



Antiviral activity of chemical compound isolated from *Artemisia morrisonensis* against hepatitis B virus *in vitro*



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Abstract

The compound *p*-hydroxyacetophenone (PHAP) isolated from *Artemisia morrisonensis* was found to have potential anti-HBV effects in HepG2 2.2.15 cells. We clarified its antiviral mode further and HBV-transfected Huh7 cells were used as the platform. During viral gene expression, treatment with PHAP had no apparent effects on the viral precore/pregenomic RNA. However, the 2.4-kb preS RNA of viral surface gene increased significantly relative to the 2.1-kb S RNA with PHAP. Promoter activity analysis demonstrated that PHAP had a potent effect on augmenting the viral preS promoter activity. The subsequent increase in the large surface protein and induce endoplasmic reticular (ER) stress has been reported previously. Interestingly, PHAP specifically reduced ER stress related GRP78 RNA/protein levels, but not those of GRP94, in treated Huh7 cells while PHAP also led to the significant intracellular accumulation of virus. Moreover, treatment with the ER chaperone inducer thapsigargin relieved the inhibitory effect of PHAP based on the supernatant HBV DNA levels of HBV-expressed cells. In conclusion, this study suggests that the mechanism of HBV inhibition by PHAP might involve the regulation of viral surface gene expression and block virion secretion by interference with the ER stress signaling pathway.

Keyword: *Artemisia morrisonensis*, chaperone, ER stress, GRP78, hepatitis B virus, HepG2 2.2.15 cell, Huh7 cell, *p*-hydroxyacetophenone

Materials and Methods

Cell Culture and Bioactivity Assays

HepG2 2.2.15 and Huh7 Cells were maintained in MEM and DMEM supplemented with heat inactivated 10% (v/v) fetal bovine serum and 1% antibiotics at 37°C in a humidified 5% CO₂ incubator. Initially, cells were seeded and grown in various cultural dishes at 80% of confluence were used in all experiments. In routine drug treatments, cells were treated with serial diluted concentrations of PHAP compound for 2 to 3 days. After treatment, the levels of viral surface antigen (HBsAg) and e antigen (HBeAg) in cultural media were measured by enzyme immunoassay (EIA). In another set of anti-viral experiments, Huh7 cells were plated in 60 mm cultural dishes, transfected with pHBV1.2 plasmid, and treated with PHAP in 2% fetal bovine serum containing medium for 48 hours. Treated cells were subjected to total cellular RNA, viral DNA, and cellular protein extraction.

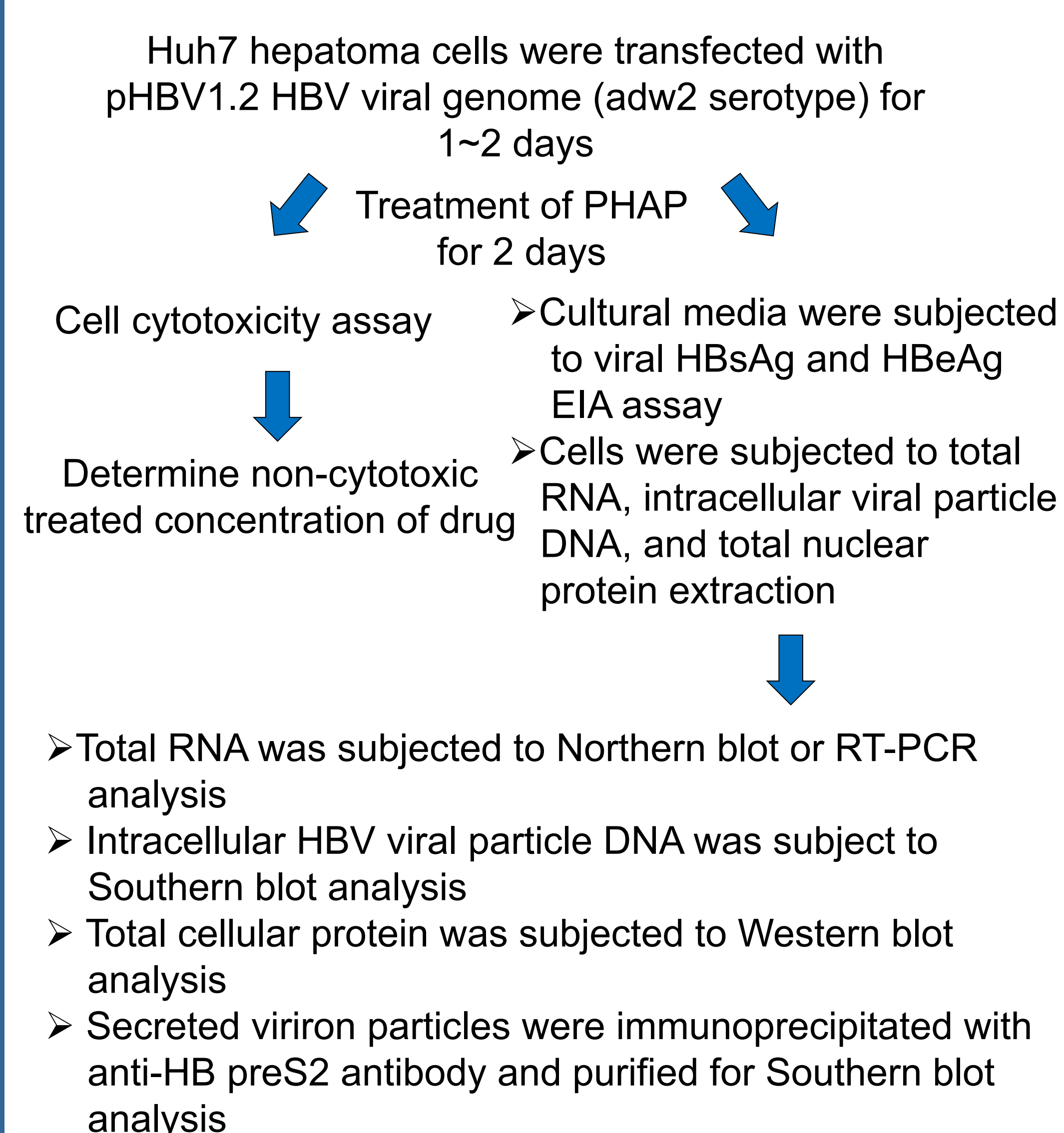
Transient Transfection and Luciferase Assay

