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# Anti-hepatitis B virus activity of wogonin in vitro and in vivo

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**Abstract**

The traditional Chinese medicine *Scutellaria radix* has been used for thousands of years, mainly for the treatment of inflammatory conditions including hepatitis. The major active constituent, wogonin (WG), isolated from *S. radix* has attracted increasing scientific attention in recent years due to its potent biological activities. However, pharmacologic studies have primarily been focused on wogonin's anti-inflammatory and anti-cancer activities. In this study, we have investigated wogonin's anti-hepatitis B virus (HBV) activity both in vitro and in vivo. In the human HBV-transfected liver cell line HepG2.2.15, wogonin effectively suppressed the secretion of the HBV antigens with an $IC_{50}$ of 4 μg/ml at day 9 for both HBsAg and HBeAg. Consistent with the HBV antigen reduction, wogonin also reduced HBV DNA level in a dose-dependent manner. Duck hepatitis B virus (DHBV) DNA polymerase was dramatically inhibited by wogonin with an $IC_{50}$ of 0.57 μg/ml. In DHBV-infected ducks wogonin dosed i.v. once a day for 10 days reduced plasma DHBV DNA level with an $ED_{50}$ of 5 mg/kg. The in vivo anti-HBV effect of wogonin in ducks was confirmed by Southern blotting of DHBV DNA in the liver. Histopathological evaluation of the liver revealed significant improvement by wogonin. In addition, in human HBV-transgenic mice, wogonin dosed i.v. once a day for 10 days significantly reduced plasma HBsAg level. Immunohistological staining of the liver confirmed the HBsAg reduction by wogonin. In conclusion, our results demonstrate that wogonin possesses potent anti-HBV activity both in vitro and in vivo. Currently, wogonin is under early development as an anti-HBV drug candidate.
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*Keywords:* Wogonin; Anti-HBV activity; Human HBV cells; DHBV; Human HBV-transgenic mice

## 1. Introduction

Wogonin (5,7-dihydroxy-8-methoxyflavone, Fig. 1) is a naturally occurring monoflavonoid isolated from the well-known traditional Chinese medicinal herb *Scutellaria radix.* This herb has been widely used in medical practice in Asia mainly for inflammatory and liver diseases for thousands of years, with an excellent safety record. The major active constituents of *S. radix* are believed to be flavonoids (Bonham et al., 2005; Chung et al., 1995; Lin and Shieh, 1996). In recent years, as a major flavonoid of this herb, wogonin has been found to have a number of pharmacologic activities, including anti-inflammatory, anti-cancer, neuroprotective, anxiolytic, anti-viral, and vascular effects (Cho and Lee, 2004; Tai et al., 2005). In particular, wogonin has a well-documented antioxidant property, which is probably a major underlying mechanism for its anti-inflammatory, anti-cancer, neuroprotective and vascular effects. Most pharmacologic studies on wogonin have been focused on its anti-inflammatory and anti-cancer activities, and have suggested that wogonin may have therapeutic potential for inflammatory diseases and cancers. With regard to wogonin's anti-viral activity, thus far there has been one report on respiratory syncytial virus (Ma et al., 2002) and one on hepatitis B virus (HBV) (Huang et al., 2000). The anti-HBV activity of wogonin was shown in vitro by suppression of secretion of the HBV antigen HBsAg from a HBV-transfected liver cell line (MS-G2), followed by confirmation with reduction of HBV DNA polymerase reaction products.

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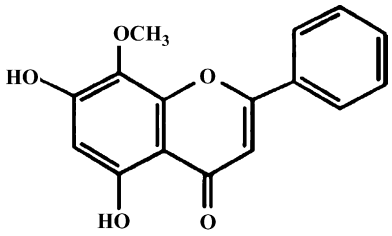


Wogonin

Fig. 1. Chemical structure of wogonin. Molecular formula: $C_{16}H_{12}O_5$; molecular weight: 284.26.

Hepatitis B remains a major public health problem worldwide, despite the available effective vaccines. There are about 400 million people with chronic HBV infection, who are at a life-long high risk of developing cirrhosis and/or liver cancer. Up to 30% of the chronic carriers will die of complications of these chronic liver diseases (Gish, 2005; Huang et al., 2000; McMahon, 2005). Several anti-viral drugs have been approved for the treatment of hepatitis B, including interferon-α and nucleoside analogues. However, unresolved significant issues remain with current drugs, such as moderate efficacy, dose-dependent side-effects, and drug resistance (Perrillo, 2005). Therefore, there exists a significant unmet medical need for safe and efficacious new anti-HBV drugs. To explore new potential clinical indication(s) for wogonin, in this study we have focused on its anti-HBV activity. Our in vitro experiments in a HBV-transfected liver cell line revealed that wogonin reduces the levels of the HBV antigens and DNA and inhibits HBV DNA polymerase with potencies much higher than previously reported (Huang et al., 2000). More importantly, we have demonstrated the anti-HBV activity in vivo in DHBV-infected ducks and in human HBV-transgenic mice.

## 2. Materials and methods

### 2.1. Wogonin and reference drugs

Wogonin (Fig. 1) was prepared at China Pharmaceutical University, Nanjing, China (purity: >99%). It was dissolved at various concentrations in physiological saline before use. For in vivo experiments in both ducks and mice, wogonin was administrated intravenously once a day. Lamivudine (3TC, from GlaxoSmithKline, UK) and foscarnet sodium (PFA, from ChiaTai TianQing Pharmaceutical, Nanjing, China) were dosed orally once a day in animal experiments.

