

Research Article

# Chlorogenic Acid Inhibition HBV Replication by Suppressed JNK Expression

Lan Lai<sup>1</sup> , Lihua Li<sup>1</sup> , Xiuji Cui , Linghua Piao<sup>\*</sup> , Zhigang Cui<sup>\*</sup> 

Key Laboratory of Tropic Translational Medicine of Ministry of Education, School of Basic Medicine and Life Sciences, Hainan Medical University, Haikou City, China

## Abstract

**Aim:** Hepatitis B virus is a primary etiological factor for various liver diseases, including cirrhosis and hepatocellular carcinoma. Hepatitis B virus is an incomplete double-stranded DNA virus, which infects hepatic cells, enters the nucleus to form a complete double-stranded DNA and products a series of functional proteins, then replicates and assembles to form a complete virus, which is released outside the cell. So far, the pathogenesis of hepatitis B virus is not clear. JNK signaling pathway is an important branch of MAPK pathway, which plays an important role in various physiological and pathological processes such as cell cycle, reproduction, apoptosis and cell stress. Study show JNK activation involves liver damage. Especially, hepatitis B virus infection promote the phosphorylation of JNK. Chlorogenic acid, as a polyphenolic compound, exhibits notable antioxidant and antiviral properties. Study revealed chlorogenic acid had ability of inhibiting hepatitis B virus replication, but the mechanisms was unknown. Here we demonstrated the antiviral mechanisms of chlorogenic acid on HBV replication. **Methods:** To investigate the effect of chlorogenic acid on HBV replication, southern blot and western blot were performed using HepAD38 cells. **Results:** Chlorogenic acid suppressed HBV replication. In this process, JNK expression was inhibited. **Conclusion:** chlorogenic acid suppressed HBV replication via inhibiting JNK expression.

## Keywords

Chlorogenic Acid, HBV, JNK

## 1. Introduction

Hepatitis B virus (HBV) infection is a major health care problem worldwide [1, 2]. Chronic infection of HBV is associated with both viral and host factors, and persistent HBV infection is the leading cause of chronic liver disease, including cirrhosis and hepatocellular carcinoma [3, 4]. HBV is a partially double-stranded DNA virus, which belongs to the hepadnaviridae family. After infecting the cell, the viral nucleocapsid is transferred to the nucleus, where rcDNA is converted into covalently closed circular DNA (cccDNA),

and the formed circular DNA (cccDNA) is integrated into the hepatic nucleus, making it difficult for HBV to be cleared [5]. Widespread use of the vaccine has significantly reduced infection rates. However, current treatments for chronic hepatitis B patients have low cure rates and adverse effects. Therefore, it is necessary to explore anti-HBV therapeutic strategies.

Chlorogenic acid (CGA) is a phenolic metabolite extracted from plants, fruits, vegetables and coffee drinks, consisting of

\*Corresponding author: 1342037668@qq.com (Linghua Piao), 1229851275@qq.com (Zhigang Cui)

**Received:** 6 August 2024; **Accepted:** 28 August 2024; **Published:** 20 September 2024



Copyright: © The Author(s), 2024. Published by Science Publishing Group. This is an **Open Access** article, distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

caffeic acid and quinic acid [6]. Studies have reported that CGA has a variety of other biological properties, such as anti-microbial, anti-inflammatory and anti-diabetic activities [6, 7]. Another study has shown that CGA has a potential anti-HBV ability [8], but its possible mechanisms is unclear.

The c-Jun N-terminal kinase family is a member of the mitogen-activated protein kinase (MAPK) superfamily. JNK signaling pathway can be activated by various factors such as cytokines, growth factors and stress [9]. It has been shown that activation of JNK pathway can lead to liver injury [10, 11]. HBV infection can promote the phosphorylation of c-Jun N-terminal kinase [12]. This study demonstrates that CGA was able to inhibit HBV replication via regulating JNK signaling pathway.