Huh7 cells were transfected with various plasmids using Lipofectamine 2000 transfection reagent. Briefly, cells were plated in 24-well cultural plate (4x10⁴/ml) and transfected in DMEM supplemented with 10% FBS for 24 hours, washed with 1xPBS, incubated in DMEM supplemented with 2% FBS, and then treated with or without PHAP (294.1 μM) for 2 days. Luciferase assay was performed according to manufacturer's instructions. For promoter-reporter construct transfection, pHBV1.2 or control vector (1 μg/ml/well) was co-transfected with pRL-SV40 Renilla luciferase expression plasmid (0.02 μg/well), which was used to normalize the basal level of luciferase activity.

Statistical Analysis

Statistical analysis was performed using Student's *t*-test. *P* value less than 0.01 was considered significant.

Experimental Design



Results

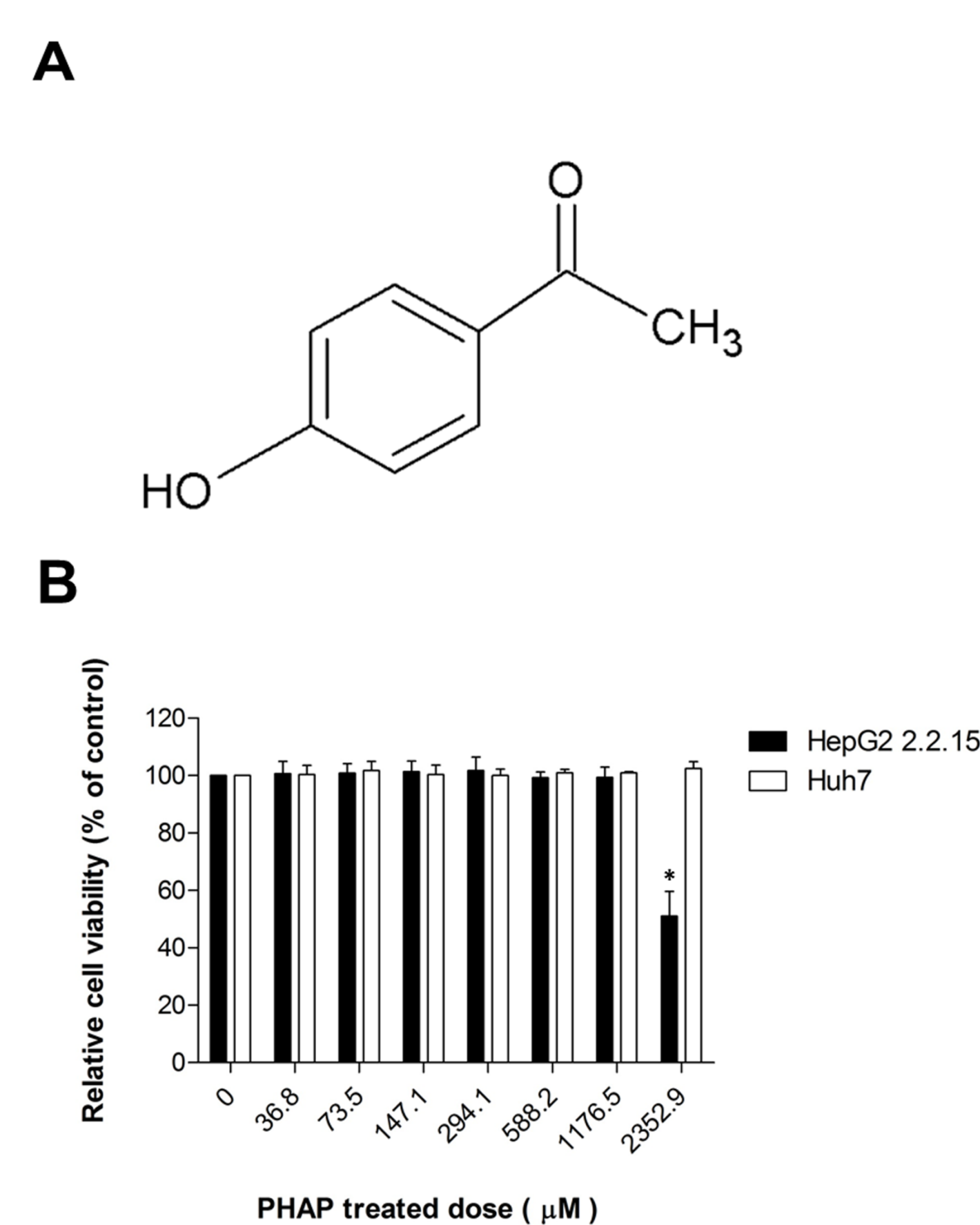


Fig. 1. (A) Chemical structures of PHAP. (B) Cytotoxic effect of PHAP on HepG2 2.2.15 and Huh7 cells.

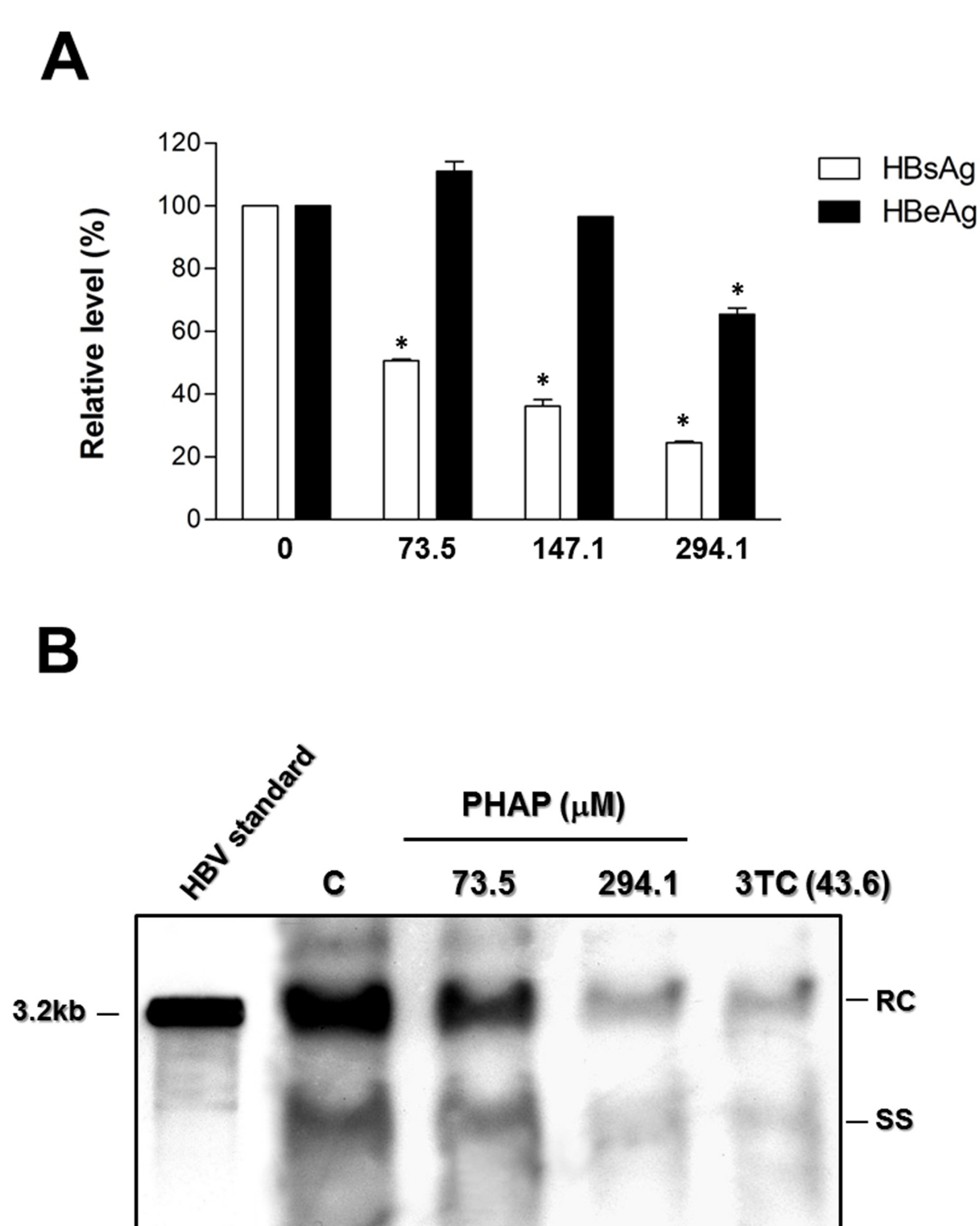


Fig. 2. The effects of PHAP on the secretion of (A) HBV viral antigen, and (B) viral particles in HepG2 2.2.15 cells.