### 2.2. Cell culture and treatment

The human HBV-transfected cell line HepG2.2.15 (Sells et al., 1987) was maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FCS, 100 unit/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (all from Invitrogen, USA). Cells were treated with wogonin at various concentrations for a specified period in DMEM supplemented with 10% FCS, 100 unit/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine in 24-well plate at a density of $2 \times 10^4$ per well.

### 2.3. Animals

DHBV-positive (from vertical transmission) female ducks were maintained under normal daylight and fed with a standard commercial diet and water ad libitum. The ducks were used at an age of about 12 months with a body weight of 900–1100 g.

Human HBV-transgenic mice from lineage 1.3.32 (Guidotti et al., 1995) were purchased from Canton Air Force Hospital (Guangzhou, China), and maintained under a 12/12 h light/dark cycle with a standard commercial diet and water ad libitum. They were used at an age of 5 weeks, with positive plasma HBsAg verified by ELISA (see below).

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

### 2.4. Quantification of HBsAg and HBeAg

HBsAg and HBeAg in culture supernatants of HepG2.2.15 cells were quantified using specific ELISA kits from KeHua Bio-Engineering, while HBsAg in sera of the transgenic mice was measured using a specific ELISA kit from Fudan Yueda Bio-Tech, both located in Shanghai, China.

### 2.5. Measurement of HBV DNA by quantitative polymerase chain reaction (PCR)

For HBV DNA from HepG2.2.15 cells, the DNA was extracted from culture supernatants using DNA Extraction Kit (CASarray, Shanghai, China), and real-time quantitative PCR was performed in Lightcycler (Bio-Rad, USA) using HBV Fluorescent Quantitative PCR Detection Kit (PiJi Biotechnology Development, Shenzhen, China).

For real-time quantitative PCR of duck serum HBV DNA, the DNA was extracted with DNA Extraction Kit (CASarray). The sense and antisense primers and the TaqMan probe used were 5′-TCG GAT TAC TGG TAA GCT T-3′, 5′-CCC GTT GTC CGT CAG ATA CAG-3′, and 5′-FAM-GGT GGA TTT CTC TCA GTT CTC CAA A-TAMARA-3′, respectively, designed with the software Primer 5 (Bio-Rad). Plasma HBV DNA was treated using Chelex 100 (Bio-Rad), and then subjected to real-time quantitative PCR in a buffer containing 10 mM Tris–HCl (pH 8.3), 2 mM $MgCl_2$, 0.2 μM dNTP, the sense and antisense primers each at 600 nM, the probe at 200 nM, and 1.5 U Taq DNA polymerase (all the PCR reagents were from TaqMan Core Reagent Kit, Fudan Yueda Bio-tech, Shanghai, China). After an initial denaturation (95 °C for 3 min), the samples were subjected to 42 cycles of denaturation (94 °C for 30 s) and annealing/extension (each at 60 °C for 30 s). HBV DNA was quantified using a standard curve. In this assay, the linear range was $1 \times 10^3 \sim 1 \times 10^8$ copies/ml.

### 2.6. Measurement of duck liver DHBV DNA by Southern blotting

4 g of duck liver tissues was ground in 4 ml of a buffer (10 mM Tris–HCl, pH 7.6, 0.15 M NaCl, 1.27 mM EDTA, 20 mg/ml SDS, 5 μg/ml salmon sperm DNA, and 0.5 mg/ml proteinase K) at 50 °C for 3 h, followed by centrifugation at 13,000 × *g* for 10 min. The supernatant was extracted with phenol/chloroform, and then precipitated in two volumes of ethanol containing acetic acid at 1/10. DNA was then dissolved in 800 μl of TER buffer (10 mM Tris–HCl, pH 7.5, containing 2 mM EDTA and 100 μg/ml RNase A). DNA was separated on a 1% agarose gel and analyzed by Southern blotting using a DHBV DNA probe as described (Freiman et al., 1988).

### 2.7. Assay for DHBV DNA polymerase

Viral particles were precipitated from 200 ml of duck serum by centrifugation at 185,390 × *g* for 4 h using a superspeed centrifuge (RP-83T, Hitachi, Japan). The pellet was dissolved in 1 ml of assay buffer (50 mM Tris–HCl, pH 7.5, 2% β-mercaptoethanol, 1% NP-40, 80 μCi $^{3}$H-dTTP, 10 mM $MgCl_2$, and 20 mM KCl), and treated with various concentrations of wogonin or with PFA at 37 °C for 1.5 h. After reaction, free radioactivities were separated with incorporated radioactivities by PBS washing at 185,390 × *g* for 4 h for three times. Then the signals were measured with a liquid scintillation counter (LS-6500, Beckman Coulter, USA). To reduce the interference with the accessibility of the polymerase to labeled dTTP in vitro concentrations, we have designed no drug control group (wogonin or PFA was substituted with PBS). Every group of mixture was operated parallelly and reacted in the same condition. The data in treated groups measured with liquid scintillation counter were subtracted by the data in no drug control group.

### 2.8. Histopathological examination of duck liver

DHBV-positive ducks were treated with wogonin *i.v.* and 3TC *i.g.* one time every day for 10 days. Liver tissues were obtained 24 h after last treatment. Duck liver tissues were fixed in formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy. The degrees of hepatocytic necrosis and degeneration, and periportal tract and intralobular inflammation were assessed semi-quantitatively. In addition, duck liver tissues were fixed in 3% glutaraldehyde, washed with 0.01 M PBS, dehydrated with alcohol, embedded in EPOR812 resin, sectioned, stained with uranium acetate and citromalic acid, and examined with transmission electron microscope (H-7600, Hitachi, Japan). All specimens were evaluated on a blind basis.

