neoandrographolide shows anti-inflammatory and anti-hepatoxic properties. 14-deoxy-11,12-didehydroandrographolide and 14-deoxyabdrographolide have immunostimulatory, anti-atherosclerotic, and anti-hepatotoxic activities. The hepatoprotective activities include 1) inhibiting carbontetrachloride (CCl_4), tert-butylhydroperoxide (t-BHP)-induced hepatic toxicity; 2) acting as cytochrome P450 enzymes (CYPs) inducers; 3) modulating glutathione (GSH) content; 4) influence glutathione S-transferase (GSTP) activity and phosphatidylinositol-3-kinase/Akt (PI3k/Akt) pathway; 5) synergistic effect with anti-cancer drugs induced apoptosis contributing to the bioactivities of *A. paniculata* extracts and isolated bioactive compounds. The articles reviewed suggest that the above compounds could be candidates for research and development as potential hepatoprotective drugs.

Keywords: *Andrographis paniculata*

1. Cytochrome P450 Enzymes and Liver Function

Cirrhosis may result from chronic metabolizing of xenobiotics including drugs, toxins, and chemical carcinogens in the liver. The cytochrome P450 enzymes (CYPs) are known as a superfamily of haemoproteins with a unique spectrophotometric absorbance peak at 450 nm when reduced by a reducing agent and bound by carbon monoxide [1]. Anti-hepatotoxic enzymes include cytochrome P450s (P450) super-family, or normalizing the levels of marker enzymes for the liver function test, such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) and acid phosphatase (ACP) [2]. Phase I and phase II biotransformation enzymes are involved in the metabolic activation and detoxification of various carcinogens. Phase I enzymes convert xenobiotics to active intermediates and phase II enzymes catalyze the conjugation of these active intermediates with endogenous cofactors to increase their water solubility and facilitate their excretion through urine or bile. Glutathione S-transferase (GST) is one of phase II enzymes [3].

P450s, CYP1A1 and CYP1A2 have been shown to be the major enzymes in the metabolism of potential procarcinogens such as polycyclic aromatic hydrocarbons (PAHs) and aryl and heterocyclic arylamines. CYP1A1 is expressed constitutively in several extrahepatic tissues. But, while CYP1A1 expression has been demonstrated in liver after inducer treatment, CYP1A2 is constitutively and inducibly expressed only in the liver. CYP1B1 is a relatively new member of family 1 which is constitutively expressed in steroidogenic tissues, but is not detected in liver, kidney and lung [4]. Cytochrome P450 2C19 (CYP2C19), is a major hepatic CYP isoform involved in metabolism of many clinical drugs such as *S*-mephenytoin [5]. Human cytochrome P450s are concentrated in the liver, the major isoforms include CYP 1A2 (13%), CYP 2C9 and 2C19 (20%), CYP 2E1 (7%), CYP 2A6 (4%), CYP 2D6 (2%) and CYP 3A4 (30%). The 5 CYP isoforms 3A4, 2D6, 2C9, 1A2 and 2C19 account for almost 70% of all drug clearance [6].

[*]WWC and BFL searched the literature and drafted the manuscript. All authors read and approved the final version of the manuscript.
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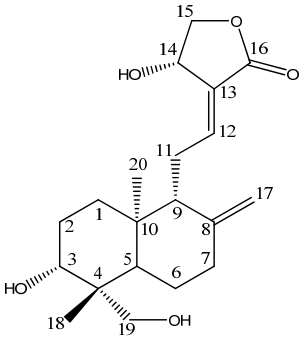
2. Bioactive Constituents from *Andrographis paniculata*

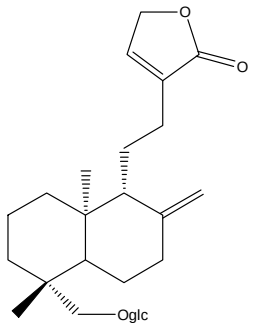
Andrographis paniculata (Burm.f.) Nees (Acanthaceae) has been used as a traditional medicine in Taiwan, China, India and Thailand [7-9]. In traditional Chinese medicine, it is an important cold property herb used for antipyretic properties. And it is commonly used to prevent and treat the common cold [10].

