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ANTIVIRAL ACTIVITY OF *DIANTHUS SUPERBUSN L.* AGAINST HEPATITIS B VIRUS *IN VITRO* AND *IN VIVO*

Wei-Guo Li*, He-Qun Wang

Department of Infectious Disease, Zhumadian Center Hospital, Zhumadian, Henan ,463000, China

***Corresponding Author E-mail:** liweiguo101@hotmail.com

Abstract

Background: Hepatitis is a viral infection of hepatitis B virus (HBV). Limitations of drug used in the management of it opens the interest related to alternative medicine. The given study deals with the antiviral activity of *Dianthus superbusn L.* (DSL) against HBV *in vitro* & *in vivo.*
Material and Methods: *In vitro* study liver cell line HepG2.2.15 was used by transinfected it with HBV. Cytotoxicity stduy was performed by using different concentrations of DSL such as 50, 100, 200, 500 & 1000 µg/ml. Anti HBV activity of DSL was estimated by assesing the concentration of HBsAg and HbeAg in cell culture medium by using ELISA. Whereas *in vivo* study was performed on ducklings and antiviral activity of DSL (100, 200, 400 mg/kg) was confirmed by estimating the serum concentration of HBV DNA and histopathology study of hepatocytes in HBV infected ducklings.
Result: Result of the study suggested that >500 µg/ml concentration of hydroalcoholic extract of DSL was found tobe cytotoxic. It was also observed that DSL significantly ($p<0.05$) reduces the concentration of antigenes in cell culture media as per the concentration and days of treatment dependent. Moreover in vivo study confirms the anti viral activity of DSL (200 & 400 mg/kg) as it significantly ($p<0.05$) decreases the serum concenetration of HBV DNA in HBV infected dukling compared to control group. Histopathology study was also reveals the hepatprotective effect of DSL in HBV infected ducklings.
Conclusion: The given study concludes the antiviral activity DSL against HBV by *in vitro* and *in vivo* models.

Key words: *Dianthus superbusn* L., Hepatitis B virus, HepG2.2.15, HBsAg and HbeAg

Introduction

Diseases caused due to viral infactions are one of the major cause of mortality thoughout the world such as aquired immunodificiency syndrom, hepatitis and respiratory diseases. Hepatitis B is a inflamation of liver by the infection of causitive organism Hepatitis B virus (HBV). Prevalance of HBV infection occur higher in developing countries like Asia and Africa (Liaw, 2002). Chronic infection of HBV may reult into the development of hepatic cirrhosis and carcinoma. HBV is a envoloped double strand DNA genome virus of hepadnaviridae family (Ganem and Varmus, 1987). World health organisation approved the combination therapy of antiviral drugs for the managment of chronic hepatitis B (Ocama et al., 2005; Mailliard and Gollan, 2006). Combination therapy shows synergestic effect despite of that drug resistance HBV and unresponsiveness of INF-a remain issues in the management of hepatitis B (Tenney et al., 2004; Lee et al., 2006; Fischer et al., 2001). Therefore lots of effort put into the research for the identification of antiviral agent (anti HBV agent) from the natural origin.

Traditionally in different part of the world many plants are used for management of various diseases including viral infections. In the recent years various study recognises several phytochemical consistuient that controls the viral infactions (Yamasaki et al., 1998; Abad et al., 2000). *Dianthus superbus* L. (family: Caryophyllaceae) is chinese herbal medicine used as diuretic and in the management of hepatotoxicity, inflamation and carcinoma (Oshima et al., 1984). Several report suggested that DSL contains few saponins that poseses haptoprotective and anti-inflamatory activity. The isolated compounds like 4-methoxydianthramide B, dianthin E, cyclic peptide and dianthramide poseses cytotoxic activity on cancer cell line (Hep G2) (Hikino et al., 1984). On the virtue of these pharmacological properties present investigation evaluates the antiviral activity of DSL.

Material and Methods

Extraction of Plant

Dianthus superbus L leaves were collected from the local suplier and authontified from Guangxi Traditional Chinese Medicine University. Leaves of *Dianthus superbus* L were shed dried at room temprature and choped into small pieces. Small piceses of leaves were defated with petrolium ether and then kept it in a container with water: alcohol (1:1) for the period of 3 days. The extract was obtained by the help of rotavapor apparatus at low temprature and pressure by seperating methanolic solvent completely. The extract of

DSL was kept in dsiccator till the completeion of experimental procedure. The percentage yield of methanolic extract was found to be 8.1% w/w.