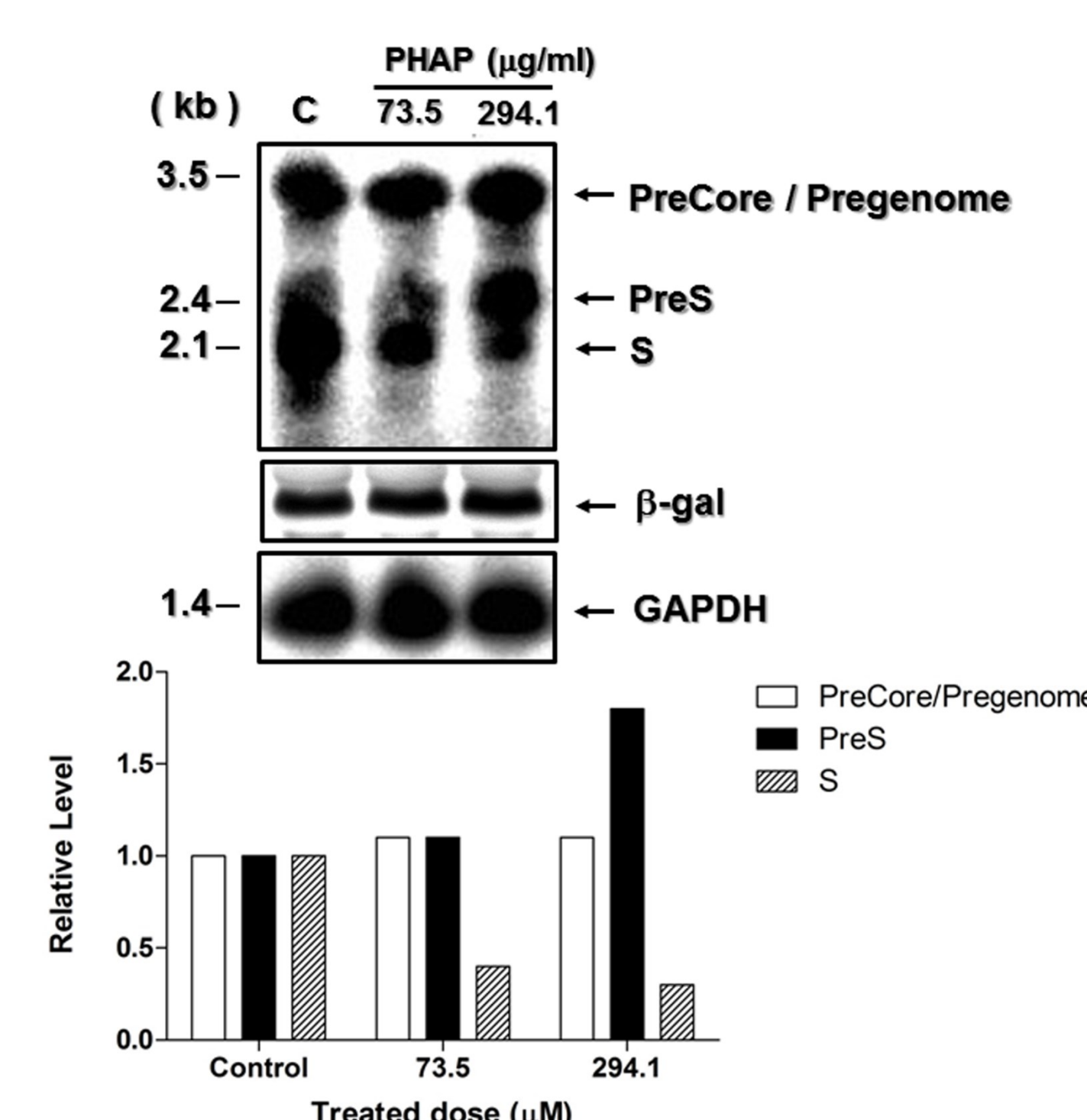


Fig. 3. The effects of PHAP on HBV gene expression in Huh7 cells. Huh7 cells were transfected with the pHBV1.2 plasmid for 24 h and treated with various concentrations (73.5 and 294.1 mM) of PHAP for 2 days. Treated cells were harvested and subjected to total RNA isolation and Northern blot analysis.