A. paniculata contains diterpenes, lactones and flavonoids. Flavonoids mainly exist in the root, but also can be isolated from the leaves. The leaves contain two bitter lactone andrographolides, and kalmeghin. Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem of *A. paniculata* include over 20 diterpenoids and over 10 flavonoids [11-13]. The active constituents of *A. paniculata* are diterpene lactones, including andrographolide, 14-deoxy-11,12-didehydroandrographolide, neoandrographolide and 14-deoxyandrographolide (**Figure 1**). Andrographolide is the most active and important compound of the plant [14-19]. Neoandrographolide shows anti-inflammatory and anti-hepatoxic properties. 14-deoxy-11,12-didehydroandrographolide and 14-deoxyabdrographolide have immunostimulatory, anti-atherosclerotic, and anti-hepatotoxic activities. Two flavonoids identified as 5,7,2',3'-tetramethoxyflavanone and 5-hydroxy-7,2',3'-trimethoxyflavone were also isolated from the whole plant. Extracts of the plant have been reported to exhibit a wide range of biological activities of therapeutic effects such as anti-inflammation, anti-cancer, immunomodulation, anti-infection, anti-hepatotoxicity, anti-atherosclerosis, anti-hyperglycemic effect and anti-oxidation [20].

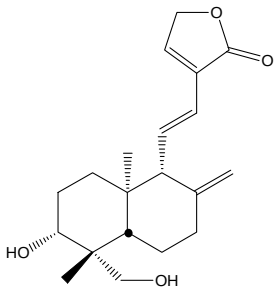
Andrographis paniculata



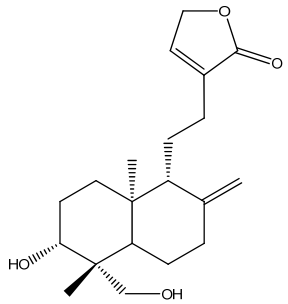
Andrographolide



Neoandrographolide



14-deoxy-11,12-didehydroandrographolide



14-deoxyandrographolide (14-DAG)

Figure 1. Chemical structures of the active compound for *Andrographis paniculata*.

Table 1. Anti-hepatotoxic mechanisms of *A. paniculata* and its bioactive compounds.