## 2. Materials and Methods

### 2.1. Cell Culture

HepAD38 cells were routinely cultured in DMEM medium containing G418 (400ug/ml), penicillin (100kU/L), streptomycin (100mg/L), doxycycline 2ug/ml, and 10% FBS. After doxycycline was removed from conventional medium, HBV could be stably expressed after culture for more than 7 days.

### 2.2. Detection of Cytoplasmic HBV DNA by Southern Blot

The cytoplasmic HBV DNA within the nucleocapsid (NC) is released by treatment with sodium dodecyl sulfate (SDS) (Sigma-Aldrich) and protease K (Thermo Fisher). First, the cells were lysed using 200 ml lysis buffer containing nonidet P (NP)-40 (Sigma-Aldrich, St. Louis, MO, USA), then centrifuged at 14,000 rpm for 5 minutes to remove the nucleolus. 18 ml of supernatant was mixed with a solution containing 20 mM ethylenediamine tetraacetic acid (EDTA), 0.5% SDS, and 0.5 mg/ml protease K, then incubated at 37 °C for 2 hours. The released core DNA dissolves on a 1.2% agarose gel and is subsequently transferred and fixed onto a nylon membrane (Millipore). The imprinted DNA was hybridized with digoxine-11-DUTP-labeled HBV-specific DNA probes, which were generated using a random primer labeling technique using full-length HBV DNA as a template according to manufacturer's instructions (Roche, Basel, Switzerland). The resulting DNA probes can hybridize to every region of HBV DNA. The probe binding HBV DNA was detected by anti-digoxine alkaline phosphatase immunoassay. After dephosphorylation by alkaline phosphatase, the chemiluminescent substrate CSPD was used for visualization. The maximum luminous wavelength of CSPD was 477 nm. The chemiluminescence signal was collected by Tanon5200 chemiluminescence imaging system, and the signal density

was quantified by Image J software. [1]

### 2.3. Western Blot

Proteins were extracted from HepAD38 cells using lysis buffer (Beyotime, China) and quantified by a BCA protein assay kit (Beyotime). A total of 15µg protein sample was separated by 12% SDS PAGE and blotted onto PVDF membrane. After blocking with 10% nonfat powdered milk in TBST for 2 hours, the membranes were then incubated with p-JNK (Thr183/Tyr185, cell signaling Technology, Danvers, MA, USA) antibody at 4 °C overnight, followed by probed with secondary antibody. The protein signals were visualized using an ECL detection system.

### 2.4. Statistic Analysis

The experiments were repeated more than three times, and the experimental data were processed using IBM SPSS Statistics 24 statistical software. The statistical data were presented as mean ± standard error (mean ± SEM). The differences between the groups were compared using a two-sample t-test. A significance level of P<0.05 was considered statistically significant.

