

## Korean Medicinal Plant Extracts Exhibit Antiviral Potency Against Viral Hepatitis

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### ABSTRACT

**Objectives:** Investigation of natural ethnopharmacologic extracts exhibiting antiviral potential may lead to the discovery of new therapeutics for the treatment of chronic viral hepatitis infections. Traditional Korean medicinal herbs have been identified that exhibit potency against hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Research on the antiviral potential of naturally derived extracts is facilitated through the use of appropriate animal and liver cell culture models for these hepatotropic pathogens. Objectives of this study were to demonstrate antiviral activity of an aqueous extract of herbal formulation KYH-1 in surrogate *in vitro* assays for HBV and HCV and identify mechanisms of action.

**Methods:** Antiviral potency of KYH-1 was measured in tissue culture systems that support replication of the woodchuck hepatitis virus (WHV), and the bovine viral diarrhea virus (BVDV). These assays serve as surrogate models for HBV and HCV, respectively. A recombinant HBV polymerase gene expression assay was used to define a molecular target.

**Results:** KYH-1 exhibited potent antiviral activity against WHV and to a lesser extent against BVDV. KYH-1 and its constituent components inhibited HBV polymerase priming *in vitro*. Additionally, KYH-1 suppressed HBV replication in a human hepatoblastoma cell line.

**Conclusion:** Evaluation of naturally derived products for antiviral activity against HBV and HCV in standardized surrogate assays provides a scientific basis for potential use as complementary or alternative medicines. This study provides significant results justifying preclinical evaluation of KYH-1 as an antiviral therapy for HBV infections.

### INTRODUCTION

Improved treatments are needed for patients with chronic viral hepatitis. Over 300 million people worldwide are chronic carriers of hepatitis B virus (HBV) and over 100 million are chronic carriers of hepatitis C virus (HCV) (Alter, 1997; Hoofnagle and DiBisceglie, 1997). Chronic hepatitis infections can progress to cirrhosis that may lead to hepatic failure or to the development of hepatocellular carcinoma (DiBisceglia and Bacon, 1999). Seroprevalence data estimates that 10%–15% of infected individuals in an endemic region may have concurrent HBV/HCV infections

(Liaw, 2001). Dual HBV/HCV infections often lead to more severe liver disease (Crespo et al., 1997).

New modes of therapy that can complement the antiviral drugs in current clinical use need to be identified. Approved drugs for the treatment of chronic viral infections include lamivudine (3TC) and adefovir dipivoxil for HBV and interferon- $\alpha$  (IFN- $\alpha$ ) and ribavirin for HCV (Anonymous, 2003; Jarvis and Faulds, 1999; McHutchison et al., 1998). However, upon prolonged treatment regimens mutant viruses develop that are resistant to antiviral treatment. Currently, combination therapy is used as an approach to prevent HBV drug resistant mutants from developing (Yang et

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al., 2002), and enhance HCV therapeutic regimens (Look et al., 1999; McHutchison et al., 1998; Schvarcz et al., 1995).

Investigation of ethnopharmacologic extracts exhibiting antiviral potential may complement conventional therapies (Bedoya et al., 2002; Liang, 1999; McCulloch et al., 2002; Schuppan et al., 1999; Seeff et al., 2001). Several herbal extracts have been used to treat Korean patients for hepatitis based on the Korean medicine book written by the king's medical doctor circa 1613 (*Donguibogam*). However, clinical studies on the effects of traditional medicines in patients with chronic liver disease during treatment are limited in scope, lack appropriate control groups, or have used inappropriate methods of randomization (McCulloch et al., 2004; Strader and Zimmerman, 2000).

