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Effects of *Rhus Coriaria* L. (Sumac) Extract on Hepatitis B Virus Replication and Hbs Ag Secretion

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A B S T R A C T

The current therapies against hepatitis B virus (HBV) infection are only effective for a number of patients. Mutant viruses that are resistant to treatment and get created due to the errors in the reverse transcriptase mechanism cause for prolonged treatments. In this regard, recent studies have been focused on new therapeutic approaches especially herbal medications to overcome the problem in resistant patients. The aim of this study was to evaluate the inhibitory effect of *Rhus coriaria L.* (Sumac) aqueous extract on hepatitis B virus replication and HBsAg secretion in HuH-7 cell line after transfection by HBV. Huh-7 cell line was transfected by a plasmid (pCH-9/3091) containing HBV genome using Lipofectamine 2000. The aqueous extract of *Rhus coriaria L.*was prepared and its cytotoxicity on transfected cells was assessed by MTT assay. The inhibitory effect of the aqueous extract of *Rhus coriaria L.*on release of HBsAg as a consequence of HBV replication was measured by ELISA. Our results showed a significant lower concentration of HBsAg after exposure to *Rhus coriaria L.*extract compared to the untreated control group and positive control group treated with Lamivudine of HBV transfected Huh-7 cell line. This study represents the inhibitory effect of sumac aqueous extract on multiplication of HBV and its antigen secretion.

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Introduction

Hepatitis B virus (HBV) has affected more than 350 million people all over the world and is considered as an important global health problem. Regarding the life-treating consequences of HBV infections such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, early and effective treatment of the patients is crucial [1-3].

There are several medications to control the HBV infection such as IFN-α, pegylated IFN-α (peg IFN-α), and the nucleoside analogues including lamivudine, adefovir, entecavir, telbivudine, and tenofovir [1-4], but there are some limitations due to their side effects, inadequate efficacy, slow mechanism of action, drug resistance and, more importantly, their cost [5, 6].

The best therapeutic approach for HBV infected patients should be short-acting, inexpensive, with low hepatotoxicity and slight side effects [7]. Herbal remedies are considered as attractive alternative treatments for liver diseases because of availability and low-toxic effects [5, 8]. There is growing evidence on the utilization of herbal medications to treat HBV infected patients especially in controlling potentially chronic infections [7]. To discover the antiviral elements, many activities have been performed in the direction of evaluating different plants [9].

Sumac or *Rhus coriaria L* belongs to the family *Anacardiaceae* and is generally used as a spice or therapeutic agent [10]. Antimicrobial, anti-fibrogenic, antifungal, anti-inflammatory, antitumor, and antioxidant properties of Sumac have been reported in some studies in the field of herbal medicine [8, 10, 11]. According to the previous studies, the antiviral effects of *Rhus Coriaria* have been shown on some viruses such as human immunodeficiency virus(HIV) and *Herpes simplex* virus(HSV)[12, 13].

The aim of this study was to evaluate the inhibitory effect of the aqueous extract of *Rhus coriaria L*. (Sumac) on multiplication of HBV and its surface antigen secretion in transfected Huh-7 cell line by HBV in vitro.

Materials and Methods

Sumac extraction

The fruits of *Rhus coriaria L.* were gathered from the Alborz Mountain, Iran and approved in the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences. After cleaning the fruits, they were dried at room temperature. The dried herb was then boiled in water for 30 min. After filtering and concentrating the extract, it was stored at 4ºC. The water content of the extract was then measured and it was comprised of 24% water. Before usage, the extract was dissolved in PBS, as described previously [8].

Cell culture and transfection

The human hepatoma cell line, Huh-7 (Provided from Pasteur Institute of Iran), was maintained in Dulbecco's Modified Eagle Medium(DMEM) (Gibco BRL, UK) with 10% fetal bovine serum (FBS) (Gibco BRL, UK), penicillin(100 U/ml) and streptomycin (100 µg/ml) (Biosera, UK). One day prior to transfection, 4.0×10^4cells were plated out in 24-well plates (40% confluence at the time of transfection) in 0.5 ml DMEM. Transfection was carried out with a plasmid (pCH-9/3091) containing HBV genome using Lipofectamine 2000 (Invitrogen, USA) according to manufacturer's instructions. pCH-9/3091 was kindly provided by Professor Michael Nassal (University Hospital Freiburg, Freiburg, Germany) [14].