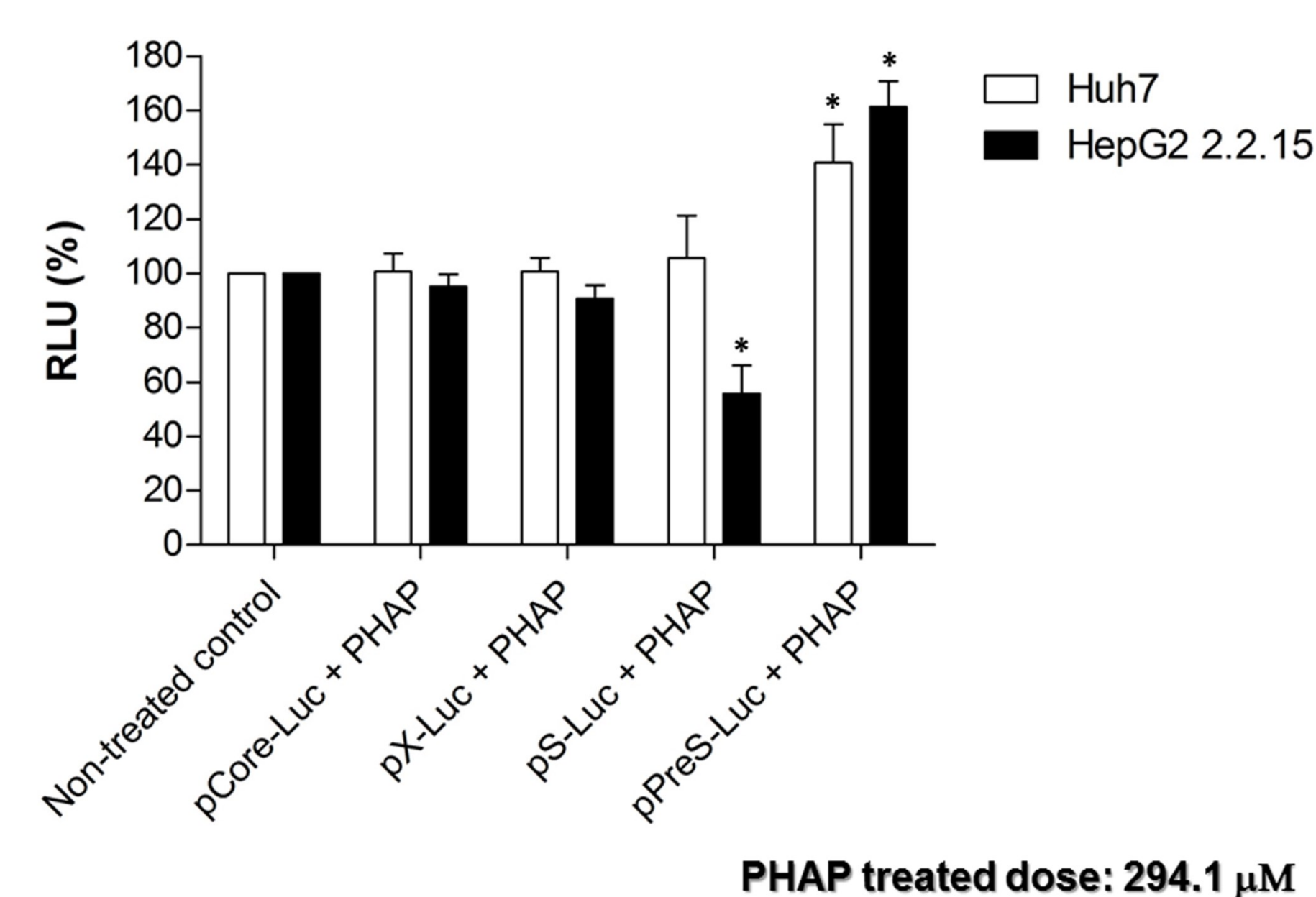


Fig. 4. The effect of PHAP on HBV gene promoter activity. Huh7 cells were seeded in 24-well plates for 24 h and transfected with either pCore-Luc, pX-Luc, pS-Luc or pPreS-Luc viral gene promoter-luciferase reporter constructs, together with pRL-SV40, for 24 h and then treated with PHAP for a further 24 h. The cellular lysates were prepared for luciferase assay. (*n* = 3) (**P* < 0.01 vs untreated cells).