A process to evaluate antiviral activity of ethnopharmacologic extracts in standardized assays provides a scientific basis for potential use as complementary or alternative medicine (Schuppan et al., 1999; Seeff et al., 2001). In this report we describe an aqueous herbal extract, termed KYH-1. The formula was optimized based upon testing individual components for *in vitro* antiviral activity. The woodchuck infected with the woodchuck hepatitis virus (WHV) is a model for investigation of the pathogenesis of human HBV infections (Tennant and Gerin, 2001) and has been instrumental in the development of antiviral drugs for chronic HBV infections (Korba et al., 2000a). HCV is a member of the viral family *Flaviviridae*. Related members of this family with similar genomic organization and replication strategies include the flaviviruses, the pestiviruses, and the HCV (Rice, 1996). The bovine viral diarrhea virus (BVDV), a pestivirus, has been developed for cell-based screening assays to assess antiviral drug activity (Baginski et al., 2000; Givens et al., 2003; Zitzmann et al., 1999).

In the rational drug design for combination therapy one ideally would like to utilize antivirals that target different components of the viral life cycle. Defining a mechanism of antiviral action may lead to increasing antiviral potency of the herbal extract by modifying the composition of its constituent components (Sato et al., 1996). Investigation into the capacity of herbal extracts to increase antiviral efficacy or reduce toxicity strengthens their use as complementary medicines (Piras et al., 1997; Yamamoto et al., 1999). Following a scientific process, improved methods to investigate natural ethnopharmacologic extracts, either alone or in combination with conventional therapies for treatment of chronic viral hepatitis will evolve (Matsuo et al., 2001; Wang, 2000).

## MATERIALS AND METHODS

### *Herbal extract preparation*

The components of KYH-1 were a formulated mixture of seven herbs, magua melon dried fruit (*Cucumis melo* L. var.

*ma-gua* Cucurbitaceae), kombu dried thallus, also known as Japanese kelp (*Laminaria japonica* Aresch; Laminariaceae), kudzu root (*Pueraria lobata* [Willd.] Ohwi; Fabaceae), hawk's beard dried root (*Youngia sonchifolia* Max.; Asteraceae), Indian lettuce dried leaf (*Lactuca indica* L. var. *laciniata* [O. Kuntze] Hara; Fabaceae), and snow thistle dried root (*Ixeris dentata* [Thunb.] Nakai; Asteraceae). The genus and species of plants used for the KYH-1 extract were certified by Dr. Jong Wook Park, Herbarium, School of Biological Sciences, Seoul National University. *L. japonica* was desalted with several washes of dH<sub>2</sub>O and air-dried at 20–25°C for 5 days. The root of *P. lobata* was separated from the whole plant and air-dried at 25°C for 20 days.

The roots of *Y. sonchifolia* and *I. dentata* were dried at 25°C for 14–21 days. The pulverized powders were combined in a ratio of 45 g (*C. melon*): 15 g (*P. lobata*): 6 g (*L. japonica*): 3 g (*Y. sonchifolia*): 6 g (*I. dentata*): 6 g (*L. indica*) and designated as KYH-1. Microbial contamination from the processed powders was reduced by dry-heat treatment at 80°C for 30 minutes and checked by streaking on LB agar plates without antibiotics. The optimized ratio of the component mixture was based on previous experiments that evaluated each component individually against HBV expression in HepG2.2.15 cells. A 10% aqueous extract was prepared by dissolving KYH-1 in phosphate buffered saline, clarified by centrifugation, and sterile filtered (0.22 μm) prior to *in vitro* assays. A heat-derived extract was prepared by heating the 10% KYH-1 solution at 90°C for 2 hours, followed by centrifugation, and sterile filtration. Heavy metal contamination in the KYH-1 mixture was evaluated at the Center for Environmental & Clean Technologies, The University of Suwon and the preparation contained 1 parts per billion (ppb) of lead and of arsenic.

### *In vitro cell culture assays*

BVDV has been used as a surrogate *in vitro* assay to screen for compounds effective against HCV (Baginski et al., 2000; Givens et al., 2003; Zitzmann et al., 1999). A bovine cell line, designated NCL, was generated via immortalization of primary bovine uterine cells with the SV40 large T antigen oncogene by established procedures (Dobranski et al., 1999) and is permissive to cytopathogenic BVDV isolates. This assay determines the compound's effective concentration: (1) to inhibit BVDV-induced cell killing by 50% (EC<sub>50</sub>); (2) to reduce the release of progeny virus by 90% (EC<sub>90</sub>); and (3) at which 50% of normal uninfected cells were killed as a result of cytotoxic effects of the drug (CC<sub>50</sub>). Linear regression equations were used to determine effective concentrations (SigmaPlot8.0; SPSS Scientific, Chicago, IL). Ribavirin (Virazole<sup>RF</sup>; Viratek, Inc., Covina, CA) and IFN-α (rHuB/DαIFN; Novartis, Basel, Switzerland) served as positive antiviral controls in this assay.