Pharmacological properties	References
1. Against CCl_4 and tBHP	
Kalmegh leaf extract ↓ CCl_4 (5 ml/kg) induced hepatic toxicity	[22]
Andrographolide, andrographiside and neoandrographolide (100 mg/kg, *i.p.*) ↓ CCl_4 or tBHP induced hepatic toxicity in serum MDA, GPT, ALP contents in mice	[25]
A.paniculata methanol extract ↓ CCl_4 induced plasma lipid peroxidation, ALT and AST contents	[26]
Andrographolide ↓ acetaminophen induced liver damage in rats	[27,28]
Andrographolide prevents CCl_4 induced acute liver injury via ↑ HO-1 and ↓TNF-α in mice	[29]
A. paniculata (100 - 200 mg/kg) ↓ paracetamol induced hepatotoxicity in mice	[30]
2. Act as CYPs inducers	
A. paniculata extract ↑ mouse hepatic CYP1A1 and CYP2B	[31]
Andrographolide ↑ CYP1A1 and CYP1A2 mRNA expression levels	[32]
Andrographolide ↑ CYP1A1 and CYP1B1 mRNA expression	[33]
A. paniculata 60% ethanol extract or andrographolide ↓ CYP3A, CYP2C9 *in vitro* and CYP2C11 *in vivo*	[34,35]
Andrographolide plus 3-MC synergistically ↑ CYP1 family gene in male B6 mice	[36]
14-deoxy-11,12-didehydroandrographolide and andrographolide ↓ CYP1A2, CYP2D6 and CYP3A4 expression in HepG2 cells	[37]
A. paniculata ethanol and methanol extracts ↓ CYP3A4, CYP2C9; andrographolide ↓ CYP3A4	[38]
Andrographolide and 14-deoxy-11,12-didehydroandrographolide co-treatment with BNF ↑ CYP1A1 expression; but, neoandrographolide co-treatment with BNF ↓ CYP1A1 expression	[39]
Andrographolide (1, 10, 100 μM) ↓ CYP3A4 mRNA and protein levels in Caco-2 cells	[40]
3. Modulate GSH content	
Andrographolide interaction with GSH ↑ BNF induced CYP1A1 mRNA expression in B6 mouse	[41]
A. paniculata ethanol extracts ↓ CYP2C19 activity	[42]
4. Influence GSTP activity and PI3k/Akt pathway	
A. paniculata ethanol, EtOAc extracts and andrographolide ↑ GSTP expression in rat primary hepatocytes	[43]
Andrographolide ↑ GSTP expression is mediated by the PI3k/Akt pathway	[44]
5. Synergistic effects with anti-cancer drugs	
Andrographolide and 5-FU combination treatment ↑ apoptosis in SMMC-7721 cells	[45]
Andrographolide combined with D-penicillamine ↓ copper toxicosis	[46]
Andrographolide (50, 100, 200 mg/kg, *i.p.*) ↓ serum ALT, AST, TNF-α, IL-1β and Bax, cytochrome c in BDL SD rats	[47]
Andrographolide ↑ doxorubicin induced apoptosis via ↓ STAT3 pathway in HepG2 cell	[48]
14-DAG ↓ TNF-α mediated apoptosis in primary rat hepatocytes	[49]
Andrographolide ↑ BSO improved the inhibition of tumor growth in nude mice bearing xenografted Hep3B	[50]
Andrographolide ↓ Con A induced liver injury	[54]

3. *A. Paniculata* and Pure Compounds Anti-Hepatotoxic Mechanisms (Table 1)

3.1. Against Carbontetrachloride (CCl_4) and Tert-Butylhydroperoxide (t-BHP)

It is well known that CCl_4 is converted by cytochrome P450 mixed function oxygenases in smooth endoplasmic reticulum of liver into toxic metabolite, mainly trichloromethyl radical ($CCl_3^{\bullet}$). In the presence of oxygen this free radical can induce peroxidation of lipids on target cells resulting in extensive damage [21]. Choudhury and Poddar reported that oral administration of kalmegh leaf extract (500 mg/kg) or its bitter compound, andrographolide (5 mg/kg), to adult male albino rats produced no significant change in nicotinamide adenine dinucleotide phosphate (NDAPH)-induced hepatic microsomal lipid peroxidation. The data suggests that kalmegh leaf has greater protective effect on carbontetrachloride (5 ml/kg)-induced hepatic toxicity than andrographolide [22].

t-BHP has often been used as a model to investigate the mechanism of cell injury initiated by acute oxidative stress [23]. t-BHP can be metabolized to free radical intermediates by cytochrome P450 (in hepatocytes) or hemoglobin (in erythrocytes), which can subsequently initiate lipid peroxidation [24] and affect cell integrity. Pretreatment of mice with andrographolide, andrographiside and neoandrographolide (100 mg/kg, *i.p.*) reduced CCl_4 or t-BHP-induced malondialdehyde (MDA) levels and release of GPT and ALP in the serum, as effective as the known hepatoprotective agent silymarin [25]. Oral treatment of rats with the *A. paniculata* methanol extract followed by CCl_4 administration restored plasma lipid peroxidation, alanine transaminase (ALT) and aspartate transaminase (AST) levels [26]. Oral or *i.p.* pretreatment with andrographolide was also protective against galactosamine-induced liver damage in rats. On the other hand, protection was also observed when rats were treated with andrographolide post acetaminophen challenge and on an *ex vivo* preparation of isolated rat hepatocytes by increasing the viability of the hepatocytes after parcacetamol-induced toxicity [27,28]. Ye *et al*. demonstrated that andrographolide prevents acute liver injury induced by CCl_4 via induction of heme oxygenase-1 (HO-1) and inhibition of an inflammatory response such as tumor necrosis factor-α (TNF-α) production in mice [29]. Oral administration of *A. paniculata* (100 - 200 mg/kg) exerted a significant dose dependent protection against paracetamol-induced hepatotoxicity in mice [30].