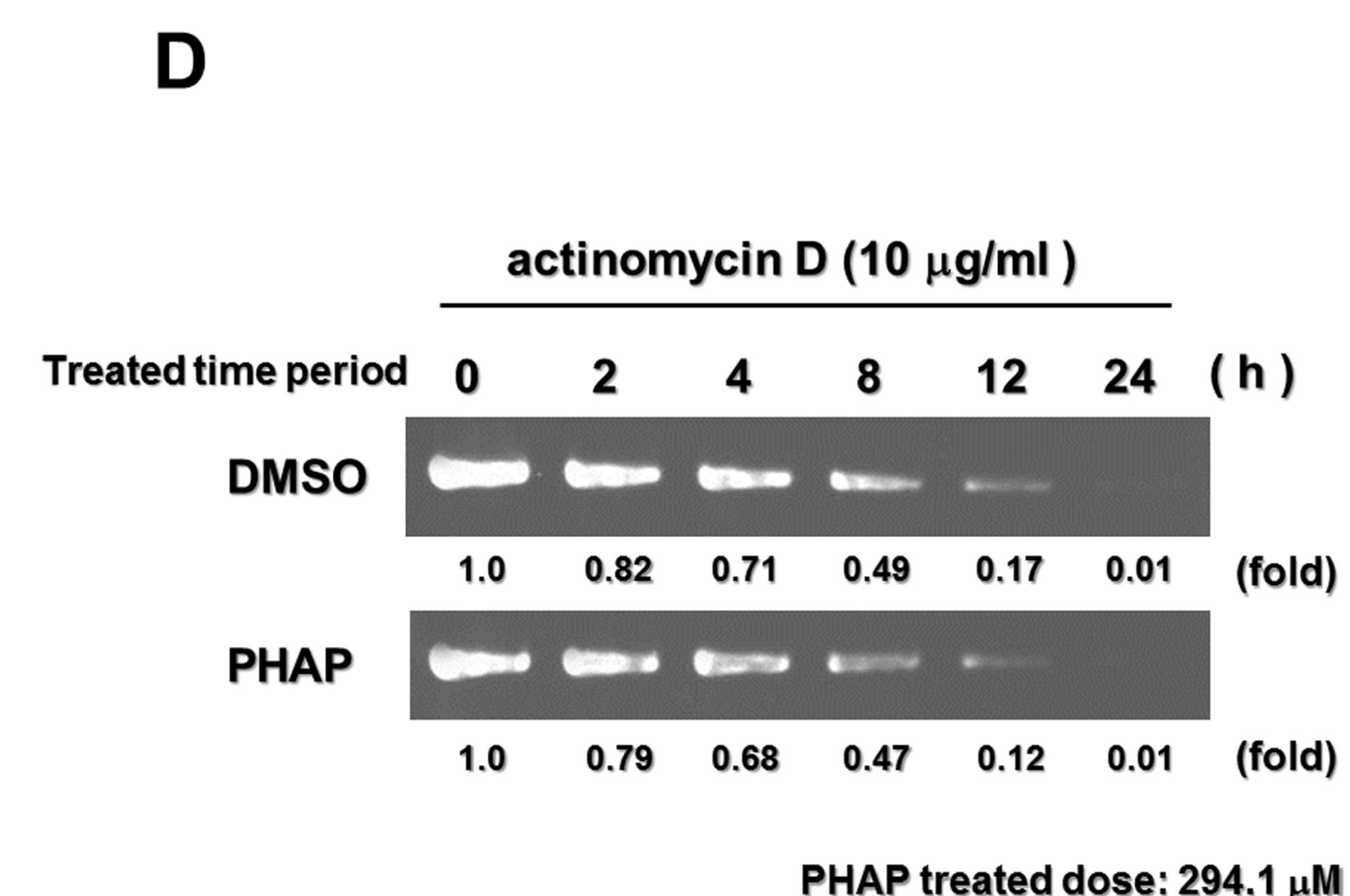
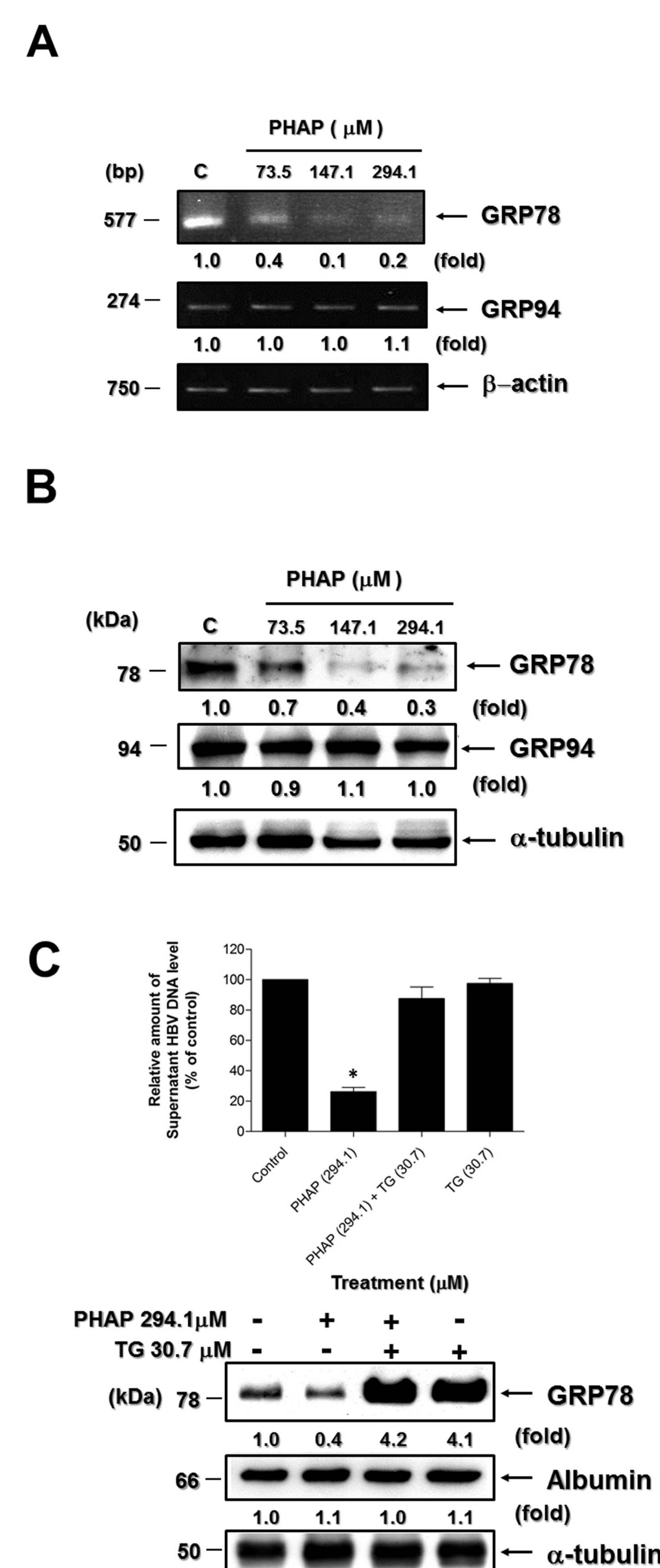


