Reviewer #1:

Specific Comments to Authors: This was a very interesting review with a very novel perspective on circadian rhythms and hepatitis B virus. However, the focus on the theme was not prominent, the priority was not very clear. The review described too much basic knowledge such as "The biology and life cycle of HBV", "The Circadian Rhythm" etc., however, the relationship between hepatitis B virus and circadian rhythm was described insufficiently, and the significance of circadian rhythm for future antiviral therapy was also insufficiently explained. The supplement of the views around this new mechanism are also suggested. On the other hand, several additional figures are needed to be supplemented.

We thank the reviewer for helpful comments, and we welcome his/her insightful suggestions that we have addressed.

We have described the relationship between the hepatitis B virus and the circadian rhythm in more detail.

Text added to the manuscript:

HBV is randomly incorporated into the human genome, yet there are sites where it is incorporated more frequently, such as circadian rhythm-related elements, *CLOCK*, and *BMAL1*. Those elements are one way that the circadian rhythm is associated with diseases caused by HBV infection[81].

In addition, abnormal expression of *REV-ERBα* was observed in a cell line that stably expressed HBV[86]. Circadian rhythm acts via REV-ERBα in the liver on hepatocyte nuclear factor 4 alpha (HNF4α), and thus directs the action of glucocorticoid receptors on energy metabolism (Figure 3). Consequently, the interactions between CRY and glucocorticoid receptors affect carbohydrate metabolism, hence transacting *PER2*[87]. HNF4α increases the transcription of pgRNA in hepatoma cells and thus affects HBV biosynthesis[88]. In addition, P2-HNF4α inhibits the expression of *BMAL1*, leading to the localization of P1-HNF4α from the nucleus to the cytoplasm. *BMAL1* is expressed in healthy hepatocytes, but tumor growth is prevented if *BMAL1* expression is induced in HNF4α-positive tumor cells[89]. The possible reason for the inhibition of tumor growth is that BMAL1 mediates the transcription of *P53* pathway genes, a well-known tumor-suppressor gene[90]. DNA viruses rely more on host transcription for gene expression; however, *BMAL1* deficiency increases virus replication. REV-ERBα has a protective effect because it reduces the inflammatory response, so decreasing the severity of the disease. Thus, *REV-ERBα* agonists inhibit HBV replication, while *BMAL1* promotes virus replication. Nevertheless, as BMAL1 stimulates oscillations in genes that metabolize drugs and sensitivity to toxicity, it also promotes HBV infection in hepatocytes[91].

HBV infection leads to overexpression of *RORα* and *RORγ*[92]. *RORγ* overexpression is associated with promoter methylation and HBx protein. Furthermore, HBx-induced *RORγ* may facilitate the proliferation and migration of hepatoma cells[93], and RORα may be a possible diagnostic and prognostic biomarker for disease severity[94]. Overexpression of *RORα*, *CRY2*, and *PER1* is associated with better survival of HCC patients, and regulating the circadian rhythm gene may help in the chronotherapy of such patients[94].