### 2.9. Immunohistological examination of HBsAg in HBV-transgenic mouse liver

HBV-positive mouse were treated with wogonin *i.v.* and 3TC *i.g.* one time every day for 10 days. Liver tissues were obtained at 24 h after last dosing, fixed in 4% formaldehyde solution overnight at room temperature, and embedded in paraffin. For immunohistological analysis (Fukuda et al., 1998), paraffin-embedded sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% $H_2O_2$ for 30 min. Non-specific binding was blocked with normal sheep serum. Subsequently, the sections were incubated with a 1:500 diluted mouse polyclonal antibody against human HBsAg (Fudan Yueda Bio-Tech, Shanghai, China) for 30 min at room temperature. Immunoreaction signals were detected using Vectastain ABC Kit (DingGuo Bio-Tech, Beijing, China). Liver sections were counter-stained with hematoxylin. Normal serum from non-immunized mice was used as a control.

## 3. Results

### 3.1. Anti-HBV activity of wogonin in HepG2.2.15 cells

Treatment of HepG2.2.15 cells with wogonin at various concentrations for 3 days resulted in significant reduction of HBsAg secretion in a dose-dependent manner, with an $IC_{50}$ value of 2.56 μg/ml. After treatment for 6 or 9 days, wogonin still significantly reduced HBsAg secretion, albeit to slightly less degrees (Fig. 2A). For HBeAg secretion, the time course of the inhibitory effect of wogonin was different. Thus, at day 3 there was a minimal inhibitory effect. However, at day 6 or 9, like HBsAg, the secretion of HBeAg was significantly reduced by wogonin in a dose-dependent manner, with an $IC_{50}$ value of 4 μg/ml on day 9 (Fig. 2B). Note that for both HBsAg and HBeAg wogonin was more potent than 3TC and was highly efficacious, achieving maximal (80–100%) inhibition at 20 μg/ml.

In HepG2.2.15 cells, wogonin showed no inhibitory effect on cell proliferation up to 20 μg/ml, as analyzed by MTT assay. At 50 and 200 μg/ml, wogonin inhibited HepG2.2.15 cell proliferation at 29 and 48%, respectively (data not shown).

To further confirm the anti-HBV activity of wogonin in HepG2.2.15 cells, the effect of wogonin treatment on HBV DNA level was evaluated. Consistent with the inhibitory effect on HBsAg and HBeAg secretion, 50 μg/ml wogonin treatment led to a statistically significant reduction in extracellular HBV DNA compared with the no drug control (Fig. 3).

### 3.2. Inhibition of DHBV DNA polymerase by wogonin

To elucidate mechanism of the anti-HBV action of wogonin, effect of wogonin on DHBV DNA polymerase was examined. As shown in Fig. 4, wogonin exhibited a potent inhibitory activity on the DNA polymerase. The inhibition was dose-dependent, with an $IC_{50}$ value of 0.57 μg/ml, and with 33% inhibition at the lowest concentration tested: 0.2 μg/ml.

### 3.3. In vivo anti-HBV activity of wogonin in ducks

DHBV-infected ducks were treated with wogonin at various doses or with 3TC at 50 mg/kg once a day for 10 days, and then plasma DHBV DNA levels were measured by real-time quantitative PCR. As shown in Fig. 5, wogonin significantly reduced plasma DHBV DNA levels in a dose-dependent manner, the

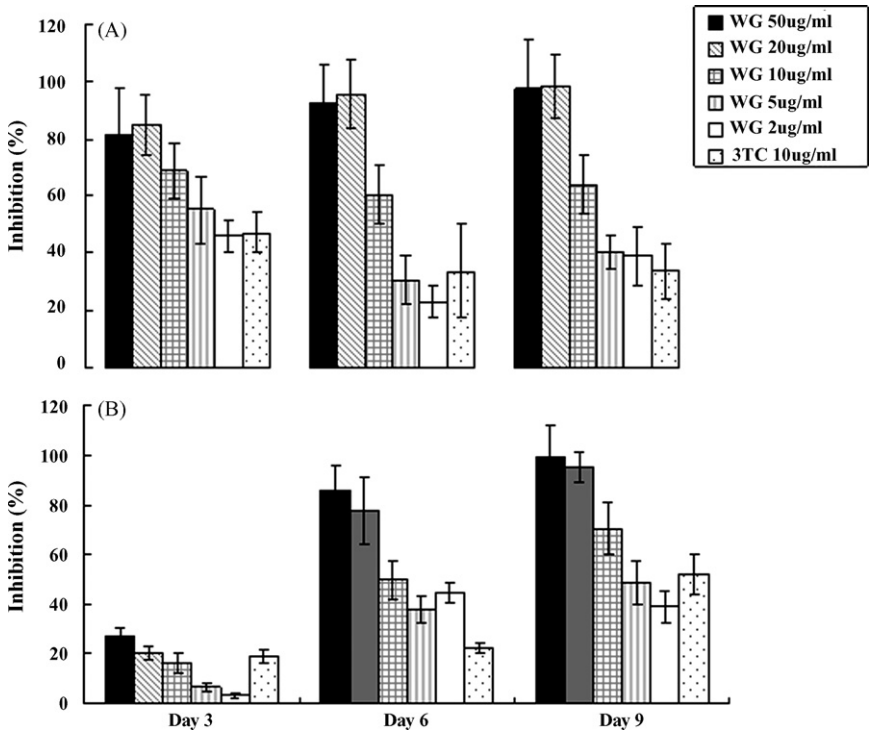


Fig. 2. Inhibitory effect of wogonin on secretion of HBsAg (A) and HBeAg (B) from HepG2.2.15 cells. HepG2.2.15 cells were cultured in the presence of wogonin (WG) at various concentrations or of 3TC at 10 μg/ml for 3, 6 or 9 days, and then HBsAg and HBeAg in the supernatants were quantified using specific ELISA kits. The experiments were performed three times, and data are presented as mean ± S.D. of all experiments. $^{**}P < 0.01$, $^{*}P < 0.05$ and compared with the no drug control group.