Establishment of primary woodchuck hepatocyte cultures (1°WCH) expressing WHV has been previously described (Jacob et al., 1994). Hepatocytes were isolated from chronic WHV-carrier animals (Cornell University, Ithaca, NY) and plated at a density of  $5 \times 10^5$  cells/cm<sup>2</sup>. Growth media (Life Technologies, Grand Island, NY) was changed at regular 2-day intervals, including addition of test compounds. Recombinant IFN- $\alpha$  served as a positive antiviral (Korba et al, 2000b) control in these experiments, and was used at a concentration of  $2 \times 10^6$  IU/mL ( $5 \mu\text{g/mL}$ ). Untreated 1°WCH served as control cultures.

At the end of treatment, nucleic acids were extracted from cultures using commercially available kits (Qiagen, Valencia, CA) and the replicative state of WHV analyzed by molecular techniques. Hybridizations were performed in Church buffer (0.5 mmol NaPO<sub>4</sub>, 7% sodium dodecyl sulfate [SDS], 1 mmol/L ethylenediaminetetraacetic acid [EDTA], and 100  $\mu\text{g/mL}$  yeast tRNA) at 60°C. The 3.3-kb *EcoRI* fragment of pWHV-2 was used to generate WHV-specific probe by random primed, DNA synthesis (Life Technologies, Gaithersburg, MD) incorporating [<sup>32</sup>P]dCTP (3000 Ci/mmol, NEN). Hybridization signals were analyzed using Phosphor-Imager 830 (Molecular Dynamics, Sunnyvale, CA) (Jacob and Tennant, 1996).

The HepG2.2.15 cell line constitutively expressing HBV via an integrated HBV genome has been used for drug screening (Korba and Milman, 1991). Each individual component of KYH-1 was evaluated in this assay to optimize the final aqueous KYH-1 extract. The nucleoside analogue lamivudine (3TC) served as a positive antiviral control and viral replication was quantified by molecular techniques (Korba and Gerin, 1992; Korba and Milman, 1991). Inhibition of HBV secretion from HepG2.2.15 cells was monitored by PCR. The forward primer sequence (nt157–171): 5'-GGG-GGA-ATT-CAT-GGA-GAA-CAT-CAC-ATC-AGG-ATT-C-3' and reverse primer (nt837–814): 5'-GGG-CTG-CAG-TTA-AAT-GTA-TAC-CCA-AAG-ACA-AAA-3' amplifies a 680 nt product, and after 30 cycles analyzed by electrophoresis through 1% agarose gels.