Experimental Animals

Ducks (one day old) were procured from the Avian Disease Research Center, Sichuan Agricultural University, China. All animals were kept for 12 hr light/dark cycle under CPCSEA guidelines. Protocol of the experiment was permitted by Institutional ethical committee.

Cell Culture and Experimental Design: *In Vitro*

HBV infected HepG2 2.2.15 human cell line was procured from CCTCC, China. In a 5% CO_2 humidified incubator at 37 37 °C cells lines were incubated. Dulbecco's Modified Eagle's Medium was used with (10% v/v) fetal bovine serum as a culture medium for the present study. Cells were cultured by seeding it in to 24 well culture plate (1×10^4 cells/well) for the period of 48 h befor the start of study. In the present investigation Lamivuidine was used as a standard and all the samples with different concentrations like 50, 100, 200, 500 µg/ml were suspended in DMSO. HepG2 2.2.15 cells were merge with the known concentration of test sample. HepG2 2.2.15 cells and culture medium were taken at different time interval like day 6, 9 and 12^{th} day of protocol. The concentration HBsAg and HbeAg were estimated using ELISA in the collected cell culture medium. The effect of DSL on inhibition was calculated by the below given formula (Li et al., 2005).

% of control = (Number of cell adjusted optical density of test drug)/(Number of cell adjusted optical density of control) × 100%

Cytotoxicity Assay

Cytotoxicity of hydroalcoholic extract of DSL was estimated by using MTT assay as given by Wang et al., 2009.

Estimation of Anti-HBV Activity of DSL: *In Vivo*

Viral DNA (5.7×10^6) was injected I.V. to infect the ducklings. A week after injection all the ducks were seperated into 5 groups (n=10) as given below: Control group treated with normal saline, The standard group trated with lamivudine, 50 mg/ kg, and DSL 100, 200 and 400 mg/kg, p.o., treated groups. All the groups were treated with the drug given as per the protocol for the period of 14 days. Blood samples were withdrawan at 7^{th} and 14^{th} day of treatment protocol.

Analysis of Vermia

Viremia was estimated during the protocol via detection of HBV-DNA in the serum. On the nitrocellulose filter 50 µl of the serum was spotted. HBV DNA labeled with 32P were hybridised with filter. Scintillation counter was used for counting the spot (Guiqin et al., 2009).

Evaluation of Histopathology of Hepatocytes

At the termination of protocol, liver was isolated and fix it with 5% of formaline solution. Liver tissue was dehydrated with 90% of ethanol, kept in parafin and haematoxylin-eosin dye was used for the staining for photomorphic determination at 40X (Li et al., 2005).

Statistical Analysis

All the value of experiments were articulated as mean ± SD and statistical analysis of data was done by one-way ANOVA (Dunnett post hoc test). $p<0.05$ was considered significant statistically.

Result

Cytotoxic Effect of *Dianthus Superbus* L.

Effect of DSL was assessed for its cytotoxicity on the cell viability by MTT assay as shown in Fig. 1. DSL >500 µg/ml treated samples has not showing any significant differences compared to control group, but DSL at the concentration of 1000 µg/ml or more posses cytotoxicity.