Toxicity measurements (MTT assay)

Cells were grown in culture plates at $5x10^3$cells/well. They were incubated with *Rhus coriaria* L. extract at six concentrations (31.25, 62.5, 125, 250, 500, 1000µg/ml). Lamivudine (Exir Pharmaceutical Co.) at 50 µg/ml was used as positive control. After three days, the culture media was removed. Then MTT solution (10 µl/100 µl medium) was added to the wells. After incubating at 37 °C for 4h, 100 µL DMSO was added to dissolve the dark blue crystals. Conversion of a soluble tetrazolium salt, 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide

(MTT) to an insoluble formazan was precipitated by viable cells is the basis of this assay(15). Then, the absorbance was read by microplate reader at the wavelength of 540 nm. Cell viability was calculated via the following equation:
Percent cell viability= (OD sample-OD background)/ (OD control -OD background) x 100% Four concentrations of 250, 125, 62.5 and 31.25 µg/ml of *Rhus coriaria* L. extract and Lamivudine at 50 µg/ml were used for incubation with the cells to measure HBsAg using ELISA.

HBs Ag production in cell culture

After 24 hours of transfection, the different concentrations of *Rhus coriaria L.*Extract and Lamivudine at 50 µg/ml were added to Huh-7 cells. Then, the supernatants were collected at 24h, 48h, and 72h after treatment, respectively. They were stored at -20°C for determination of HBsAg until the last supernatant was collected. The determination of HBsAg (DIA.PRO, Milano, Italy) was performed using ELISA according to the manufacturer's instructions. The measurement of HBsAg was repeated two times and in each assay, it was performed in duplicate. The absorbance was measured at 450 and 620 nm for detecting the HBsAg in the samples.
Statistical Analysis: Statistical analysis was performed using Prism 5 (Graphpad Software Inc., La Jolla, CA, USA) software. The mean and Standard Deviation was calculated for the cell viability and HBsAg variables. T-test was used to compare the means of one quantitative variable in two independent groups. The P value less than 0.05 were considered as significant.

Results

MTT assay was used to evaluate Huh-7 cell viability after being incubated with various concentrations of *Rhus coriaria L.* extract and Lamivudine at 50 µg/ml. Figure 1 shows cell viability of *Rhus coriaria L.* on Huh-7 cells in the presence of DMEM without FBS. The percentages of cell viability of *Rhus coriaria L.* at concentrations of 1000, 500, 250, 125, 62.5and 31.25 µg/ml and Lamivudine at 50 µg/ml are shown in figure 1. Four wells of tissue culture plates were checked for each concentration. Figure 2 shows the same experiments in the presence of plasmid and FBS.
Absorbance values that indicate the concentration of HBsAg in the supernatant of Huh-7 cells transfected with pCH-9/3091 in 48 h after treatment with Sumac's aqueous extract and Lamivudine as positive control are shown in figure 3. The values show the average +/- SD of duplicate experiments. HBsAg concentration in supernatants treated with 31.25, 62.5, 125, and 250µg/ml concentrations of the extract were significantly reduced ($P< 0.05$ for the first three concentrations, $P< 0.001$ for 250 µg/ml of the extract) in 48 h. The same tests were performed at 24 and 72 h posttransfection. The same trend was observed, although, after 72 h, p values showed significant changes in the HBsAg concentration at 125 and 250 µg/ml concentrations of the extract. Figure 4 shows the comparison of HBsAg concentrations after 24, 48 and 72 h of treatment with the Sumac extract and Lamivudine at 50 µg/ml.
Results indicated that the concentration of HBs Ag in cells incubated with the extract was reduced compared to the control group (the cells not incubated with *Rhus coriaria* L. extract*)* and positive control group according to the duration of incubation and concentration of the extract.

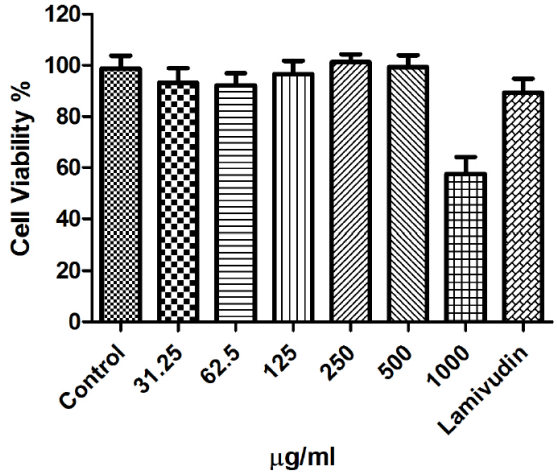

Fig. 1. Measurement of Huh-7 cell viability at various concentrations of *Rhus coriaria L.* extract on Huh-7 cells without plasmid and in FBS-free DMEM. Lamivudine at 50 µg/ml was used as positive control.

