

# Anti hepatitis B activity of methanolic extracts of *Achyranthes aspera* leaves

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## Abstract

*The present study aims to investigate the anti hepatitis B activity of Achyranthes aspera. Achyranthes aspera is well known medicinal plant and it is widely distributed in India. Hepatitis B infection is very common disease and it is a major global disease which often leads to liver failure including liver cirrhosis and liver carcinoma. Conventional treatment options are always causes severe post treatment clinical consequences and this limit the use of high doses of drugs for treatment. Natural remedies are preferred to combat different diseases and world health organization (WHO) recommends the use of active principles extracted from natural sources as they are well tolerated by human system. In this study we investigated the anti viral efficacy of Achyranthes aspera leaves extract on hepatitis B virus. Different parts of Achyranthes aspera extracts are reported for antimicrobial activities however, there is no reports are available on anti hepatitis B activity Achyranthes aspera collected from Tamil Nadu. In this study Achyranthes aspera leaves extract showed considerable anti hepatitis B activity at different concentration tested. The anti viral activity of A. aspera leaves extract was found to be dose dependent manner and it is comparable with reference drugs.*

**Key words:** *Achyranthes aspera; Hepatitis B; Extract; Dose dependent*

## Introduction

The hepatitis B virus (HBV), a member of the Hepadnaviridae family, contains a partial double-stranded circular DNA genome of 3.2 kb. Its genome has a compact organization, with four overlapping reading frames running in one direction and no non-coding regions. This unconventional genome structure reflects the unconventional mode of its replication, which involves reverse transcription of an RNA pregenome of 3.5 kb as a first step (Arbuthnot et al. 2005). Approximately 20% of people with cirrhosis will then develop liver failure, and 5% will develop liver cancer, both of which can be fatal (Thomas, 2013; Mohd Hanafiah et al. 2013). Although there are many effective therapeutic drugs available, they have certain limitations. While interferon (IFN- $\gamma$ ) has a high incidence of adverse effects and non response, long-term therapy with nucleotide analogues has risks of emergence of drug resistant viral mutants (Lacombe et al. 2010). To over these obstacles finding a drug formulation with lesser side effects is highly warranted. many effective natural or plant products have been investigated against hepatotoxin induced liver damages (Alqasoumi and Abdel-Kader, 2008; Dandagi et al. 2008; Kinjo et al. 2006; Ganie et al. 2013; Al-Said et al. 2012). In addition, numerous active phytoproducts or phytochemicals (flavonoids, polyphenolic tannins, terpenoids, lignans, saponins, alkaloids, and anthraquinones) of diverse geographic origin and based on local cultural practices have been also reported effective against HBV infections *in vitro* or/and *in vivo* (Wohlfarth and Efferth, 2009; Liu et al. 2001). More than 40% of all currently prescribed drugs are derived from chemicals that have been initially identified in plants. Keep these facts in mind in this study we explored the anti viral activity of *Achyranthes aspera* collected from tamilnadu. The active principles of medicinal plant varied depending on climate, soil condition, water availability, environmental stress etc. Anti-microbial activity of *A. aspera* is reported (Lakshmi Naidu et al. 2006) however; efficacy of *Achyranthes aspera* on HBV is remaining elusive. To address this issue, we study the anti hepatitis role of *Achyranthes aspera* on HBV. To the best of our knowledge this is the first report on anti hepatitis efficacy of *A. aspera*.

## Materials and Methods

During April – June, 2014, *Achyranthes aspera* (Figure 1) was collected from Thambathukonam, Nagercoil, Kanyakumari District. The impurities were removed by rinsing in sterile distilled water and authenticated at the Department of Botany, Scott Christian College, Nagercoil. The leaves of the *Achyranthes aspera* were shade dried, powdered and stored at room temperature until use. Methanol extract of *Achyranthes aspera* was prepared by adding 50 gm of leaf powder in 500 ml of methanol and then filtered using Whatman filter paper (No. 1). The filtrate was allowed to evaporate for about 2-3 days. Then dried filtrate was collected, weighed and it was stored at 40°C until use (Dinesh et al., 2011)

Equal volume of pre-titrated HBV and varying concentration of methanolic extract was mixed and incubated at 37°C for 5 days. The mixture was assayed on day 5 for the presence of bound/unbound HBsAg using ELISA kit (Hepanostika HBsAg kit). Controls included in the experiment are drug positive control (Elan-PA001) and drug negative control (Nonoxynol-9). Other controls included the kit positive and negative controls. ELISA was performed as per the manufacturer protocol. Briefly, to the anti HBsAg antibody precoated plates extract treated HBV virus was added and incubated for 1 hour at 37°C. Then the plates were washed and secondary antibody-HRP conjugate was added and further incubated for 1 hour at 37°C. Then the plates were washed and TMB substrate was added and incubated at room temperature for 30 minutes. To this stop solution was added and the plates were read at 450 nm in ELISA reader (BioTek). Experiments were done 3 times and one representative experiment is described. Results are represented as ELISA optical density (OD) and percentage (%) inhibition.