effect being significant at the lowest dose tested (5 mg/kg). Interestingly, even at day 20 (10 days after end of the treatment), the plasma DHBV DNA levels remained lower than control level. Moreover, the rebound of DHBV DNA level in wogonin-treated ducks was to a less degree as compared with 3TC-treated group.

To further confirm the in vivo anti-HBV effect of wogonin in ducks, DHBV DNA levels were examined by Southern blotting in livers obtained at day 5 after end of the treatment. Consistent with the inhibitory effect on plasma DHBV DNA level, wogonin treatment dose-dependently reduced both the relaxed circular and linear forms of DHBV DNA in the liver (Fig. 6). Densitometric analysis of the autoradiographic signals showed 54, 37 and 26% inhibition by wogonin at 20, 10 and 5 mg/kg, respectively, and 66% inhibition by 3TC at 50 mg/kg.

### 3.4. Histopathological examination of duck livers

Typical photographs of liver sections by light microscopy are shown in Fig. 7. Results of evaluation of all slides are summarized in Table 1. Liver sections were evaluated primarily in terms of hepatocytic degeneration and necrosis, and inflammatory cell infiltration. Ten-day wogonin treatment exhibited

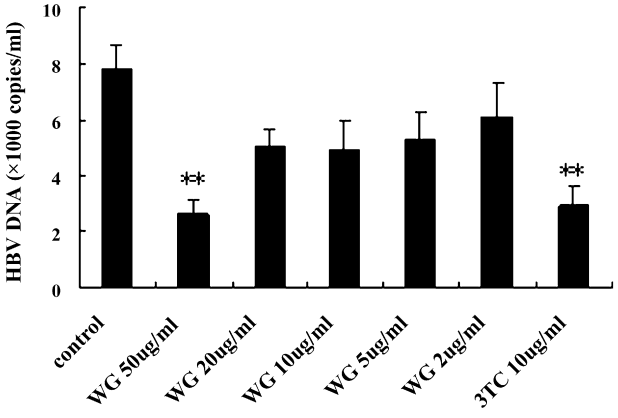


Fig. 3. Inhibitory effect of wogonin on HBV DNA level in HepG2.2.15 cells. HepG2.2.15 cells were cultured in the presence of wogonin at various concentrations or of 3TC at 10 μg/ml for 9 days, and then HBV DNA levels were quantified by real-time quantitative PCR. The experiments were performed three times, and data are presented as mean ± S.D. of all experiments. $^{**}P < 0.01$ vs. vehicle control.

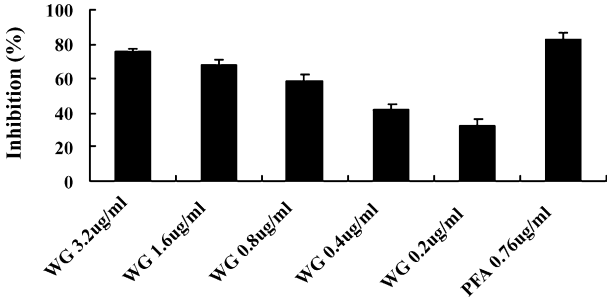


Fig. 4. Inhibition of DHBV DNA polymerase by wogonin. DHBV DNA isolated from duck blood was used as HBV DNA polymerase enzyme sample, and the enzyme activity was assayed in the presence of wogonin at various concentrations or of PFA at 0.76 μg/ml. The experiments were performed three times, and data are presented as mean ± S.D. of all experiments.

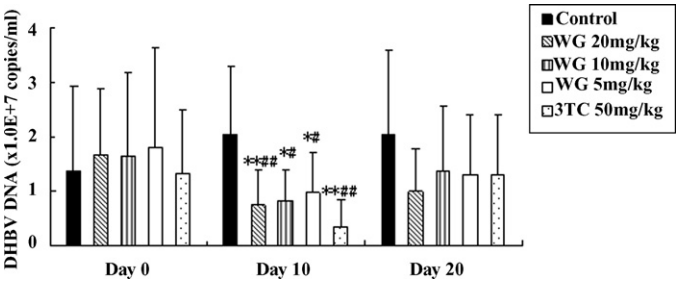


Fig. 5. In vivo inhibitory effect of wogonin on duck plasma DHBV DNA level. Ducks were treated with wogonin at various doses or with 3TC at 50 mg/kg once a day for 10 days. After the treatment, animals were maintained for additional 10 days. Plasma DHBV DNA levels were quantified by real-time quantitative PCR. The experiments were performed three times, and data are presented as mean ± S.D. of all experiments ($n = 12$). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control of the same group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ vs. day 0.