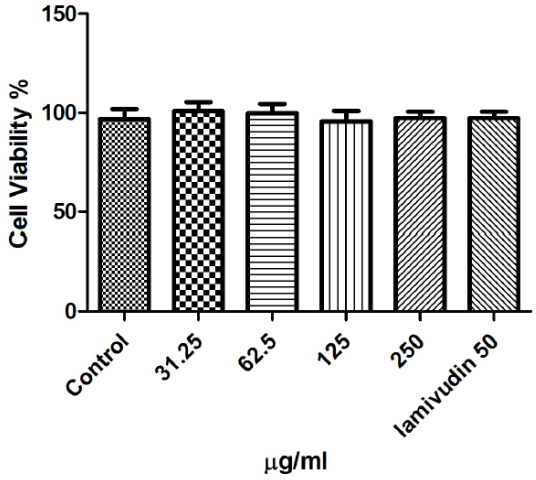

Fig. 2. Measurement of Huh-7 cell viability at various concentrations of *Rhus coriaria L*. extract on Huh-7 cells with plasmid with FBS DMEM. Lamivudine at 50 µg/ml was used as positive control.

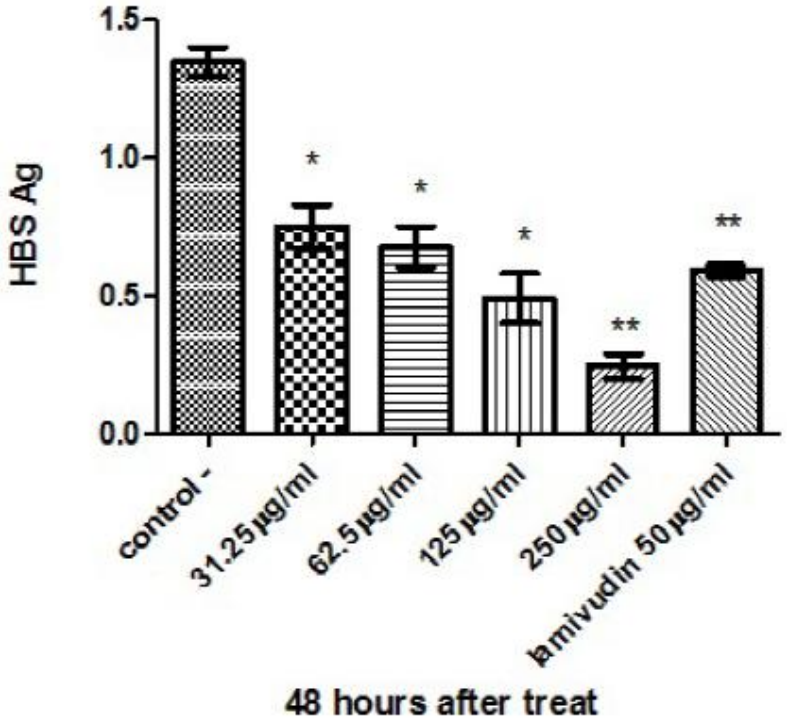

Fig. 3. Absorbance values indicating HBsAg concentration on cell supernatant of Huh-7 transfected with pCH-9/3091 in 48 h after treatment with *Rhus coriaria L*. extract or lamivudine as positive control. The value shows the average +/- SD of duplicate experiments (* p< 0.05, ** P<0.001). The same tests were performed in 24 and 72 h post transfection too and p values were calculated.

