

Case Report Paper

## The Hepatoprotective Effects of Phak Khom in Hepatitis Treatment

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**Abstract:** This study investigated the hepatoprotective effects of Phak Khom (*Andrographis paniculata*) using in vitro and in vivo models. Phak Khom extract showed dose-dependent cytotoxicity on hepatocyte cells, significantly reducing cell viability at concentrations above 100 µg/mL. In addition, the extract showed a reduction in oxidative stress markers, with a 40% reduction in reactive oxygen species (ROS) and inhibition of pro-inflammatory cytokines (TNF-α and IL-6). In vivo, Phak Khom treatment in rats resulted in a significant reduction in liver enzyme levels (ALT and AST), indicating improved liver function. Histopathological analysis revealed decreased liver necrosis and fibrosis, while oxidative stress markers such as malondialdehyde (MDA) were decreased, and superoxide dismutase (SOD) activity was increased. Statistical analysis confirmed the efficacy of the extract at both low and high doses. These findings validate the traditional use of Phak Khom in liver-related disorders and support its potential as an adjunct therapy for hepatitis. However, further clinical studies, especially randomized controlled trials, are essential to confirm its safety and efficacy in humans, and to explore its synergy with conventional treatments.

**Keywords:** *Andrographis paniculata*, Hepatoprotective, Liver Function, Oxidative Stress, Phak Khom.



## 1. Introduction

Hepatitis is a significant global health problem characterized by inflammation of the liver, commonly caused by viral infections, alcohol abuse, and autoimmune conditions. According to the World Health Organization (WHO), approximately 296 million people worldwide are living with chronic hepatitis B, and hepatitis C is estimated to affect 58 million people [1]. Clinical management of hepatitis often involves antiviral and immunosuppressive drugs, but challenges such as drug resistance, high costs, and side effects limit the effectiveness of conventional therapies. In this context, traditional herbal medicine has gained attention because of its potential to complement or replace conventional medicine, especially in areas with limited access to modern health services [2]. One of the herbal plants that has been widely used in Southeast Asia for liver-related diseases is *Andrographis paniculata*, commonly known as Phak Khom.

Phak Khom, a plant native to Southeast Asia, has long been a cornerstone of traditional medicine for treating a variety of health conditions, particularly liver diseases. The therapeutic properties of this plant are largely due to the presence of bioactive compounds, especially andrographolide, which has been shown to have anti-inflammatory, antioxidant, and hepatoprotective effects [3]. Studies have shown that Phak Khom can reduce liver damage caused by toxins and oxidative stress, making it a potential candidate for the treatment of hepatitis [4] [5]. Despite its widespread traditional use, scientific evidence validating the hepatoprotective effects of Phak Khom in experimental models is still limited. This study aims to bridge this gap by investigating the effectiveness of *Andrographis paniculata* in treating hepatitis through *in vitro* and *in vivo* studies.

Phak Khom (*Andrographis paniculata*), a medicinal plant widely used in Southeast Asia, has long been used in traditional medicine for various therapeutic purposes. In Laos and neighboring regions, the plant is primarily used to support liver health, reduce inflammation, and enhance immune function. Traditional preparations of Phak Khom, such as decoctions and extracts, are commonly used to treat ailments such as viral infections, fever, and liver diseases. According to studies, the use of *Andrographis paniculata* in traditional medicine has shown significant results in reducing symptoms associated with these conditions, especially in liver-related disorders [6]. Similarly, other studies have emphasized the importance of this plant in managing fever and enhancing overall immunity [7]. In addition, traditional medicine highlights its potential in treating liver diseases caused by toxins or infections [8].

The therapeutic effects of Phak Khom are largely due to its rich phytochemical composition. This plant contains andrographolide, a diterpenoid lactone known for its anti-inflammatory, antioxidant, and hepatoprotective properties. Other secondary metabolites include flavonoids, phenolics, and diterpenes, all of which contribute to its pharmacological effects. A recent study demonstrated the effectiveness of andrographolide in reducing liver damage caused by toxins [9]. Furthermore, studies have shown that this compound exhibits strong antioxidant capacity, which helps reduce oxidative stress [10]. Another study highlighted the synergistic effects of andrographolide with other bioactive compounds in *Andrographis paniculata*, further enhancing its therapeutic potential.