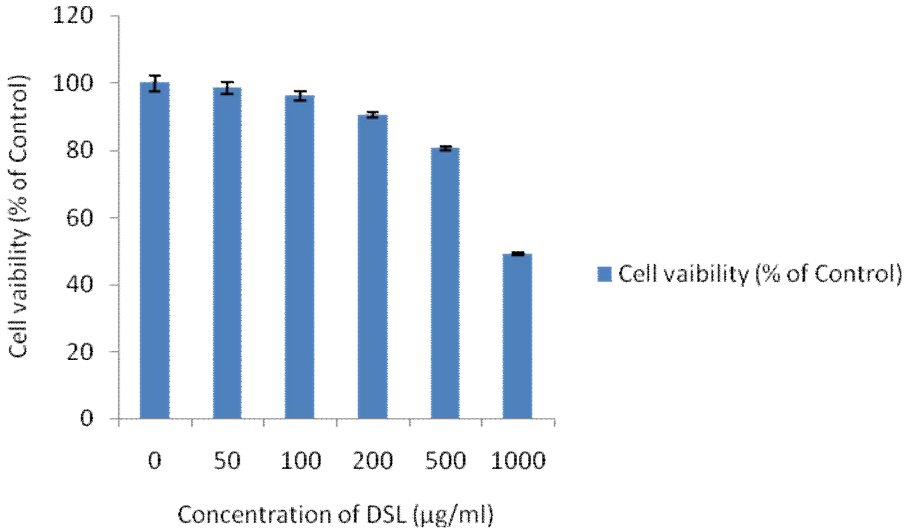

Figure 1: Cytotoxic effect of *Dianthus superbus* L.on HepG2 2.2.15 human cell line (MTT assay)

Effect of *Dianthus Superbus* L. on Antigens of HBV

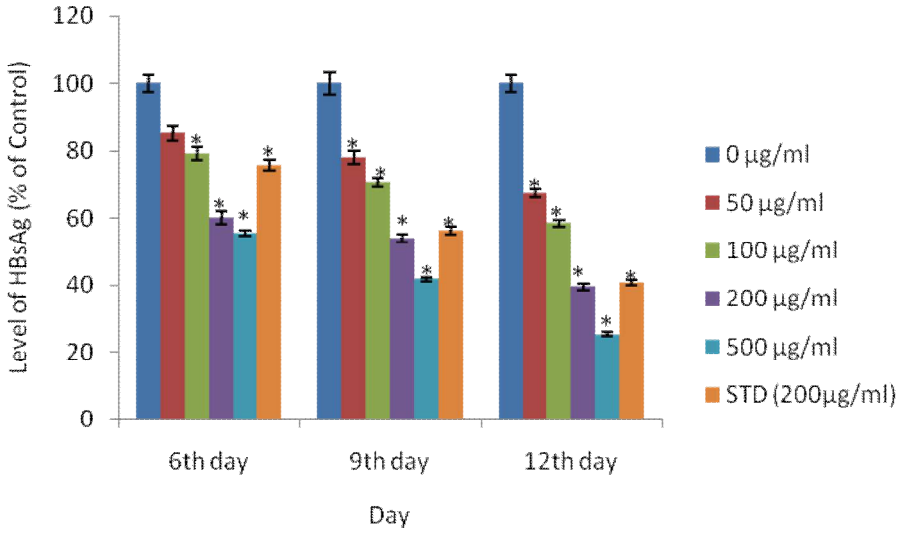

Figure 2: Effect of *Dianthus superbus* L.on the concentration of HBsAg (Antigen of HBV) in cell culture media. All the values are means ± S.D. (n=3); *p < 0.05 compared to control group (0 µg/ml)

ELISA was used for the estimation of effect of DSL on the concentration of HbsAg and HbeAg (antigen of HBV) in cell culture media after 6, 9, 12th day of protocol. Fig 2. Suggested that treatment with DSL and STD drug significantly ($p<0.05$) decrease in the level of HbsAg in the cell culture compaired to control (0 µg/ml) one. Whereas level of HbeAg were also found tobe significantly ($p<0.05$) decreases in the DSL and STD drug treated group compared to control as shown in Fig. 3. Results of the present investigation also reveals that decrease in the antigens (HbsAg and HbeAg) level were dose dependent.