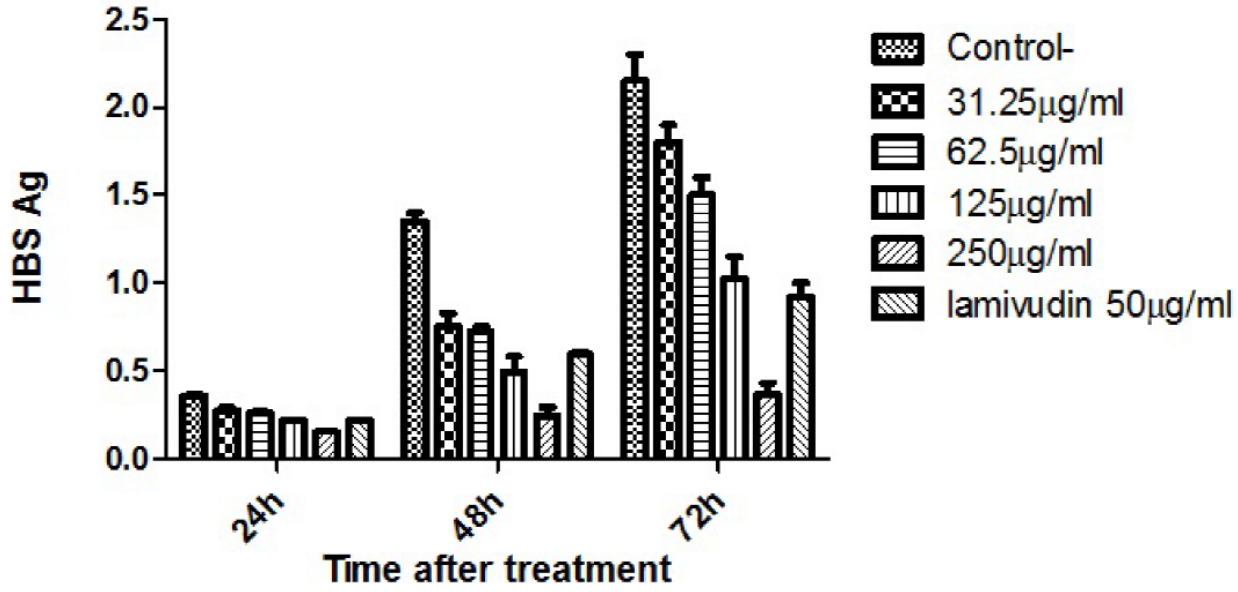

Fig. 4. Comparison of absorbance values for HBsAg in supernatant of pCH-9/3091 transfected Huh-7 cells in 24, 48 and 72 h after treatment with various concentrations of *Rhus coriaria L.* extract.

Discussion

In this study, the anti-HBV effect of *Rhus coriaria L*. (in vitro) was investigated based on the attenuation of the secretion of HBsAg in the HBV-infected Huh-7 cell line. The results of this study show a significant inhibitory effect on HBsAg secretion after treatment with *Rhus coriaria L.* extract compared to the untreated control group and positive control group of HBV transfected Huh-7 cell line.

Shedding HBsAg and HBV DNA are common indicators of HBV replication [7]. Thus, the anti-viral effect of Sumac extract was evaluated by measuring HBsAg in the supernatant of treated and untreated- HBV transfected Huh-7 cell lines. As the results revealed, the concentration of HBS-Ag showed significant difference compared with untreated group.

Reporting the anti-HBV effect of *Rhus coriaria L*. in HBV transfected Huh-7 cell line (in vitro) is being done for the first time. Chakraborty et al. showed the potent antioxidant activity of Sumac in prevention of the DNA damage caused by oxidative stress in humans [16]. Monavari et al. assessed the effect of several herbal sources on some viruses. In this study, *Rhus Coriaria* showed anti-HSV and anti-Adenovirus effect [17]. Moreover, there are several studies on the control of other infections like *Pseudomonas aeruginosa* and HIV using this plant [13, 18, 19].

The *Rhus coriaria L.* leaves have bioactive antioxidant materials and consist of flavones, tannins, anthocyanins, and organic acids. Also, recent study showed that *Rhus coriaria L.* leaves have bioactive antioxidant materials_(11). In contrast to free radicals and lipid peroxidation, the methanolic extracts of *Rhus coriaria L (in vitro)* exhibited a significant antioxidant effect in the study published by Candan *et al.* [19].

In previous study conducted by Pourahmad et al indicated that aqueous extract of *Rhus coriaria L.* had hepatoprotective activity against destructive outcomes of oxidative stress and ROS production such as cell lysis in hepatic cells [8]. In another study, the suppressive effect of aqueous extract of *Chinese Rhus*on syncytium and p24 production of HIV-infected cells reported [13].

Conclusion

In conclusion, this in vitro study established the anti-HBV activity of *Rhus Coriaria L.* on HBV transfected Huh-7 cells. After confirming the results by complementary tests, we can think of promising therapeutic approaches in future human studies.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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