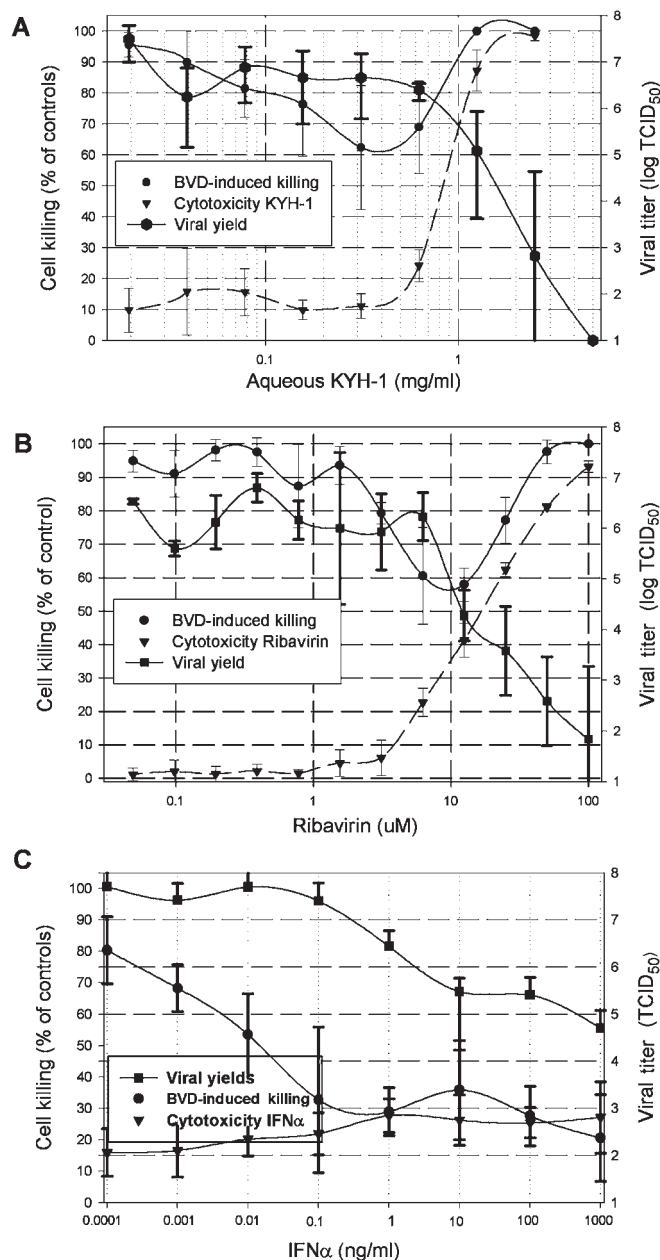
### Molecular target assay

A baculovirus system expressing the HBV polymerase gene was used to measure inhibition of polymerase activity (Lanford et al., 1995; 1997). Extracts of KYH-1 or its constituent components were added to 10 mL of Sf9 insect cell culture media and polymerase activity assayed after 48 hours. Recombinant HBV pol was precipitated from the cultures and the activity was determined by <sup>32</sup>P-dATP (Perkin-Elmer, Boston, MA) incorporation during a primer reaction monitored by autoradiography of products separated by polyacrylamide gel electrophoresis. The pol-complex sample loads were normalized by immunostaining for the recombinant Flag-HBV pol complex.

## RESULTS

### Antiviral effects of KYH-1 on BVDV replication in vitro

Antiviral activity of the aqueous KYH-1 extract was analyzed in the BVDV assay and compared to the activity of ribavirin and INF- $\alpha$  (Fig. 1). There was a dose-dependent



**FIG. 1.** Antiviral effects of KYH-1 on bovine viral diarrhea virus (BVDV) replication. Dose response of (A) KYH-1, (B) ribavirin (Virazole, Viratek, Inc., Covina, CA), and (C) interferon (IFN)- $\alpha$  on BVDV-induced cell killing (●), viral yields (■), and cytotoxicity (▼) of the drug on uninfected cells. Values are expressed as a percentage of untreated controls.

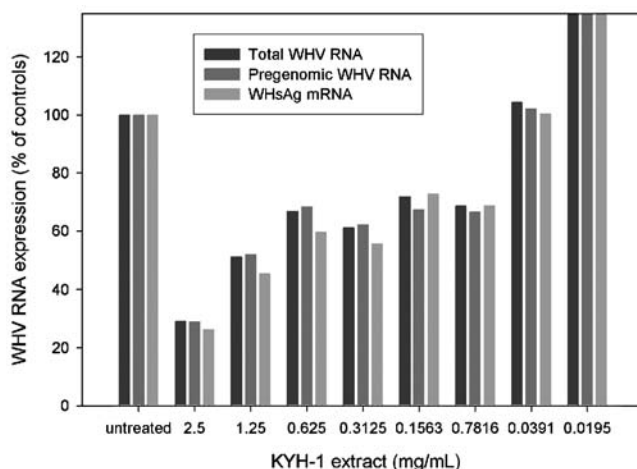
TABLE 1. ANTIVIRAL ACTIVITY OF DRUGS AGAINST BVDV-INFECTED BOVINE UTERINE NCL CELLS