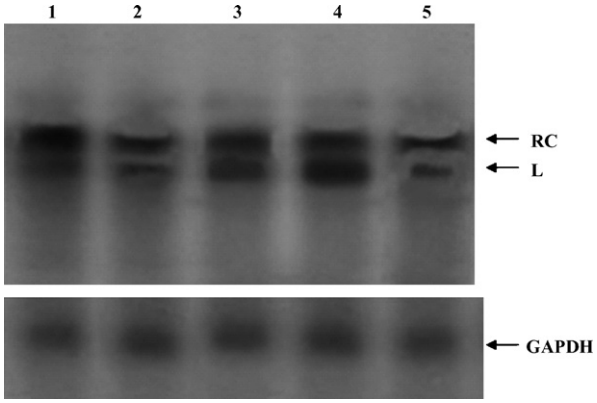


Fig. 6. In vivo inhibitory effect of wogonin on duck liver DHBV DNA level. Ducks were treated with wogonin at 0 (1), 20 (2), 10 (3) or 5 (4) mg/kg or with 3TC at 50 mg/kg (5) once a day for 10 days. At day 5 after end of the treatment, levels of both the relaxed circular (RC) and linear (L) forms of liver HBV DNA were examined by Southern blotting. The experiments were performed three times, and a representative set of data are presented.

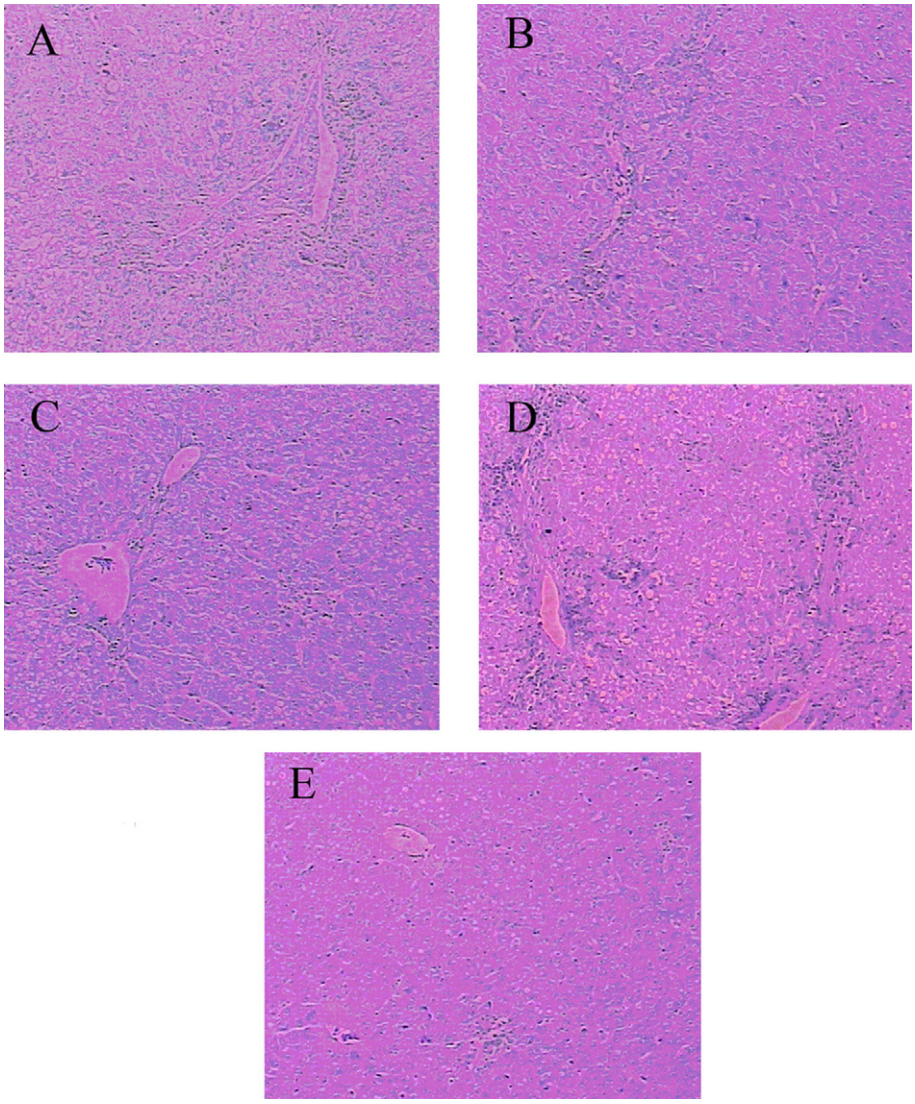


Fig. 7. Histopathological changes in duck livers. Ducks were treated with wogonin at 0 (A), 20 (B), 10 (C) or 5 (D) mg/kg or with 3TC at 50 mg/kg (E) once a day for 10 days. Then, liver sections were stained with hematoxylin and eosin, and examined by light microscopy. Representative photographs are presented (magnification: 100×).

Table 1
Summary of histopathological changes in duck livers

| Group | Severity | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Degeneration | | | | Necrosis | | | | Cell infiltration | | | |
| | − | + | 2+ | 3+ | − | + | 2+ | 3+ | − | + | 2+ | 3+ |
| Vehicle | 1 | 2 | 7 | 2 | 2 | 5 | 5 | 0 | 1 | 2 | 8 | 1 |
| Wogonin (20 mg/kg) | 4 | 5 | 3 | 0 | 6 | 5 | 1 | 0 | 6 | 5 | 1 | 0 |
| Wogonin (10 mg/kg) | 2 | 5 | 5 | 0 | 3 | 8 | 1 | 0 | 4 | 5 | 3 | 0 |
| Wogonin (5 mg/kg) | 2 | 4 | 6 | 0 | 4 | 7 | 1 | 0 | 2 | 6 | 4 | 0 |
| 3TC (50 mg/kg) | 3 | 5 | 4 | 0 | 5 | 5 | 2 | 0 | 5 | 5 | 2 | 0 |