3.2. The Role of *A. paniculata* as CYPs Inducers

A crude extract of *A. paniculata* induce mouse hepatic cytochrome P450 isoforms CYP1A1 and CYP2B via increases in ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase activities [31]. Andrographolide, a single substance extracted from *A. paniculata*, was further demonstrated to significantly up-regulate the CYP1A1 and CYP1A2 mRNA expression, as did the CYP1A inducers such as benzanthracene and β-naphthoflavone [32]. Andrographolide significantly induced the expression of CYP1A1 and CYP1B1 mRNAs in a concentration-dependent manner, and also synergistically induced CYP1A1 expression with the typical CYP1A inducers [33].

In addition, the *A. paniculata* 60% ethanol extract or andrographolide may cause herb-drug interactions through CYP3A and CYP2C9 inhibition *in vitro* or CYP2C11 inhibition *in vivo* [34,35]. A synergistically enhanced the expression of CYP1 family gene after andrographolide plus 3-methylcholanthrene (3-MC, a polycyclic aromatic hydrocarbon) treatment in male B6 mice. They demonstrated that a male hormone associated system to have a positive role in the synergistic effect [36]. 14-deoxy-11, 12-didehydroandrographolide and andrographolide have been shown to inhibit CYP1A2, CYP2D6 and CYP3A4 expressions in HepG2 cells [37]. *A. paniculata* ethanol and methanol extracts inhibited CYP3A4 and CYP2C9 activities more than aqueous and hexane extracts. On the other hand, andrographolide was found to weakly inhibit CYP3A4 activity [38]. Molecular docking analysis data supported that andrographolide and 14-deoxy-11,12-didehydroandrographolide induced CYP1A1 expression or co-treatment with CYP1A1 inducer (β-naphthoflavone, BNF) showed a synergistic increase expression of CYP1A1. In contrast, neoandrographolide suppressed BNF induced CYP1A1 expression [39]. Qiu *et al.* study the herb-drug interactions in combination therapy. They demonstrated that andrographolide (1, 10, 100 μM) significantly down regulates the mRNA level and protein level of CYP3A4 in Caco-2 cells [40].

3.3. The Modulatory Effect on Glutathione (GSH) Content

GSH is abundant in liver cells and a major protective factor against oxidative stress. Andrographolide interaction with GSH significantly enhanced the BNF inducible CYP1A1 mRNA expression in C57BL/6 mouse hepatocytes [41]. Pan *et al*. reported that *A. paniculata* ethanol extract weakly inhibited CYP 2C19 activity [42].

3.4. Influence Glutathione S-Transferase (GSTP) Activity and Phosphatidylinostol-3-Kinase/Akt (PI3k/Akt) Pathway

The π class of GST (GSTP) belongs to the cytosolic class. GSTP is not expressed in healthy liver but it is increased in both chemically induced and spontaneously arising precancerous lesions and hepatomas in experimental carcinogenesis studies [3]. Induction of drug-metabolizing enzymes is considered to be an adaptive response to a

cytotoxic environment. The *A. paniculata* ethanol extract, EtOAc extract and andrographolide induce the expression of GSTP, a phase II biotransformation enzymes involved in detoxification of various classes of environmental carcinogens, in rat primary hepatocytes [43]. Furthermore, they also demonstrated that andrographolide induced GSTP expression is mediated by the PI3K/Akt pathway in SD rat primary hepatocytes [44].