Antiviral	EC <sub>50</sub>	EC <sub>90</sub>	CC <sub>50</sub>	SI (CC <sub>50</sub> /EC <sub>50</sub> )
KYH-1 extract	530 µg/mL	630 µg/mL	840 µg/mL	1.58
Ribavirin	9.92 µmol/L	26.3 µmol/L	18.4 µmol/L	1.85
Interferon-α	0.23 ng/mL	0.013 ng/mL	> 1000 ng/mL	4348

BVDV, bovine viral diarrhea virus; EC<sub>50</sub>, effective concentration inhibiting 50% of cell-killing; EC<sub>90</sub>, effective concentration reducing viral titers 90%; CC<sub>50</sub>, cytotoxic concentration killing 50% of viable cells; SI, selectivity index.

reduction in viral-induced cell killing, with a maximum 37.3% reduction at an extract concentration of 280 µg/mL, with EC<sub>50</sub> = 530 µg/mL and reduction in viral yield EC<sub>90</sub> = 630 µg/mL (Fig. 1A). Ribavirin inhibited viral-induced cell killing with an EC<sub>50</sub> = 9.92 mmol/L and reduction in viral yields at EC<sub>90</sub> = 26.3 µmol/L (Fig. 1B). Antiviral effects of IFN-α measured in this assay showed inhibition of viral-induced cell killing at an EC<sub>50</sub> = 0.23 ng/mL and reduction in viral yield at an EC<sub>90</sub> = 0.013 ng/mL (Fig. 1C).

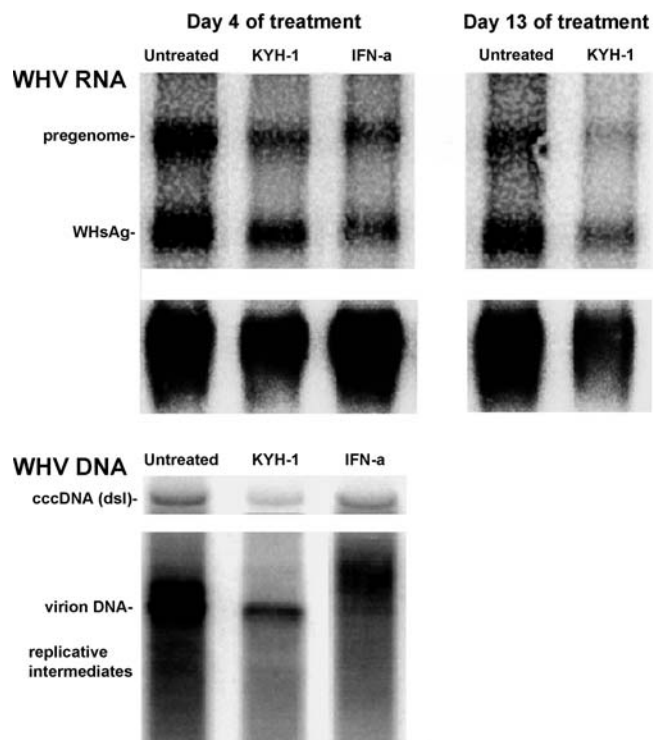
The cytotoxicity of KYH-1, CC<sub>50</sub> = 840 µg/mL, was near its EC<sub>90</sub>, and the reduction in progeny viral yields may be the result of cytotoxic effects. Unlike KYH-1, the cytotoxicity for ribavirin was CC<sub>50</sub> = 18.4 µmol/L and greater than its EC<sub>90</sub>. The decrease in progeny viral yields may be because of fewer viable cells available for viral replication. IFN-α cytotoxicity was not apparent to 1 µg/mL. A selectivity index (SI = CC<sub>50</sub>/EC) can be calculated based on a compound's effective antiviral concentration relative to its cytotoxicity, when large values indicate that the antiviral effect is removed from cytotoxic interactions. In this comparison we calculated our values on the EC<sub>50</sub>, and the SI of KYH-1 was similar to ribavirin, although IFN-α far exceeded either (Table 1).