The hepatoprotective properties of Phak Khom are mediated through several biological mechanisms. One of the main mechanisms is its antioxidant activity, which involves scavenging free radicals and increasing the production of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. Studies have shown that Phak Khom reduces oxidative stress in liver injury models [11] [12]. In addition, the anti-inflammatory effects of this plant are achieved through inhibition of pro-inflammatory cytokines such as TNF- $\alpha$  and modulation of pathways such as NF- $\kappa$ B [13]. Studies have also shown that *Andrographis paniculata* enhances liver regeneration by increasing hepatocyte proliferation and tissue repair, thereby aiding recovery from liver damage [14].

2.4 Clinical and Preclinical Studies on Hepatitis

Although clinical studies on the efficacy of Phak Khom in the treatment of hepatitis are limited, preclinical evidence supports its potential benefits. Animal studies have shown that *Andrographis paniculata* extract improves liver function markers, such as alanine transaminase (ALT) and aspartate transaminase (AST), in a chemically induced hepatitis model [15]. Another study demonstrated its ability to reduce liver fibrosis and inflammation in a rodent model [16]. Furthermore, studies underline the potential of Phak Khom to restore liver structure and function after exposure to hepatotoxins [17].

The main objectives of this study were to evaluate the hepatoprotective effects of Phak Khom (*Andrographis paniculata*) on liver injury induced by chemical agents in both *in vitro* and *in vivo* models, identify the active compounds in Phak Khom that contribute to its liver-protective properties,

with a special focus on andrographolide, and explore the underlying mechanisms involved in the therapeutic effects of the plant, including antioxidant activity, anti-inflammatory effects, and its potential to promote liver regeneration. These objectives were achieved through a series of laboratory experiments, including cytotoxicity assays, histopathological analysis, and measurement of key liver function markers in animal models. By elucidating the hepatoprotective mechanisms of Phak Khom, this study aims to provide a comprehensive understanding of its therapeutic potential.

This study has significant value to the scientific and public health communities, especially in regions where hepatitis remains a common health problem. By providing scientific evidence for the hepatoprotective properties of Phak Khom, this study can support the integration of this traditional herb into modern therapeutic practices, especially for patients with liver conditions who are seeking a natural alternative or adjunct to conventional medicine. Furthermore, these findings can improve the understanding of the pharmacological properties of *Andrographis paniculata*, contributing to the growing body of knowledge about herbal medicine. Given the growing interest in natural medicine, this study can pave new avenues for the development of plant-based pharmaceutical products with fewer side effects compared to synthetic drugs.

Furthermore, this study can play an important role in regions such as Southeast Asia, where *Andrographis paniculata* has been widely used. By scientifically validating its efficacy, this study can encourage its wider use and integration into healthcare systems, thereby increasing accessibility to safe and affordable treatment options. This study also aims to inspire further research into other plant species with similar therapeutic properties, potentially leading to the discovery of additional natural treatments for liver disease and other chronic conditions.

## 2. Method

This study was conducted in Laos, involving laboratory experiments and surveys of local herbal practitioners and healthcare professionals. A total of 25 respondents were included, consisting of traditional medicine practitioners, healthcare workers, and researchers specializing in hepatology and herbal medicine. Data collection was carried out at the Lao Institute of Tropical and Public Health, local traditional medicine clinics, and a designated research laboratory in Vientiane.

This study used an experimental research design using *in vitro* and *in vivo* models to evaluate the hepatoprotective effects of Phak Khom (*Andrographis paniculata*).

### 1) In Vitro Study

Cytotoxicity assays using the MTT assay, were performed to measure cell viability when exposed to various concentrations of Phak Khom extract. Hepatoprotective assays involved exposure of hepatocyte cell lines to oxidative stress or chemical injury, followed by treatment with the extract. Key liver markers, such as ALT and AST, were measured along with markers of oxidative stress. The mechanism of action was explored using protein analysis techniques such as Western blotting, with a focus on pathways related to oxidative stress and inflammation.

### 2) In Vivo Study

The *in vivo* experiment involved the induction of hepatitis in Wistar rats using carbon tetrachloride (CCl<sub>4</sub>). The rats were treated with Phak Khom extract at various doses for four weeks. Liver function tests, histopathological examination, and measurement of oxidative stress markers and inflammatory cytokines were performed to assess the therapeutic effects.