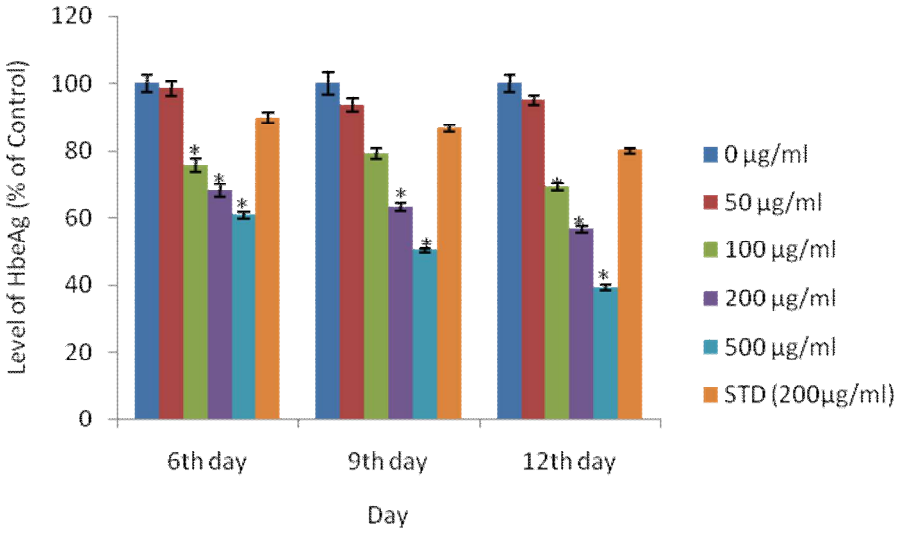

Figure 3: Effect of *Dianthus superbus* L.on the concentration of HbeAg (Antigen of HBV) in cell culture media. All the values are means ± S.D. (n=3); *p < 0.05 compared to control group (0 µg/ml)

Effect of *Dianthus Superbus* L. on Anti HBV Activity *In Vivo*

The anti HBV activity of DSL was estimated by change in the level of HBV DNA in the serum of all the ducks. It was observed that the level of HBV DNA decreases significantly (p<0.05) in the DSL treated group (200mg/kg & 400 mg/kg) compared to control group of animals (HBV infected) after 14 days of protocol. Whereas, no significant alteration in the level of HBV DNA was observed in DSL (100 mg/kg) treated group of animals as shown in Fig 4.

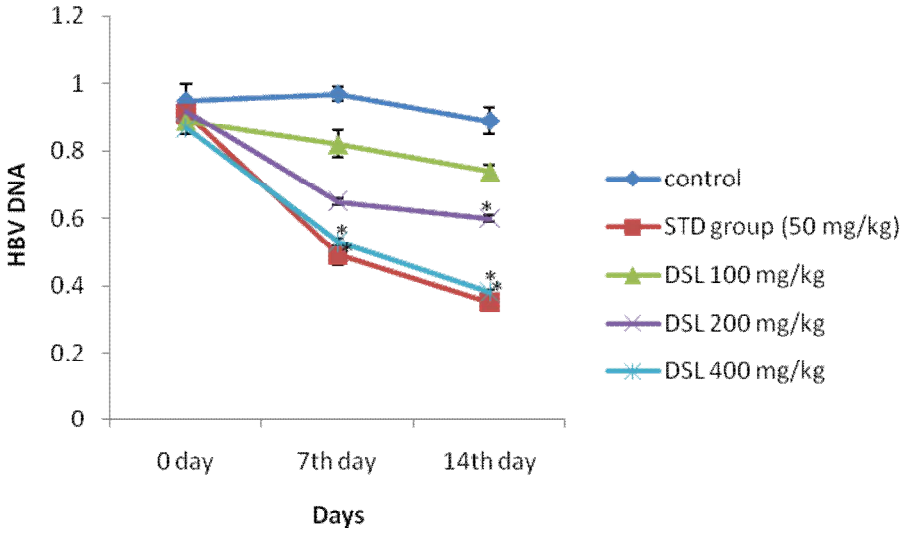

Figure 4: Effect of *Dianthus superbus* L.on the serum concentration of HBV in HBV treated ducks