**FIG. 2.** Antiviral effects of KYH-1 on woodchuck hepatitis virus (WHV) replication. Dose response of KYH-1 on expression of WHV pregenomic, WHsAg RNA and WHV RNA detected in KYH-1-treated cultures expressed as a percentage of untreated controls.

#### Antiviral effect of KYH-1 on WHV replication in vitro

A dose-response curve was established for KYH-1 in WHV-infected 1°WCH (Fig. 2). A greater than 50% reduction in WHV RNA was observed at KYH-1 concentrations greater than 1.25 mg/mL at the end of 6 days of treatment. The antiviral activity of KYH-1 was compared to that of IFN-α, which has been shown effective against WHV (Korba et al., 2000b). WHV-infected 1°WCH were treated with KYH-1 (1.5 mg/mL) or IFN-α (10<sup>6</sup> IU; 5 µg/mL) over a 2-week period. Nucleic acids were analyzed at 4 and 13 days post-treatment (Fig. 3). WHV pregenomic and WHV surface antigen (WHsAg) RNA species were reduced after treatment with



**FIG. 3.** Antiviral effect of KYH-1 on woodchuck hepatitis virus (WHV) replication in 1°WCH. (A) Relative expression of WHV RNA on day 4 and 13 of KYH-1 treatment compared to untreated and interferon (IFN)-α controls. (B) Mobility of WHV DNA on day 4 of KYH-1 treatment compared to untreated and IFN-α controls.

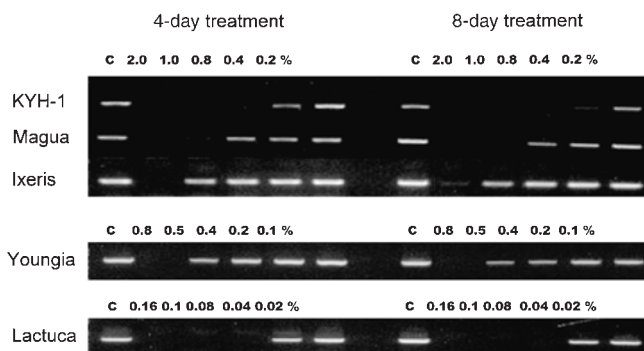
KYH-1 and by treatment with IFN- $\alpha$ , by 20% and 40%, respectively, compared to untreated control cultures after 4 days of treatment (Fig. 3A). Treatment with KYH-1 for 13 days resulted in a 20% reduction of WHV RNA compared to untreated controls at day 13, after normalizing for RNA loads.

WHV DNA is found in three forms (Fig. 3B). Nuclear WHV DNA normally exists as a covalently closed circle (cccDNA), as a result of restriction enzyme digestion the cccDNA migrates as a double-stranded linear molecule (dsl). The nuclear WHV DNA was not affected by KYH-1 or IFN- $\alpha$  after 4 days of treatment. The virion WHV DNA is a partially double-stranded genome and migrates below the dsl form. Virion DNA was significantly altered as a result of KYH-1 and IFN- $\alpha$  treatment as revealed by phosphor-image analysis after WHV DNA hybridizations. The replicative WHV DNA intermediates, migrate as a smear below the virion DNA, and were significantly diminished as a result either of KYH-1 or of IFN- $\alpha$  treatment.

#### Antiviral effect of KYH-1 extract on HBV replication in vitro

KYH-1 was evaluated in the HepG2.2.15 cell system. Effective concentrations inhibiting intracellular HBV replicative DNA intermediates ( $EC_{50}$ ) and inhibiting HBV virion DNA secretion ( $EC_{90}$ ) were determined. Both KYH-1 and a heat derived extract were compared to 3TC (Table 2). The  $EC_{50}$  and  $EC_{90}$  of the aqueous KYH-1 extract was approximately 2.8-fold greater than the activity observed in the heat-extracted KYH-1. Toxicity was not observed with the extracts in this assay, therefore, the highest concentration of drug tested was used to calculate a selectivity index ( $SI = CC_{50}/EC_{90}$ ) and an SI of 14.6 was favorable. The potency of KYH-1 was found to decrease upon heating, indicative of a heat labile substance.