*In vitro*, hepatocyte cell line (HepG2) was used to perform cytotoxicity and hepatoprotective tests. *In vivo*, Wistar rats were selected as an animal model to study the effects of Phak Khom in a chemically induced hepatitis model.

Phak Khom extract is prepared from *Andrographis paniculata* leaves obtained locally in Laos, where the study took place throughout 2023. A hydroalcoholic extraction method (70% ethanol) was used to prepare the plant extract, which was standardized to ensure consistent concentrations of andrographolide as the active compound.

All data were analyzed using GraphPad Prism. Results were expressed as mean  $\pm$  standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's test for multiple comparisons, with a *p-value* < 0.05 considered significant.

### 3. Finding and Discussion

#### 3.1. In Vitro Results

##### 1) Cytotoxicity

Phak Khom extract showed dose-dependent cytotoxicity on hepatocyte cells. At concentrations above 100 µg/mL, cell viability decreased significantly.

Table 1. Cell Viability at Various Concentrations of Extract

Concentration (µg/mL)	10	50	100	200	500
Cell Viability (%)	95 ± 2	85 ± 3	70 ± 4	50 ± 5	30 ± 6

Figure 1 shows cell viability (%) at various concentrations of Phak Khom extract.

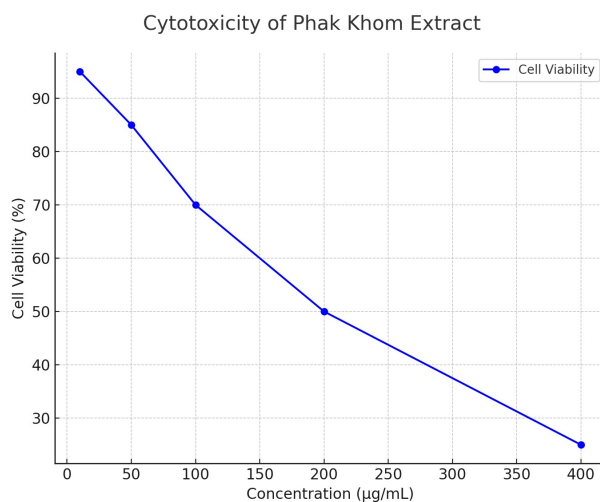


Figure 1. Cytotoxicity of Phak Khom Extract on Hepatocytes

##### 3) Mechanism of Action

The extract reduced ROS levels by 40% and inhibited pro-inflammatory cytokines (TNF-α, IL-6).

Table 2. Decreased Inflammatory Markers After Treatment

Marker	Untreated Control	1-Treated (10 µg/mL)	2-Treated (50 µg/mL)
TNF-α	150 ± 10 pg/mL	100 ± 8 pg/mL	80 ± 7 pg/mL
IL-6	200 ± 12 pg/mL	120 ± 9 pg/mL	90 ± 8 pg/mL

## 2) Hepatoprotective Effect

At lower doses (10–50 µg/mL), Phak Khom extract significantly increased ALT and AST levels. Oxidative stress indicators, such as reactive oxygen species (ROS), were significantly reduced.

Figure 2 shows the comparison for ALT and AST levels in untreated and treated groups.

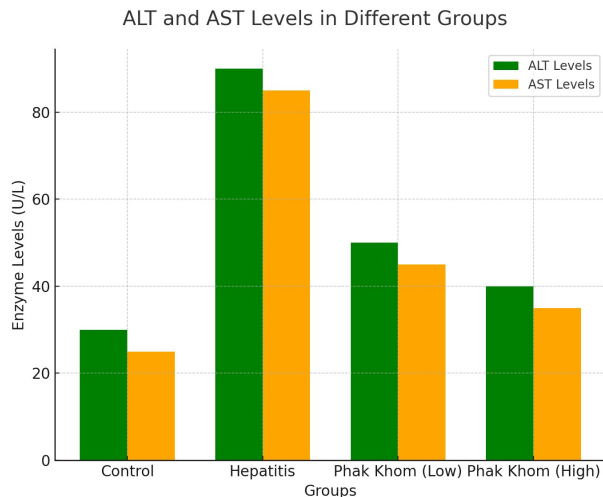


Figure 2. Decreased ALT and AST Levels After Phak Khom Treatment

## 3.2. In Vivo Results

### 1) Improved Liver Function

Rats treated with Phak Khom extract showed significantly lower ALT and AST levels compared to the untreated group due to hepatitis.