3.5. The Role in Apoptosis Pathway

The combination of andrographolide and anti-cancer drug 5-fluorouracil (5-FU) could enhance the apoptosis in hapatocellular carcinoma (SMMC-7721) cells through a caspase-8 dependent mitochondrial pathway involving p53, Bax, cytochrome c, caspase-9 and caspase-3 [45]. Copper accumulation within the hepatocyte results in oxidative stress and promotes apoptosis. One study demonstrated that andrographolide combined with D-penicillamine could decrease caspase-8 activation and upstream events of caspase-3 activation and inflammatory cytokines production for treatment of copper toxicosis [46]. Bile duct ligation (BDL) could induce hepatic fibrosis. The BDL SD rats treated with andrographolide (50, 100, 200 mg/kg, *i.p.*) significantly reduced serum ALT, AST, TNF-α and interleukin-1β (IL-1β) levels and Bax, cytochrome c [47]. Zhou *et al.* reported that andrographolide promoted anti-cancer drug doxorubicin-induced apoptosis in HepG2 cell, indicating that andrographolide enhances cancer cells to anticancer drug doxorubicin *via* signal transducers and activators of transcription-3 (STAT3) pathway suppression [48]. 14-deoxyandrographolide (14-DAG), a bioactive compound of *A. paniculata*, was shown to protective on TNF-α-mediated apoptosis. Pre-treatment of primary rat hepatocytes with 10 nM 14-DAG accentuated microsomal Ca-ATPase activity through induction of NO/cGMP pathway and desensitizes hepatocytes to TNF-α-mediated apoptosis through the release of tumor necrosis factor receptor 1 (TNFR1) [49]. In nude mice bearing xenografted Hep3B tumors, buthionine sulfoximine (BSO, an inhibitor of cellular GSH biosynthesis) improved the inhibition of tumor growth by andrographolide [50]. Concanavalin A (Con A)-induced hepatitis model was widely used for the investigation of immune mediated acute liver injury [51-53]. Shi and coworkers demonstrated that andrographolide attenuated Con A-induced liver injury through reducing oxidative stress and inhibited hepatocyte apoptosis [54].

4. Conclusion

The major compounds andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide and 14-deoxyandrographolide of *A. paniculata* are most well studied for both bioactivities and functional mechanisms. They exert anti-hepatotoxic actions, such as 1) inhibiting CCl_4, t-BHP induced hepatic toxicity; 2) acting as CYPs inducers; 3) modulating GSH content; 4) influencing GSTP activity and PI3k/Akt pathway; 5) synergistic effects with anti-cancer drugs inducing apoptosis are suggested to be the bioactivities of *A. paniculata* extracts and isolated active compounds. With more *in vitro* and *in vivo* studies, the *A. paniculata* extracts and purified active compounds might be developed into potential anti-hepatotoxic drug that warrant further research and development in the future.

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Abbreviations

CYPs: cytochrome P450 enzymes;
GPT: glutamate pyruvate transaminase;
GOT: glutamate oxaloacetate transaminase;
ALP: alkaline phosphatase;
ACP: acid phosphatase;
GST: Glutathione S-transferase;
PAHs: polycyclic aromatic hydrocarbons;
CCl_4: carbontetrachloride;
tBHP: tert-butylhydroperoxide;
NDAPH: nitotinamide adenine dinucleotide phosphate;
MDA: malondialdehyde;
ALT: alanine transaminase;
AST: aspartate transaminase;
HO-1: heme oxygenase-1;
TNF-α: tumor necrosis factor-α;
3-MC: 3-methylcholanthrene;
BNF: β-naphthoflavone;
GSH: glutathione;
GSTP: glutathione S-transferase;
PI3k/Akt: phosphatidylinositol-3-kinase/Akt;
5-FU: 5-fluorouracil;
BDL: Bile duct ligation;
IL-1β: interleukin-1β;
STAT3: signal transducers and activators of transcription-3;
14-DAG: 14-deoxyan-drographolide;
TNFR1: tumor necrosis factor receptor 1;
BSO: buthionine sulfoximine;
Con A: concanavalin A