Fig. 5. Effect of PHAP on Chaperon (A) RNA, (B) protein, (C) chaperon mediated HBV DNA replication, and (D) chaperon RNA stability in HBV transfected Huh7 cells.

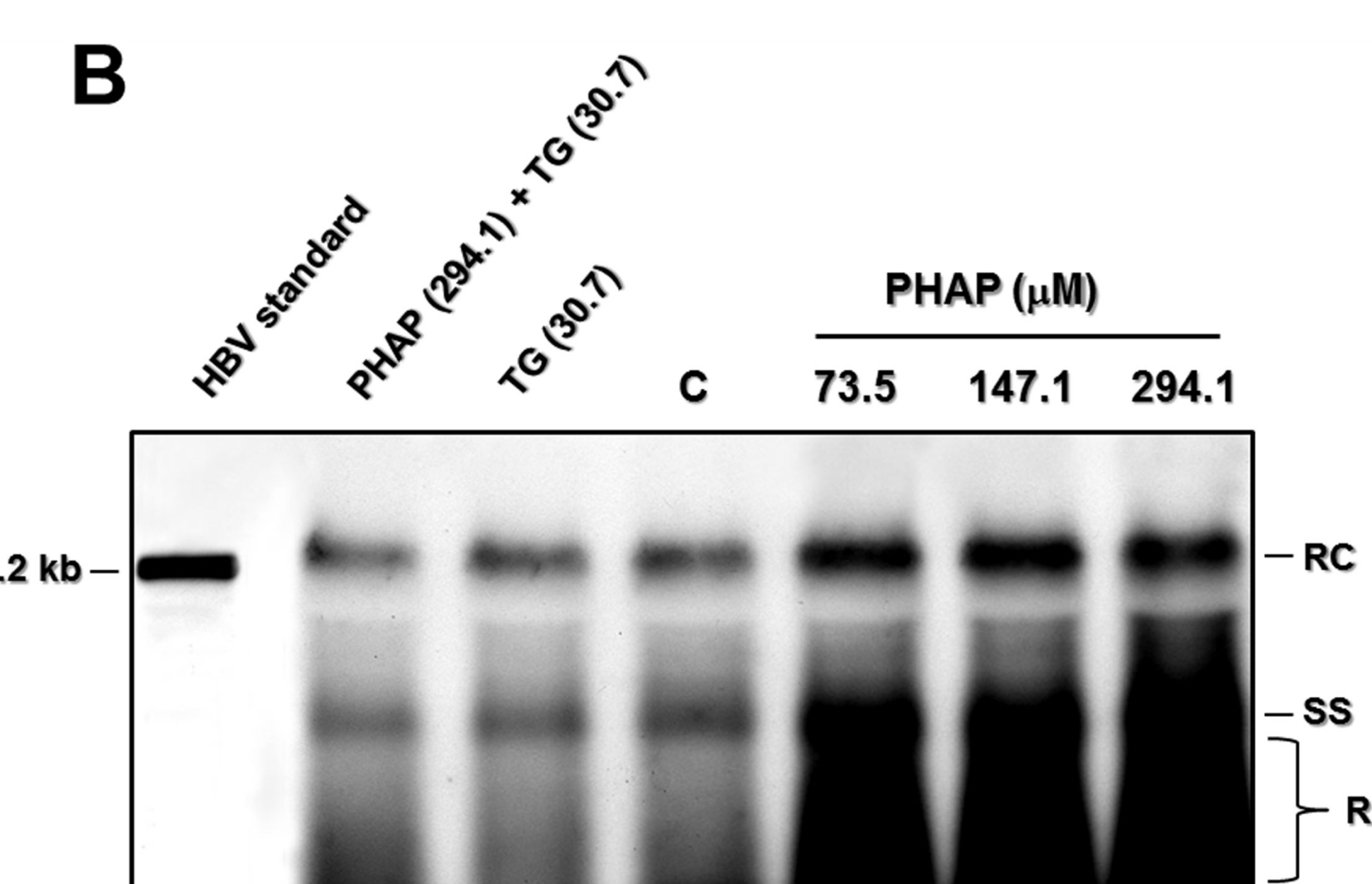
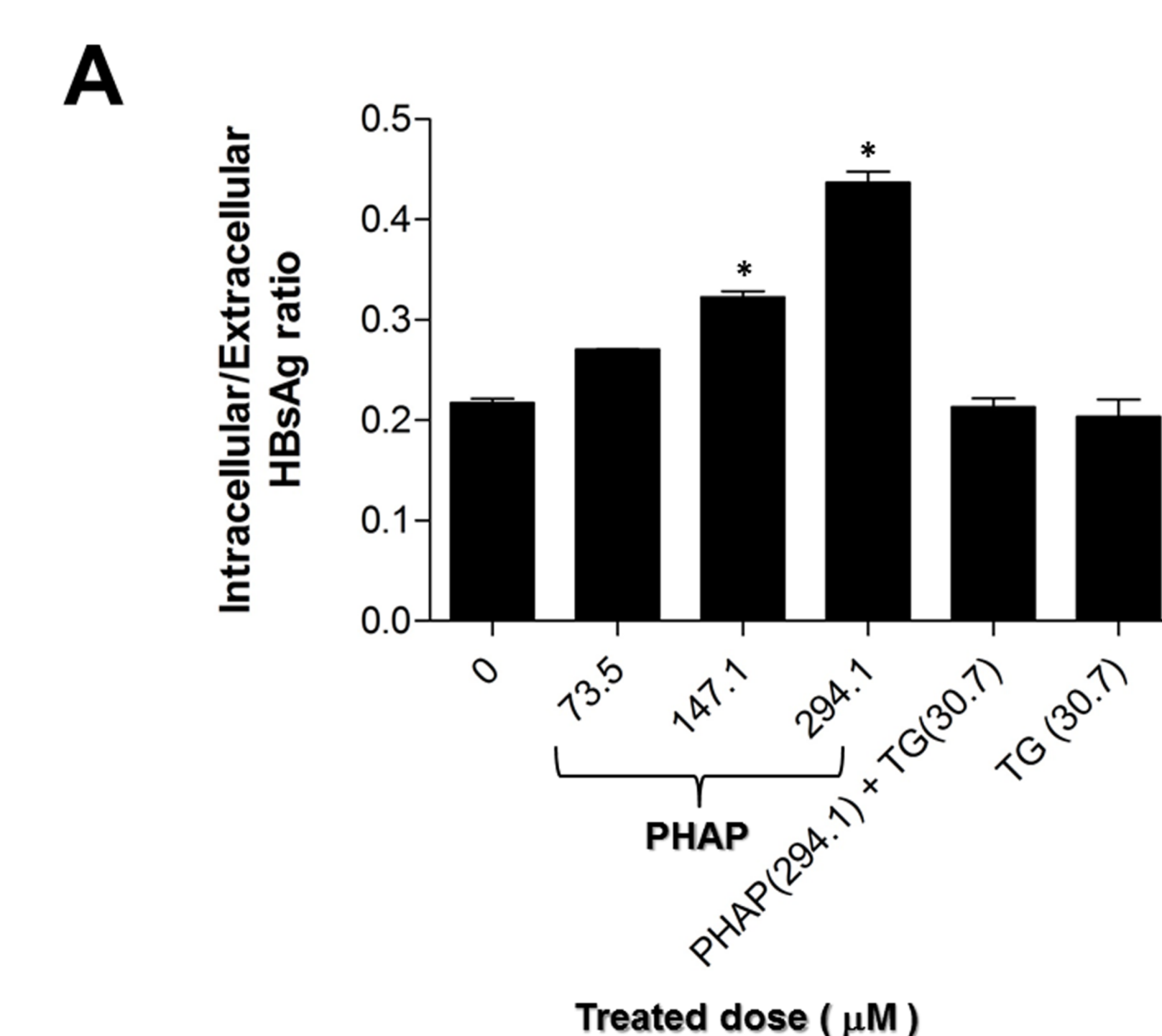


Fig. 6. Effect of PHAP on the (A) HBsAg and (B) HBV viral particle intracellular accumulation in HBV transfected Huh7 cells.

Summary

- Production of HBV viral HBsAg, HBeAg and viral DNA in HepG2 2.2.15 cells was significantly reduced by PHAP in a dose-dependent manner.
- The levels of HBV 3.5-kb precore/pregenomic RNA was not reduced by PHAP, but the 2.1-kb surface antigen RNA were strongly inhibited by PHAP. In addition, the expression of 2.4 kb preS RNA was significantly increased during treatment.
- Treatment with PHAP strongly suppressed major S gene promoter but also increased preS promoter activities in HBV expressed Huh7 cells.
- PHAP treatment specifically inhibited chaperone GRP78 gene expression and its effect on HBV propagation.
- Treatment of PHAP significantly inhibited ER stress related chaperone protein expression and caused HBV viral particle accumulation in hepatoma cells.

Acknowledgments

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