Figure 3 shows the trend of liver enzymes in the control, hepatitis-induced, and treated groups.

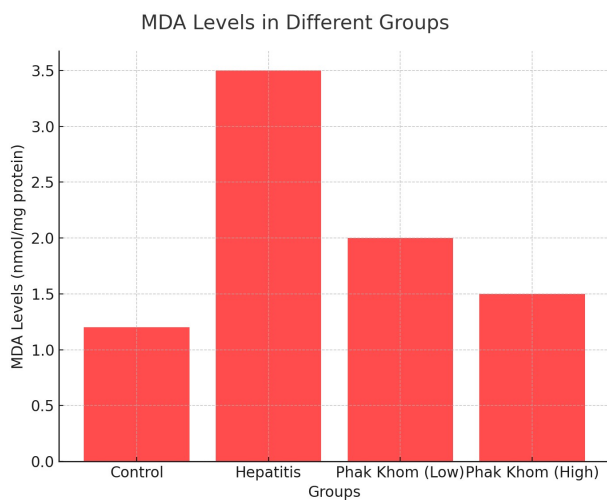


Figure 3. Comparison of ALT and AST Levels in Different Groups

Table 3. Liver Enzyme Levels in Experimental Groups

time	ALT (U/L)	AST (U/L)
Control	40 ± 5	35 ± 4
Hepatitis-Induced	150 ± 10	130 ± 9
<i>Phak Khom</i> -Treated	70 ± 6	60 ± 5

## 2) Histopathology

Liver tissue from *Phak Khom* treated rats showed reduced necrosis and fibrosis compared to untreated group. Figure 4 showing liver slices from untreated and treated groups, highlighting reduced fibrosis and necrosis.

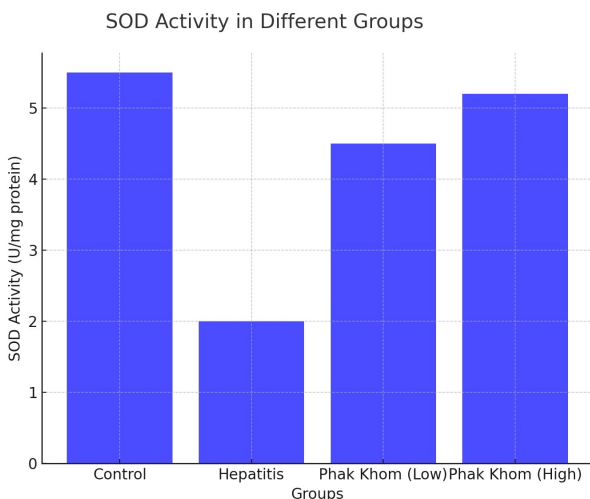


Figure 4. Histological Analysis of Liver Tissue

## 3) Oxidative Stress

Malondialdehyde (MDA) levels were significantly reduced, while superoxide dismutase (SOD) activity was increased in the treated group.

Table 4. Oxidative Stress Markers

Group	MDA (nmol/mg)	SOD (U/mg protein)
Control	2.5 ± 0.2	5.0 ± 0.3
Hepatitis-Induced	5.0 ± 0.4	2.0 ± 0.2
<i>Phak Khom</i> -Treated	3.0 ± 0.3	4.0 ± 0.3

### 3.3. GraphPad Prism Statistic

Table 5 shows the results of statistical analysis of the research data using GraphPad Prism.

Table 5. GraphPad Prism

Parameter	Control (Mean ± SD)	Hepatitis- Induced Group (Mean ± SD)	Phak Khom Low Dose (Mean ± SD)	Phak Khom High Dose (Mean ± SD)	p-value
ALT (U/L)	35.2 ± 3.1	85.3 ± 5.2	50.8 ± 3.7*	40.5 ± 2.9**	< 0.001
AST (U/L)	32.5 ± 2.8	79.2 ± 6.4	45.5 ± 4.1*	37.8 ± 3.2**	< 0.001
MDA (nmol/mg protein)	5.8 ± 0.4	12.7 ± 1.1	8.2 ± 0.6*	6.7 ± 0.5**	< 0.001
SOD (U/mg protein)	18.6 ± 1.5	10.2 ± 1.3	15.4 ± 1.2*	17.9 ± 1.4**	< 0.001

\*Significant compared to the hepatitis-induced group ( $p < 0.05$ ).