Hepatic degeneration and necrosis, and inflammatory cell infiltration were examined in lobular and periportal tract regions. The degree of degeneration was determined as the percent of hepatocytic edema and fatty degeneration, and scored as follows: (−) none; (+) <25%; (2+) <50; (3+) ≥50%. Necrosis was scored as follows: (−) none; (+) spotty necrosis; (2+) piecemeal necrosis; (3+) bridging or widespread necrosis. Cell infiltration was scored as follows: (−) none; (+) mild infiltration; (2+) moderate infiltration; (3+) massive infiltration or lymphoid nodules. For other details, see legend to Fig. 7.

dose-dependent improvements in all three parameters, with improvements in necrosis and cell infiltration better than that in degeneration. It is worth noting that wogonin at 20 mg/kg resulted in more significant improvements than that with 3TC at 50 mg/kg did (Table 1).

To evaluate pathological changes at the subcellular level, liver sections from the above various treatment groups were examined under transmission electron microscope. In vehicle control group, there was significant edema at endoplasmic reticulum, indicating significant DHBV expression (Fig. 8). The 10-day

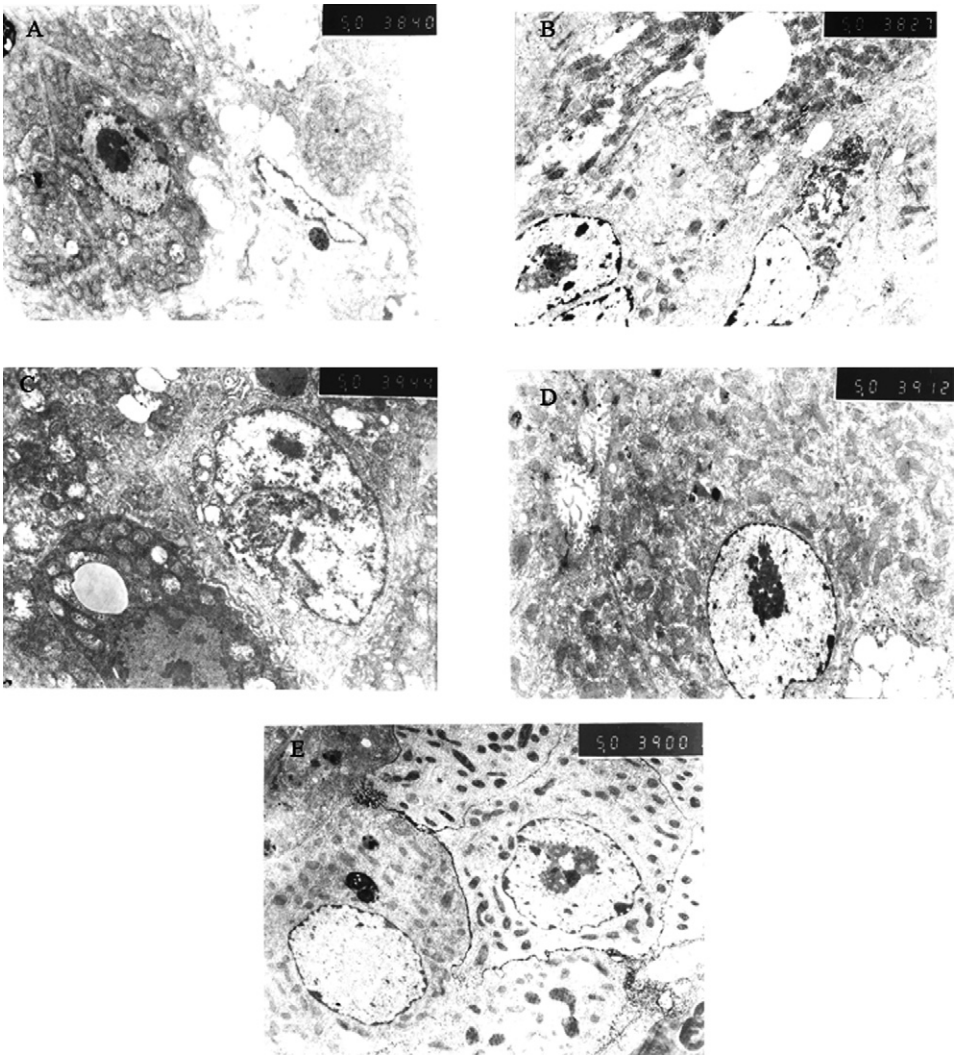


Fig. 8. Subcellular changes in duck livers. Ducks were treated with wogonin at 0 (A), 20 (B), 10 (C) or 5 (D) mg/kg or with 3TC at 50 mg/kg (E) once a day for 10 days, and then liver sections were examined by transmission electron microscopy. All slides were evaluated blindly by two reviewers independently. When evaluation results were different between the reviewers, the slide was re-evaluated by two other reviewers. Representative photographs are presented (magnification: 5000×).

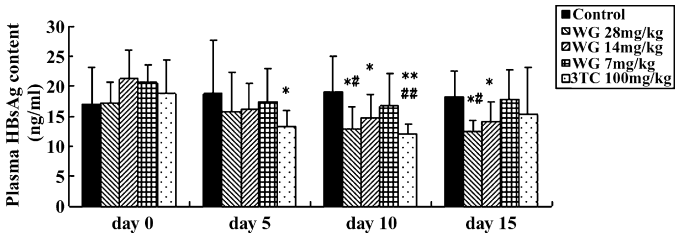


Fig. 9. In vivo inhibitory effect of wogonin on serum HBsAg in human HBV-transgenic mice. Human HBV-transgenic mice were treated with wogonin at various doses or with 3TC at 100 mg/kg for 10 days. Sera were collected at days 0, 5, 10 and 15 (5 days after termination of treatment), and then HBsAg was quantified using a specific ELISA kit. The experiments were performed three times, and data are presented as means ± S.D. of all experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control of the same group; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ vs. day 0.

treatment with wogonin at all doses, particularly 20 mg/kg, significantly improved the endoplasmic reticulum edema.