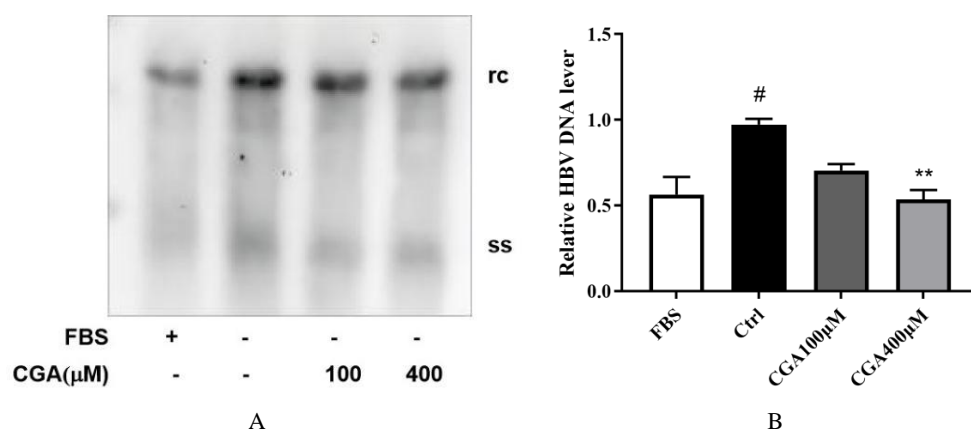
## 3. Results

### 3.1. Chlorogenic Acid Suppresses HBV Replication

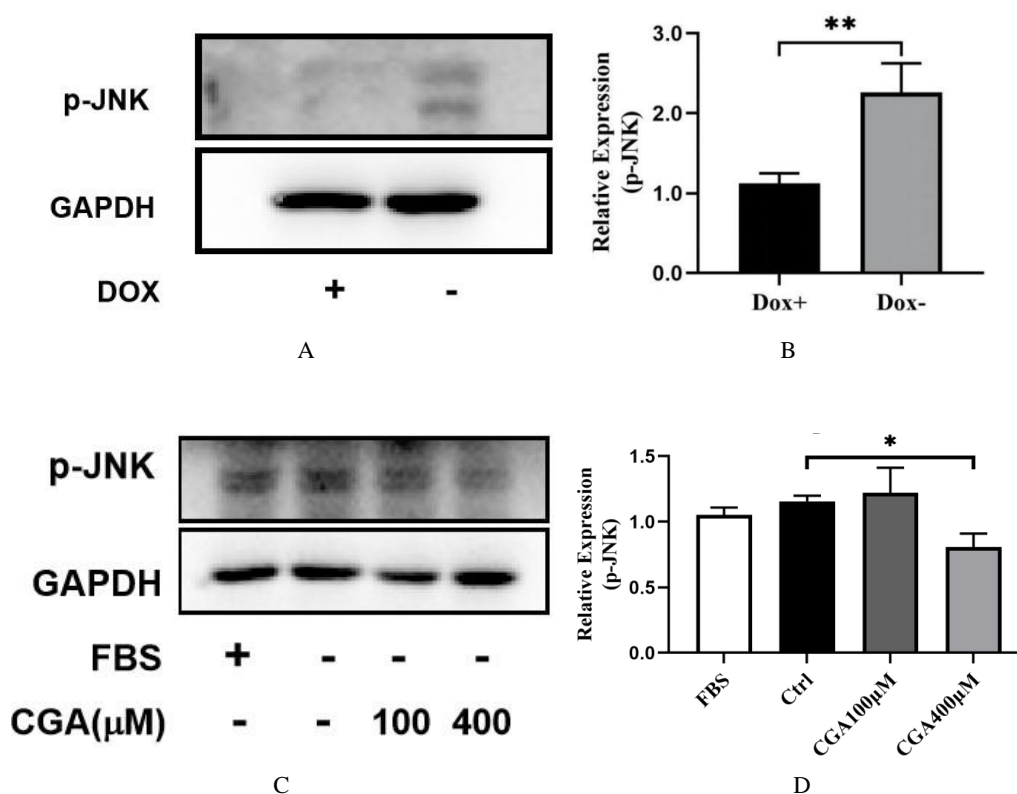
To investigate the anti-HBV effect of CGA, we assessed the cytoplasmic HBV DNA levels in HepAD38 cells using northern blot analysis. The HepAD38 cells were cultured for at least 7 days without doxycycline and then treated with 100µM or 400µM CGA for 1 hour. The results suggested that 400µM CGA had distinctly inhibiting effects on HBV-DNA replication (Figure 1A).

### 3.2. Chlorogenic Acid Suppresses HBV Replication Via Inhibiting JNK Expression

To assess the impact of HBV infection on c-Jun N-terminal kinase (JNK) phosphorylation, we examined p-JNK expression through western blot analysis in HepAD38 cells. The cells were cultured at least 7 days with or without doxycycline. Our findings revealed that the JNK phosphorylation was increased without doxycycline (figure 2A). Subsequently, we treated HepAD38 cells without doxycycline with either 100µM or 400µM CGA for 1 hour. Interestingly, our results demonstrated a significant reduction in p-JNK expression upon treatment with CGA, particularly at the concentration of 400µM (figure 2C).