Constituent components of the KYH-1 formula were examined for their capacity to inhibit release of HBV particles from HepG2.2.15 cells (Fig. 4). Culture medium was collected from cells after 4 and 8 days incubation with increasing concentrations of either the aqueous KYH-1 extract or its constituents. In the presence of KYH-1 at more than 0.8%, HBV was not detected in the media after 4 days of treatment. KYH-1 at more than 0.4% inhibited viral release after 8 days of treatment. Concentrations at which constituent components of KYH-1-inhibited release of HBV were as follows; *C. melo* var. *ma-gua* more than 1%; *I. den-*



**FIG. 4.** Effects of KYH-1 and its constituent components on the release of hepatitis B virus (HBV) viral particles from HepG2.2.15 cells. Agarose gel of polymerase chain reaction (PCR) products amplified from culture media on day 4 and 8 of KYH-1 treatments. Lane C represents untreated control culture samples followed by samples from cultures incubated with decreasing concentrations (2.0%–0.02%) of KYH-1, *Circumis melo* var. *ma-gua* (magua), *Ixeris dentata* (Ixeris), *Youngia sonchifolia* (Youngia), and *Lactuca indica* (Lactuca).

*tata* 2%; *Y. sonchifolia* at 0.8%; and *L. indica* var. *laciniata* >0.08%. Extending the time of treatment (8 days) did not alter these results.

#### Antiviral effect of KYH-1 extract on HBV polymerase priming

Constituent components of the aqueous KYH-1 extract were examined for their capacity to inhibit priming by the HBV polymerase. Each component was tested at a concentration equal to that present in the aqueous KYH-1 extract. KYH-1 showed 60%–70% inhibition of HBV polymerase priming compared to untreated control, based upon intensity of the autoradiographic image (Fig. 5A). Inhibition of HBV polymerase primer activity by the constituent components of KYH-1 were as follows; *C. melo*, *L. indica*, and *L. japonica* more than 50%; *I. dentata* and *P. lobata* less than 50%; and *Y. sonchifolia* no inhibition.

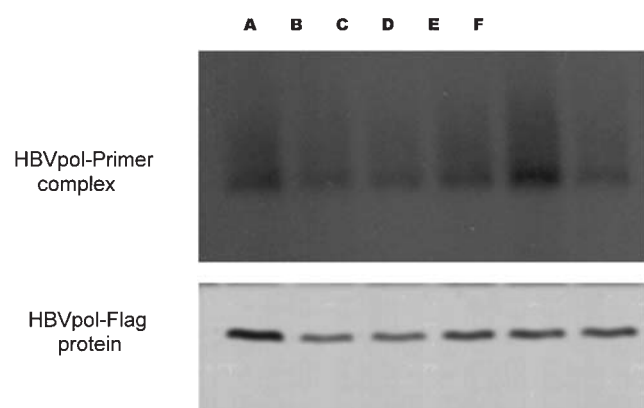
## DISCUSSION

There is substantial lag time between drug discovery and clinical application. Factors that influence the time differen-

TABLE 2. ANTIVIRAL ACTIVITY OF DRUGS AGAINST HBV EXPRESSED IN HEPG2.2.15 CELL

Antiviral	$EC_{50}$	$EC_{90}$	$CC_{50}$	SI ( $CC_{50}/EC_{50}$ )
KYH-1 extract	90 $\mu$ g/mL	254 $\mu$ g/mL	> 3700 $\mu$ g/mL	14.6
KYH-1 heat extract	259 $\mu$ g/mL	697 $\mu$ g/mL	> 1000 $\mu$ g/mL	1.4
3TC	0.06 $\mu$ mol/L	0.166 $\mu$ mol/L	2165 $\mu$ mol/L	13042

3TC, lamivudine.