### 3.5. *In vivo anti-HBV activity of wogonin in transgenic mice*

Human HBV-transgenic mice were treated with wogonin at various doses for 10 days. At days 0, 5, 10 and 15 (5 days after termination of treatment), serum HBsAg levels were quantified by ELISA. At day 5, wogonin exhibited a trend of reducing HBsAg level, although the reductions were not statistically significant. However, at day 10 wogonin at 14 and 28 mg/kg significantly reduced the HBsAg level (Fig. 9). 3TC at 100 mg/kg had a more significant effect on HBsAg than that of wogonin. Interestingly, however, the in vivo anti-HBV activity of wogonin persisted for at least 5 days after termination of treatment, whereas there was a more rapid rebound of serum HBsAg level with 3TC treatment.

To further confirm the in vivo anti-HBV activity of wogonin in transgenic mice, immunohistological analyses was performed in livers from the above various treatment groups. In vehicle control group, HBsAg was detected (stained brownish yellow) in the cytoplasm of more than half of all hepatocytes viewed. Wogonin treatment reduced the HBsAg level in a dose-dependent manner. At 28 mg/kg of wogonin, HBsAg expression was nearly undetectable, similar to the result with 3TC at 100 mg/kg (Fig. 10).

## 4. Discussion

Thus far, there is only a single report on the anti-HBV activity of wogonin (Huang et al., 2000). In that report, the anti-HBV activity was shown in vitro in two assays in a HBV-transfected liver cell line (MS-G2): HBV antigen secretion, and HBV DNA polymerase reaction. In our present study, we set out to confirm the in vitro inhibitory effect of wogonin on HBV antigen secretion. In HBV-transfected HepG2.2.15 cells, wogonin exhibited a potent inhibitory activity on HBsAg secretion, with an $IC_{50}$ value of 2.56 μg/ml, after 3-day treatment. Consistent with the previous report (Huang et al., 2000), 3-day treatment with wogonin did not have a significant effect on HBeAg release. However, by 6- or 9-day treatment, wogonin inhibited HBeAg secretion with potencies similar to those for HBsAg inhibition. It is worth noting that the potency of wogonin was higher than that of 3TC, and that wogonin was highly efficacious, achieving 80–100% inhibition (Fig. 2). For the mechanism of 3TC on deducing HBsAg, it is phosphorylated inside HepG2.2.15 cells and is subsequently incorporated into nascent viral DNA by the HBV polymerase during replication. 3TC incorporation results in the termination of DNA elongation by virtue of its lack of a 39 hydroxyl group Therefore, the expected result would be that 3TC would not directly affect the transcription or translation of HBV gene products from nuclear DNA because it acts downstream of these events (Cammack et al., 1992). Consistent with its widely reported anti-cancer activity, wogonin exhibited a weak inhibitory effect on proliferation of HepG2.2.15 cells, with about 30 and 50% inhibition at 50 and 200 μg/ml, respectively. However, at 20 μg/ml wogonin showed no inhibitory effect on proliferation of the cells (data not shown), suggesting that the inhibition of HBV antigen secretion might be a specific anti-HBV activity. The anti-HBV activity of wogonin was further confirmed by its inhibitory effects on HBV DNA level in HepG2.2.15 cell culture (Fig. 3) and on duck HBV DNA polymerase activity (Fig. 4). Thus, wogonin may exert its anti-HBV activity via, at least in part, inhibition of HBV DNA polymerase. Taken together, our data clearly demonstrate that wogonin has a potent anti-HBV activity. The difference in potency of wogonin between the present study and the previous report might be due to differences between the different cell lines used (Cho and Lee, 2004; Tai et al., 2005).

Furthermore, the present study demonstrates, for the first time, that wogonin has a potent anti-HBV activity in vivo. The in vivo anti-HBV activity was investigated in DHBV-infected ducks (Guha et al., 2004; Schultz et al., 2004; Zoulim, 2001) and human HBV-transgenic mice (Akbar and Onji, 1998; Chisari, 1996; Guha et al., 2004; Morrey et al., 1998), both being well-established animal models for pharmacologic anti-HBV studies. In DHBV-infected duck, wogonin administered for 10 days significantly reduced plasma (Fig. 5) and liver (Fig. 6) DHBV DNA levels at the lowest dose tested: 5 mg/kg. And there were significant differences in every dose groups of wogonin at day 10, even at day 20, the plasma HBV DNA levels remained lower than control level (Fig. 6). The in vivo anti-HBV activity was confirmed by histopathological improvement (Fig. 7 and Table 1) and further by improvement at the subcellular level (Fig. 8) in the liver. It is worth noting that the histopathological examination revealed more significant improvement by wogonin at 20 mg/kg than 3TC at 50 mg/kg. In addition, the in vivo anti-HBV activity of wogonin was also shown in human HBV-transgenic mice. Wogonin administered at 7–28 mg/kg for 10 days reduced plasma (Fig. 9) and liver (Fig. 10) HBsAg levels in a dose-dependent manner. At the highest dose (28 mg/kg) of wogonin, liver HBsAg was reduced to a nearly undetectable level. To investigate the reduction of mice HBsAg expression was caused by antiviral effect of wogonin but not the loss of the HBV transgene, we had detected the viral DNA level in no drug control mouse sera before and after the experiment. The results showed that the level of DNA remains no change. Moreover, we have detected the cytotoxicity of wogonin on the primary cultured liver cells and found that the tested concentration of wogonin exerted little growth inhibition (data not shown). From these results we concluded that the