**Figure 1.** Effect of CGA on HBV replication. (A) HBV DNA level were analyzed by Northern blot. HepAD38 cells were cultured 7days without deoxyline, then the cells were treated CGA 100μM or 400μM in 1h. (B) the band intensity of HBV DNA level were quantified by densitometry. # control, \* $P < 0.05$  as compared with the control.



**Figure 2.** Effects of CGA on JNK phosphorylation. (A) the phosphorylation of JNK were analyzed by western blot. (C) HepAD38 cells were cultured 7days without deoxyline, then the cells were treated CGA 100μM or 400μM in 1H. (B.D) the band intensity of p-JNK were quantified by densitometry and normalized to GAPDH. \* $P < 0.05$  as compared with the control.

## 4. Conclusion

HBV infection is the leading cause of primary liver cancer in China. HBV is a kind of incomplete DNA double-stranded virus, which enters hepatocytes, uses the enzyme system of hepatocytes to synthesize the complete DNA double-stranded, and integrates with the hepatocyte nucleus, leading to the difficulty of HBV clearance. On the other hand, HBV pro-

duces functional proteins that cause intracellular signal transduction, affect processes such as apoptosis, autophagy and cell cycle, and promote its own replication. In this study, HepAD38 cells were used as a cell model to investigate the mechanism of action of chlorogenic acid against HBV. Studies have found that chlorogenic acid inhibits HBV replication through JNK signaling pathway. JNK signaling pathway is strongly correlated with apoptosis, autophagy and cell cycle processes. Therefore, based on this study, the effect of

chlorogenic acid on HBV replication through JNK pathway will be further explored in the future.

## 5. Discussion

HBV is an important infectious disease-causing pathogen that endangers human health. The mechanism of HBV replication in hepatocytes to induce recurrent infection is unclear. Research has shown that chlorogenic acid exhibits antiviral activity against hepatitis B virus (HBV), although its specific mechanism remains unclear. Therefore, our study primarily aims to investigate the mechanism by which chlorogenic acid inhibits HBV replication. The response of liver cells following HBV entry is complex and diverse, involving various processes such as autophagy and apoptosis. JNK, an important downstream signaling protein in the MAPK protein family, participates in numerous intracellular responses. Our study results demonstrate that chlorogenic acid can reduce HBV replication by decreasing JNK phosphorylation. This discovery sheds light on a novel mechanism for the antiviral action of chlorogenic acid. HBx is a functional protein produced by HBV. It can regulate a variety of cellular processes in HBV-infected hepatocytes, such as autophagy and apoptosis [13, 14]. Our experiments suggest that chlorogenic acid may reduce HBx protein expression and subsequently act on the JNK signaling pathway to inhibit HBV replication. Further experiments are required to elucidate the specific cellular response processes involved, such as autophagy and apoptosis. Studies have shown that exosomes produced by HBV infection of hepatocytes are involved in the pathological mechanism of hepatic fibrosis [15]. Therefore, it was expected whether the effect of inhibition of HBV replication by chlorogenic acid could regulate the production of exosomes and subsequently affect the formation of liver fibrosis.

## Abbreviations

HBV	Hepatitis B Virus
HBX	Hepatitis B Virus X Protein
CGA	Chlorogenic Acid
JNK	c-Jun N-terminal Kinase
MAPK	Mitogen Activated Protein Kinase

## Research Involving Human Participants and/or Animals

This article did not contain study with human participants or animals.

## Funding

This work was supported by Hainan Provincial Natural

Science Foundation of China (Grant No. 821MS0781) for ZGC, The National natural Science Foundation of China (Grant No. 81660335, 82060365) for XJC.

## Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- [1] Xiao, Y., et al., *Discrepant results of hepatitis B virus genotype determination by PCR and DNA sequencing*. Journal of Virological Methods, 2022. 303: p. 114503. <https://doi.org/10.1016/j.jviromet.2022.114503>
- [2] Korkmaz, P., et al., *New Treatment Options in Chronic Hepatitis B: How Close Are We to Cure?* Infectious Diseases & Clinical Microbiology, 2023. 5(4): p. 267. <https://doi.org/10.36519/idcm.2023.265>
- [3] Acharya, C., & Bajaj, J. S. *Chronic Liver Diseases and the Microbiome-Translating Our Knowledge of Gut Microbiota to Management of Chronic Liver Disease*. Gastroenterology, 2021, 160(2), 556–572. <https://doi.org/10.1053/j.gastro.2020.10.056>
- [4] Zhang, M., et al., *Persistent steatosis correlates with decreased fibrosis regression during anti-HBV treatment in patients with chronic HBV infection*. Journal of medical virology, 2023, 95(10), e29156. <https://doi.org/10.1002/jmv.29156>
- [5] Jose-Abrego, A., et al., *Host and HBV Interactions and Their Potential Impact on Clinical Outcomes*. Pathogens, 2023, 12(9), 1146. <https://doi.org/10.3390/pathogens12091146>
- [6] Huang, J., et al., *Chlorogenic acid: a review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems*. Frontiers in pharmacology, 2023, 14, 1218015. <https://doi.org/10.3389/fphar.2023.1218015>
- [7] Shi, H., et al., *Chlorogenic acid protects against liver fibrosis in vivo and in vitro through inhibition of oxidative stress*. Clinical Nutrition, 2016. 35(6): p. 1366–1373. <https://doi.org/10.1016/j.clnu.2016.03.002>
- [8] Wang, G.-F., et al., *Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro*. Antiviral Research, 2009. 83(2): p. 186–190. <https://doi.org/10.1016/j.antiviral.2009.05.002>
- [9] Chatzifrangkeskou, M., et al., *JNK regulates ciliogenesis through the interflagellar transport complex and actin networks*. The Journal of cell biology, 2023, 222(11), e202303052. <https://doi.org/10.1083/jcb.202303052>
- [10] Li, X., et al., *JNK/c-Jun pathway activation is essential for HBx-induced IL-35 elevation to promote persistent HBV infection*. Journal of Clinical Laboratory Analysis, 2023. 37(5). <https://doi.org/10.1002/jcla.24860>

- [11] Wang, L., et al., *Orexin A ameliorates HBV X protein-induced cytotoxicity and inflammatory response in human hepatocytes*. Artificial Cells, Nanomedicine, and Biotechnology, 2019. 47(1): p. 2003-2009. <https://doi.org/10.1080/21691401.2019.1614014>
- [12] Zhong, L., et al., *Reactive Oxygen Species-Mediated c-Jun NH<sub>2</sub>-Terminal Kinase Activation Contributes to Hepatitis B Virus X Protein-Induced Autophagy via Regulation of the Beclin-1/Bcl-2 Interaction*. Journal of Virology, 2017. 91(15). <https://doi.org/10.1128/JVI.00001-17>
- [13] Zhang, C., et al., *C-terminal-truncated hepatitis B virus X protein promotes hepatocarcinogenesis by activating the MAPK pathway*. Microbial Pathogenesis, 2021. 159: p. 105136. <https://doi.org/10.1016/j.micpath.2021.105136>
- [14] Wang, F., et al., *Role of hepatitis B virus non-structural protein HBx on HBV replication, interferon signaling, and hepatocarcinogenesis*. Frontiers in microbiology, 2023, 14, 1322892. <https://doi.org/10.3389/fmicb.2023.1322892>
- [15] Yin, M., et al., *Exosomes from hepatitis B virus-infected hepatocytes activate hepatic stellate cells and aggravate liver fibrosis through the miR-506-3p/Nur77 pathway*. Journal of biochemical and molecular toxicology, 2023, 37(10), e23432. <https://doi.org/10.1002/jbt.23432>