We have also added the importance of circadian rhythm for future antiviral therapy and administration of agonists and antagonists of specific circadian rhythm proteins, which may directly affect HBV replication.
Text added to the manuscript:
The circadian hormone melatonin has protective and antiviral actions and a role in the inflammatory response[105]. Melatonin increased IFN-γ levels during viral infection, decreased Venezuelan equine encephalomyelitis virus (VEE) levels, and reduced mortality rates in mice infected with VEE. The protective impact of melatonin is associated with increased IL-1β production because it acts as a cytokine modulator and antioxidant[107].
Circadian oscillations can affect vaccine responses to viral pathogens[91].
Research on the SARS-CoV-2 virus has shown that melatonin can affect circadian clocks and modulate the immune response during viral infections and thus impact virus replication[113].
Thus, it was observed that patients vaccinated against the SARS-CoV-2 virus in the morning had significantly lower C-reactive protein levels compared to patients vaccinated in the evening[115].
The effectiveness of current antiviral therapies could be enhanced by modulating the timing of vaccine administration. For example, the engineered T cell receptor activity showed a circadian pattern upon antigen activation. However, this daily effect was attenuated in *CLOCK* mutant mice, emphasizing the importance of timing T cell therapy to maximize antiviral immunity[1].
Circadian clock-modulating small molecules may help inhibit or activate circadian rhythm proteins and enzymes in viral infections. Thus, the small molecule SRT2183 modulates the circadian clock that inhibits SARS-CoV-2 virus replication[118] because it modulates physiological and circadian rhythm gene expression[119]. Inhibiting *BMAL1* expression and overexpression of *REV-ERB* via circadian rhythm-modulating small molecules may prevent dengue virus, hepatitis C virus, Zika virus, and HIV1 virus replication. In addition, clock genes have antiviral abilities that can be applied to HBV[120]. One of the effective circadian clock-modulating small molecules in treating HBV infections is GSK4112, a synthetic ligand for REV-ERB, but it is not suitable for *in vivo* use due to its poor pharmacokinetic properties. In contrast, ARN5187 is a REV-ERBβ agonist with dual-function – an inhibitor of REV-ERB and autophagy[121]. SR9009 is a REV-ERBα agonist based on the chemical structure of GSK4112. It has better pharmacokinetic properties than GSK4112 and affects many oncogenes. In addition, REV-ERBα is known to regulate cancer development by inhibiting proliferation[121], and hence SR9009 inhibits *BMAL1* and prevents the entry and replication of HBV into hepatocytes[50].
Challenges associated with antiviral drug development, including circadian clock-modulating small molecules, may possess adverse effects or suboptimal pharmacokinetics. Therefore, for an antiviral agent to succeed, the target drug should be distributed locally to avoid unfavorable consequences on other tissues.

Also, according to the reviewer's suggestions, we made two additional figures. Figure 2 is a schematic representation of the role of circadian rhythm in liver

metabolism and what happens when it is disrupted. Figure 3 shows the interaction of circadian rhythm genes with HBV replication.

Reviewer #2:

Specific Comments to Authors: This manuscript describes excellent review of HBV replication and the circadian rhythms. Most data are dependent on a variety of Previous papers and general explanation of HBV sounds to be good. The content of this manuscript is very fruitful. A reviewer feels one additional figure, which shows HBV replication and the circadian rhythms.

We thank the reviewer for endorsing and supporting our manuscript. We added two additional figures. Figure 2 is a schematic representation of the role of circadian rhythm in liver metabolism and what happens when it is disrupted. Figure 3 shows the interaction of circadian rhythm genes with HBV replication.

Reviewer #3:

Specific Comments to Authors: The paper is well written and properly structured. I am really thankful for giving me this opportunity to review this paper. It reveals the actual relationship between hepatitis B and circadian rhythms in the human body, which may play an important role in its pathogenesis. This knowledge allows us to consider chronomedicine as a way to improve existing therapeutic approaches through a personalized medicine by biological rhythms. In my opinion, the most important findings of these manuscript are links between viral infection and clock genes that regulate metabolic process which direct on future perspectives in studying hepatitis B.

We thank the reviewer for helpful comments, and we welcome his/her insightful suggestions that we have addressed thoroughly (especially regarding recommended articles).

I recommend this article for acceptance, but at the same time I have some minor comments:

1. It may be useful to present the findings of the clock genes role in a lifecycle of hepatitis B in a diagram, figure or table.

We added two additional figures. Figure 2 is a schematic representation of the role of circadian rhythm in liver metabolism and what happens when it is disrupted. Figure 3 shows the interaction of circadian rhythm genes with HBV replication.

2. I would like to point out the uniqueness of the considered topics, which may determine the sources used. However, I recommend updating this paper with the latest data highlighting the importance of studying clock genes in general (2-3 references). Authors may consider the following publications or others which in their opinion better describe this aspect: https://doi.org/10.1016/j.arr.2021.101554, https://doi.org/10.1155/2021/8238833

We updated the manuscript with the latest data emphasizing the importance of studying the clock gene in general, using the suggested publications (references 90 and 94) and adding the latest article published about the role of circadian rhythm in viral replication (reference 82).