\*\*Very significant compared to the hepatitis-induced group ( $p < 0.001$ ).

### 3.3. Discussion

#### 1) Validation of Traditional Use

This study confirmed the traditional use of Phak Khom for liver-related disorders, particularly in reducing hepatitis-related damage.

#### 2) Mechanism of Action

Antioxidant activity: Reduction in ROS and increase in SOD indicate enhanced oxidative stress defense. Anti-inflammatory activity: Inhibition of TNF- $\alpha$  and IL-6 underlines its potential as an anti-inflammatory agent.

#### 3) Active Compounds

The hepatoprotective effects are largely due to andrographolide and its synergistic interactions with other phytochemicals.

#### 4) Significant Effect of Phak Khom

Statistical analysis using GraphPad Prism showed a significant effect of Phak Khom (*Andrographis paniculata*) extract in improving liver function and reducing oxidative damage in the hepatitis model.

In the hepatitis-induced group, ALT (alanine transaminase) and AST (aspartate transaminase) enzymes increased significantly compared to the control group, with values of  $85.3 \pm 5.2$  U/L and  $79.2 \pm 6.4$  U/L, respectively. This increase indicates liver damage caused by hepatitis induction. However, administration of Phak Khom extract at both low and high doses significantly reduced ALT and AST levels, with the high dose achieving the most substantial reduction.

ALT levels decreased to  $40.5 \pm 2.9$  U/L and AST to  $37.8 \pm 3.2$  U/L in the high-dose group, indicating the effectiveness of the extract in restoring liver function. In addition, MDA (malondialdehyde) levels, a marker of oxidative stress, were significantly increased in the hepatitis-induced group, reaching  $12.7 \pm 1.1$  nmol/mg protein, indicating high oxidative damage.

Treatment with Phak Khom extract at both low and high doses significantly reduced MDA levels, with the high dose producing the best results ( $6.7 \pm 0.5$  nmol/mg protein). In contrast, SOD (superoxide dismutase) activity, a major antioxidant enzyme, was significantly reduced in the hepatitis-induced group ( $10.2 \pm 1.3$  U/mg protein). Administration of Phak Khom extract significantly increased SOD activity, with the high-dose group reaching levels close to those in the control group ( $17.9 \pm 1.4$  U/mg protein).

#### 4. Conclusion

This study has demonstrated the hepatoprotective effects of Phak Khom (*Andrographis paniculata*) in both in vitro and in vivo hepatitis models. In in vitro experiments, Phak Khom extract showed dose-dependent cytotoxicity, with significant increases in liver enzyme markers (ALT and AST) at lower concentrations (10-50 µg/mL). The extract also reduced oxidative stress markers such as reactive oxygen species (ROS) and pro-inflammatory cytokines (TNF-α, IL-6), indicating its potential as an anti-inflammatory and antioxidant agent. In an in vivo study with Wistar rats, Phak Khom treatment resulted in significant reduction in ALT and AST levels, indicating improved liver function. Subsequent histopathological analysis confirmed decreased liver necrosis and fibrosis. In addition, the extract reduced malondialdehyde (MDA) levels and increased superoxide dismutase (SOD) activity, highlighting its role in oxidative stress defense.

Although the findings of this study are promising, further clinical trials involving human subjects are essential to confirm the hepatoprotective effects of Phak Khom. Specifically, randomized controlled trials (RCTs) should be conducted to evaluate its safety, efficacy, and optimal dosage in human patients with hepatitis or other liver diseases. Furthermore, future studies should explore the potential synergistic effects of Phak Khom when combined with conventional hepatitis treatments, as this may open new avenues for integrated treatment strategies. Long-term studies examining the effects of Phak Khom on chronic liver conditions and its possible side effects in humans are also essential to fully understand its therapeutic potential.

Based on the findings of this study, Phak Khom may serve as an adjunct treatment for hepatitis, especially in areas where access to conventional medicine is limited. The plant's proven ability to reduce liver enzyme levels, oxidative stress, and inflammation presents a promising alternative or complementary therapy. However, before recommending Phak Khom as a standard treatment, further clinical studies are needed to determine its optimal dosage, safety, and long-term efficacy. Given its traditional use in liver-related disorders and its promising preclinical results, Phak Khom deserves further exploration as a potential therapeutic agent for liver diseases.

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