**FIG. 5.** Effect of KYH-1 and its constituent components on recombinant hepatitis B virus (HBV)-pol primer activity in Sf9 cells. Top panel: autoradiograph of Pol-primer-complex reaction after incubation in the presence of KYH-1 extracts. Lanes: (A) untreated control activity; (B) 1.0% KYH-1 extract; (C) *Circumis melo* var. *ma-gua*; (D) *Ixeris dentata* (E) *Youngia sonchifolia*, and (F) *Lactuca indica* var. *laciniata*. Bottom panel: immunoblot for Flag-pol protein used to normalize data for sample loads.

tial in this process are the available targets for drug screening, relatedness of *in vitro* assays to disease, and animal models for preclinical testing. The initial judgment on efficacy of ethnopharmacologic extracts is based upon individual clinical case histories. A scientific basis for pursuing such traditional medicines is required before larger, double blind, and placebo controlled clinical trials are pursued. The isolation of a single active compound is not necessarily the objective in this pursuit. Demonstrating functional activity, a possible mechanism of action, and testing for safety and efficacy in preclinical models are, however, important objectives.

A criticism of surrogate assays lies in the relevance of the system to the interaction between infectious agent and the human host. In our case we employed BVDV as a surrogate model of HCV. The two drugs approved for clinical use against HCV, ribavirin and IFN- $\alpha$ , were effective in our BVDV model. By analogy, other compounds tested in this system that exhibit similar or greater antiviral activity may inhibit HCV.

KYH-1 exhibited an antiviral profile *in vitro* against BVDV similar to ribavirin, as inhibition of viral-induced cell killing and a commensurate decrease in viral yield was observed. The generation of reactive oxygen free-radicals contributes to viral-induced cytopathic effects (Schweizer and Peterhaus, 1999). We have examined several antioxidants (mycophenolic acid, N-acetylcysteine, and  $\alpha$ -lipoic acid) and observed inhibition of viral-induced cell killing, but without a commensurate decrease in viral yield (data not shown). Several phytochemicals have been isolated from the component plants used in KYH-1 and will be the focus of future studies in defining specific antiviral mechanisms attributed to compounds in this aqueous extract (Ooi et al.,

2004; Prasain et al., 2003; Vouldoukis et al., 2004; Wang et al., 2003).

KYH-1 inhibited the HBV polymerase gene in a molecular target assay and a dose response effect against HBV in a cell-based replication assay was also shown. Data from these HBV-specific assays lend credence to reports on reduction of HBV viremia in patients upon treatment with KHY-1. Experiments in the WHV culture model confirm a similar mechanism of action. The hepdanavirus (WHV and HBV) polymerase is similar to retroviral reverse transcriptases (Pugh, 1990; Scaglioni et al., 1996) and converts the viral RNA pregenome into the replicative intermediates. These replicative intermediates are converted into the virion DNA by the viral polymerase. The virion DNA is packaged into infectious particles. Upon reinfection, virion DNA is converted into cccDNA in the nucleus by cellular enzymes. This nuclear DNA serves as the template for cellular RNA polymerases that generate the viral pregenomic and surface antigen RNA transcripts.

Our data indicated that KYH-1 inhibits the formation of replicative intermediates leading to decreased virion DNA, although nuclear WHV DNA would be unaffected directly by this action against the viral polymerase. The observed 20% decrease in WHV RNA may reflect action on other steps of the replication cycle. In regards to IFN- $\alpha$ , viral replication can be inhibited through one of two mechanisms, either destruction of RNA molecules or by inhibition of protein translation (Vilcek and Sen, 1996). Induction of the pathway leading to destruction of RNA was reflected in the significant decrease in WHV RNA after 4 days of IFN- $\alpha$  treatment. Furthermore, some influence by IFN- $\alpha$  on the RNA priming mechanism that led to alteration in virion DNA size was observed.

We postulate that the antiviral activity of KYH-1 toward WHV operates through a similar mechanism of inhibition as demonstrated against the HBV polymerase. In this regard testing the safety and efficacy of KYH-1 for treatment of chronic HBV infections in the woodchuck model is a rational progression. Initial investigation on phyllanthus (*Phyllanthus amarus* Schumach. and *Phyllanthus niruri* L.; Euphobiaceae), a plant with purported therapeutic benefit for liver disease, followed similar experimental approach (Venkateswaran et al., 1987) and is being critically evaluated through meta-analysis of randomized controlled studies (Liu et al., 2001).

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