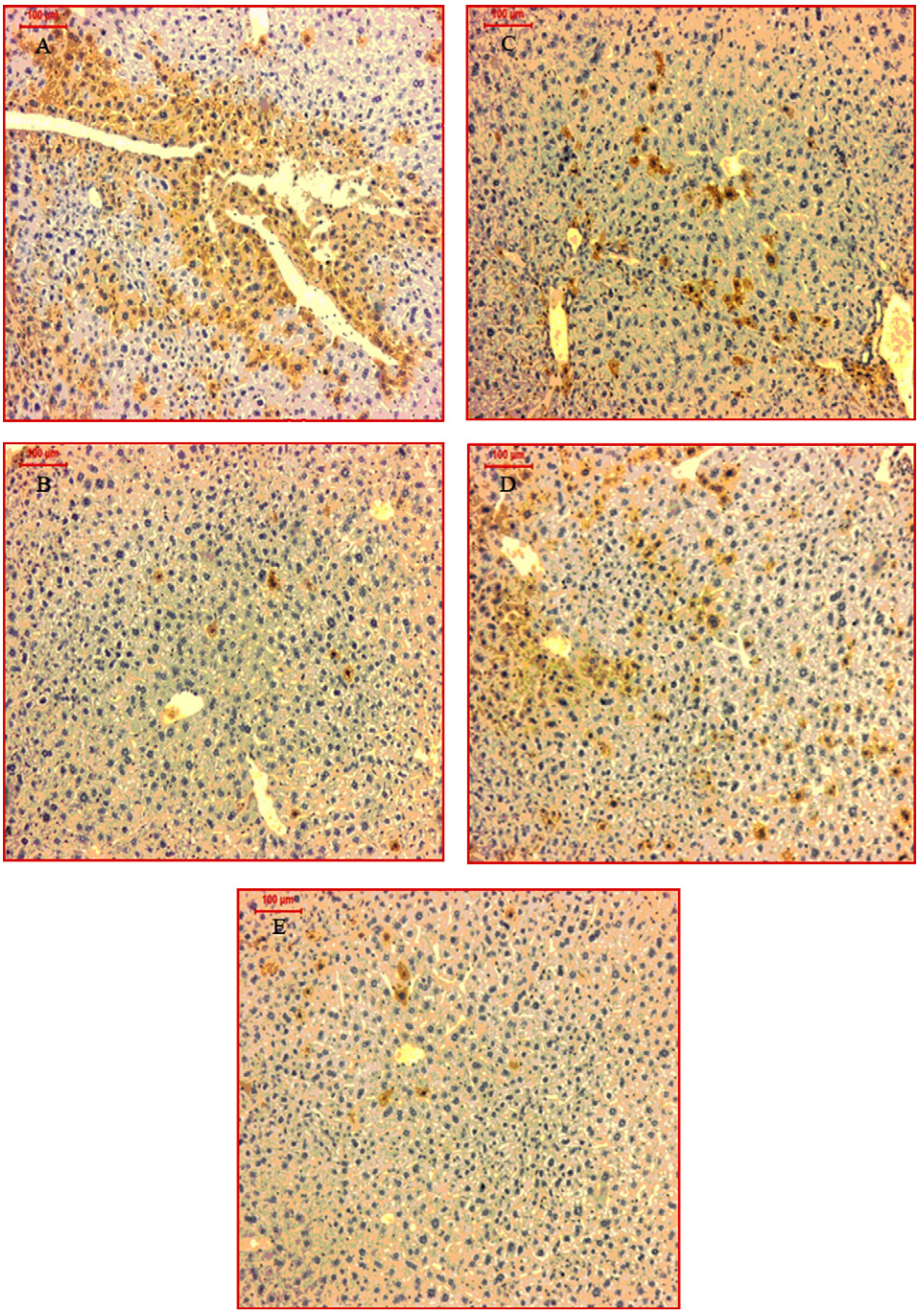


Fig. 10. Immunohistological analysis of transgenic mouse livers. Human HBV-transgenic mice were treated with wogonin at 0 (A), 28 (B), 14 (C) or 7 (D) mg/kg or with 3TC at 100 mg/kg (E) for 10 days. Then, liver sections were subjected to immunohistological analysis of HBsAg. Representative photographs are presented (magnification: 100×).

reduction of HBsAg expression was the effect of antiviral but not liver toxicity.

Interestingly, the anti-HBV effect of wogonin, lasted for at least 10 and 5 more days after termination of treatment in ducks and transgenic mice, respectively, indicating the in vivo anti-HBV effect of wogonin was relatively long-lasting in both ducks (Fig. 5) and transgenic mice (Fig. 9) as compared with the effect of 3TC. The relatively rapid rebound of plasma HBV DNA level in 3TC-treated ducks has been reported previously (Marion et al., 2002). This long duration of wogonin's activity may have significant clinical implications, and further supports the significance of developing wogonin into an anti-HBV drug.

In conclusion, the present study demonstrated that wogonin has potent anti-HBV activity in vitro and in vivo. The in vivo potency, efficacy and safety of wogonin support the necessity of developing this natural product into a potential therapeutic agent for better management of hepatitis B.

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