Percentage inhibition =  $(\text{OD of Test} - \text{OD of the control}) / \text{OD of the control} \times 100$ .

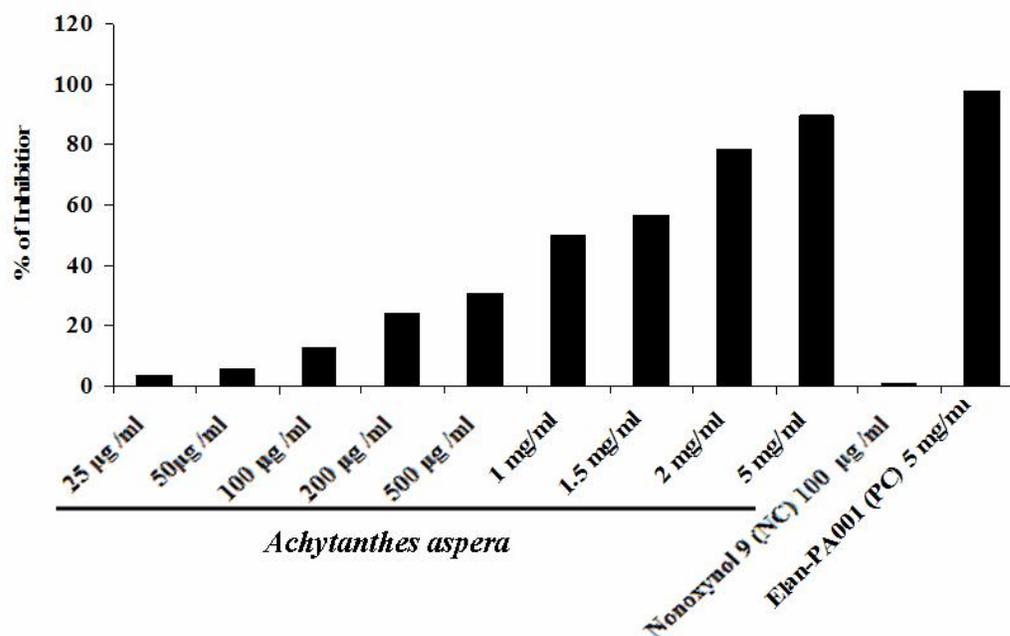
## Results and Discussion

Liver injury accounts for approximately one-half of the cases of hepatic failures, including all forms of acute and chronic liver diseases (Kaplowitz, 2004). In most of such cases, toxins and drugs are involved in oxidative stress-induced hepatotoxicity (Bala et al. 2012; Kus et al. 2005). Liver infection with hepatotropic viruses, including hepatitis viruses, is characterized by acute and chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinomas. Of these, hepatitis B virus (HBV) infection continues to be an important cause of morbidity and mortality worldwide (Locarnini et al. 2013). Plant derived natural products playing a pivotal role in developing many drugs and it is strongly recommended by world health organization (WHO) due to their less toxic nature in our study we explored the anti viral potential of methanolic extract of *Achyranthes aspera* against HBV. In this study we found dose dependent anti HBV of activity of *A. aspera* (Figure 2) and it is comparable with standard drugs such as Elan-PA001. The mechanism of anti viral activity of methanolic extract of *Achyranthes aspera* is remaining elusive however it is suggested that active principles *Achyranthes aspera* inhibits the HBV multiplication. Basically methanolic extract comprised of polar molecules which includes alkaloids, flavanoids, and other active ingredients which contains hydroxyl groups. Polar active moieties of active principles might interfere with HBV replication. Our data forms the rationale for isolating active molecules from methanolic extract of *Achyranthes aspera* which possesses anti HBV activity. Further complete characterization of active principles of *Achyranthes aspera* and its anti viral mechanism should be studied in detail.

**Figure 1: *Achyranthes aspera***



**Figure 2: Anti HBV activity of methanolic extract of *Achyranthes aspera* leaves**



NC: Negative control

PC: Positive control

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