Values are means ± SD (n=10); *p < 0.05 compared to contol group of animals

Estimation of Histopathological changes

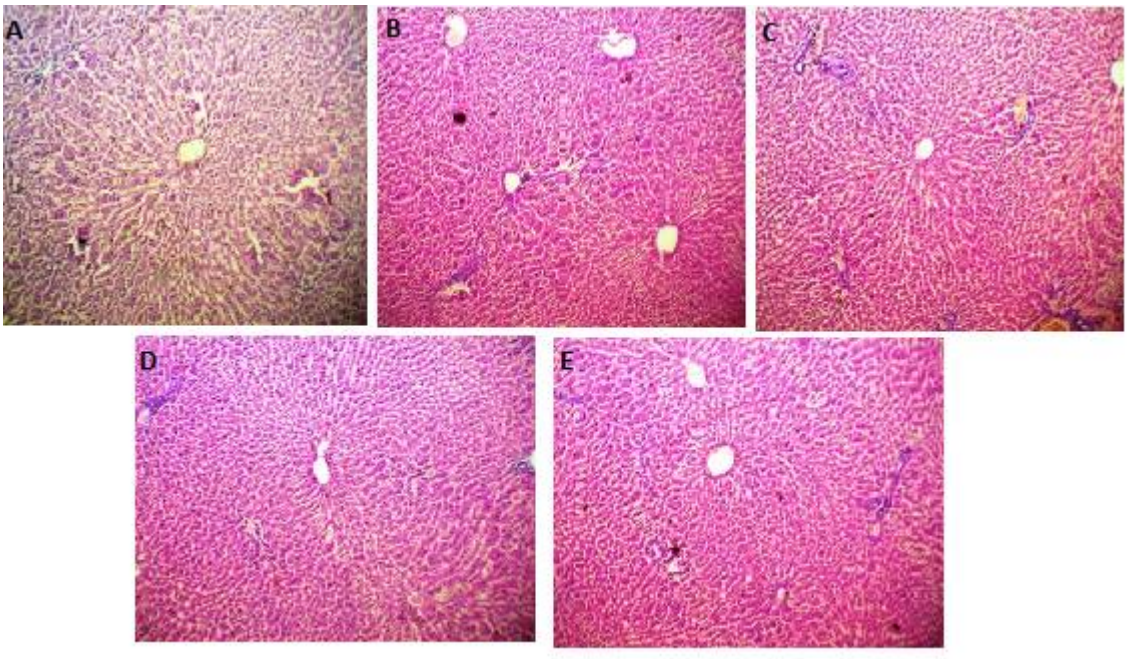

Figure 5: Effect of *Dianthus superbus* L on the histopathology of liver in HBV infected ducks
A.TS of liver tissues of HBV infected duck (Control group) B. TS of liver tissues of STD (50 mg/kg) + HBV infected duck (STD group) C. TS of liver tissues of DSL (100 mg/kg) + HBV infected duck D. TS of liver tissues of DSL (200 mg/kg) + HBV infected duck E. TS of liver tissues of DSL (400 mg/kg) + HBV infected duck

Histopathology of HBV infeacted ducklings liver revealed the swelling of hepatic cytoplasm, steatosis and necrosis of liver tissue. Effect of DSL (200 mg/kg & 400 mg/kg) treatment found tobe protects the liver cells in HBV infected ducks as it significantly improves the articular structure of hepatic cell and reduces the necrosis compared to TS of liver of HBV infected ducks as shown in Fig. 5.

Discussion

Hepatitis is a epidimological disorder, mostly affect the population of Africa, Asia and China. Due to limitations of different therapies that are used for the management of it, now a days research was focused on the alternative medicine. Previous literature suggested that many of Chinese herbs posses the anti HBV activity such as Geranium carolinianum, Scutellaria radix and Phyllanthus urinaria show its anti HBV effect by assessing it via *in vitro* and *in vivo* study (Guo et al., 2007; Li et al., 2008). The present investigation also evaluates antiviral activity of DSL by in vitro and in vivo activity.

Cytotoxicity of DSL was estimated at concentration of 50, 100, 200, 500 and 1000 µg/ml by using MTT assay. It was observed that DSL at a concentration below 500 µg/ml found tobe non-cytotoxic. All the non cytotoxic concentrations of DSL were used for the assessment of reduction of antigen HBV (HbsAg and HbeAg) in HepG2 2.2.15 cells. HBV antigens like HbsAg and HbeAg level decreases depends on the concentration and duration of the treatment. The present study suggested that the decrease in the antigen level proves that DSL posses anti HBV activity in the cellular model i.e. *in vitro* (Shin et al., 2005).

However in vitro study prove the anti HBV activity of DSL but it is important confirm the same effect by animal experimentation. There were several reports confirms the antiviral effect of drug by using various animal models for example ducklings, mice, immunocompromised rat model (Lin-lin et al., 2007; Xing et al., 2013). In this study the serum concentration of HBV DNA were determined in HBV infected ducks. It was observed that treatment with DSL (200 mg/kg & 400 mg/kg) significantly decreases the serum concentration of HBV DNA in the HBV infected ducks. Literature suggested that decreaed concentration of HBV DNA confirms the anti viral activity of drugs (Xing et al., 2013).

Conclusion

The present investigation concluded that DSL possesses the antiviral activity by both *in vitro* and *in vivo* study. Illustriation of possible mechanism of action of its anti HBV activity will improve the perception of gene expression of virus in future.

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