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ORIGINAL ARTICLE

Antiviral Activity of Extract of Neem (Azadirachta Indica) leaves: An *in vivo* study

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ABSTRACT

Background: HCV in mainly replicate in liver cell and cause liver damage. The replication of HCV may be inhibited by leaves extract of neem (Azadirachta Indica).
Aim: To find the antiviral activity of extract of neem (Azadirachta Indica) leaves.
Methods: Thirty five hepatitis C positive and disappointment of conservative treatment were studied. Effect of neem (Azadirachta indica) leaves was seen in serum of patient. Extract of Azadirachta indica leaves was prepared and given orally to patients. The seropositivity of hepatitis C was estimated prior and after the leave extract of neem by HCV- RNA quantitative analysis by Polymerase chain reaction. Level of ALT, AST and total protein were estimated by standard kit methods.
Results: A high HCV seropositivity was observed in patients before taking neem extract. We observed that after using the leave extract of Azadirachta indica or neem, the HCV seropositivity was significantly decreased. Levels of enzymes ALT and AST were also significantly decreased after taking neem extract. However, the level of serum protein was not changed.
Conclusion: Extracted leaves of Azadirachta indica may serve as valuable regimen against hepatitis C virus as it has an ability to inhibit the protease responsible for replication of virus.
Keywords: HCV seropositivity, Azadirachta indica leaves, transaminases

INTRODUCTION

Hepatitis C (HCV) is a health issue worldwide and common in all ages. The chronic infection with HCV is related with cirrhosis of liver and may lead to failure of liver function and carcinoma[1]. HCV in mainly replicate in liver cell and cause liver damage, however it is found that virus is not hepatotoxic and injury of liver cell is due to cell mediated immune reaction against infected cells of liver[2].

A protein known as NS3-4A of HCV shows protease/ helicase activity and has an important role in the viral replication, processing of viral polyprotein, replication of RNA and formation of virion. This protease also board particular proteins of cells and thereby inhibits distinctive immune pathway and modulating the signaling of growth factor[3]. It is proposed that precise inhibitor of protease NS3-4A meaningfully recovers sustained rate of virological response in patients with HCV when using interferon-alpha with ribivarin[4].

Number of antiviral drug is proposed as therapeutic treatment of HCV , but due to viral resistance, side effect, high cost cannot be used by ordinary people. Due to the non-availability of vaccine of HCV, there is a need of antiviral herbs having bioactive components which may help to cure against the infection of HCV[5].

Azadirachta indica or neem is used to treatment number of diseases in many countries of Asia. Leaves extract of neem has shown antiviral activity and help in inactivation of virus[6,7]. The effectiveness of this hepatoprotective herb is estimated by finding the level of enzymes of liver like AST or aspartate transaminase and ALT or alanine transaminase and the concentration of proteins. However the prolong use of herb may have toxic effect, it is therefore a need of proper dose with limited time period[8].

Phytochemicals of leaves of neem shows antiviral activity against protease (NS3-4) of HCV via the methods of simulation. It is found that a component of leaves of neem known as 3-Deacetyl-3-cinnamoyl-azadirachtin have an ability to bind with NS3 protease of HCV and act as inhibitor of this protease[1].

The therapeutic effect of interferon alone or with ribivarin is failed to completely remove viral infection in most of the patients infected with HCV and these therapies also have side effects. It is therefore a need to develop more effective drugs.

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The purpose of this study is goal of this study is to find out the antiviral action of leave extract of neem in a group of HCV positive patients.

MATERIAL AND METHODS

Thirty five HCV patients and failure of conservative treatment were studied. Patients were taken from local Hospital Lahore. Duration of study was six months i.e. from July 2019 to October 2019. Effect of neem leaves was seen blood of HCV patients. Extract of Azadirachta indica leaves was prepared and given orally to patients. The seropositivity of hepatitis C viurs was estimated prior and after the usage of neem leaves extract by HCV- RNA quantitative analysis by Polymerase chain reaction. Level of ALT, AST and total protein were estimated by standard kit methods.

Study was accepted from Ethical Review Board of Institute. Letter of consent was taken from each patient. Patients infected with any other type of viral infection and with liver disease were excluded from the study.

Preparation of Extraction of Azadirachta indica leaf extract: Fresh leaves of Azadirachta indica were collected from Garden of Shaikh Zayed Hospital Lahore. About 10-12 leaves neem were soaked in a glass of water for whole night and extract was prepared by boiling water till the amount of water (neem extract) is only one table spoon. Extract was used by the patients for the period of 2.0 weeks in fasting condition. Seropositivity of HCV was estimated before & after the usage of leave extract by reverse transcriptase Polymerase chain reaction (RT-PCR).

Statistical Analysis: Data was analyzed by SPSS 20. All quantitative variables are expressed as means±SD. The difference between the titer HCV, AST, ALT and serum protein before and after using the extract of neem was evaluated by Student's t-test. P ≤0.05 was considered significant.

RESULTS

HCV sero positivity and liver enzymes in HCV patients before and after the neem extract is given in table. A high HCV seropositivity was observed in patients before taking neem extract. We observed that after using the leave extract of Azadirachta indica or neem, the HCV seropositivity was significantly decreased (P<0.001). Levels of enzymes ALT and AST were significantly decreased after taking neem extract. However, the level of serum protein was not changed.

Table : HCV sero positivity and liver enzymes in Hepatitis C positve patients before and after the leave extract of neem

Patients (35)	HCV- sero positivity (IU/ml)	ALT (U/L)	AST (U/L)	Serum protein (gm/l)
Before using extract	78.5±35.5	15.84±17.50	13.8±13.5	6.40±1.01
After using extract	26.5±14.8**	8.5±3.5*	7.5±3.9*	6.60±2.01

**P < 0.001= highly significant *P < 0.05 = Significant

DISCUSSION

Herbal medicine used traditionally as an alternative treatment for number of disease including liver diseases. The reason may be that due to harmful side effects of drugs, these are nowadays more popular[8].

A high HCV seropositivity was observed in patients before taking leaves extract of *Azadirachta indica*. We observed that after using the HCV seropositivity was significantly decreased. Number of studies found significant effect of the usage of leaves of neem for the handling of liver disease. It is reported that *Azadirachta indica* have shown hepatotropic, due to its anti-inflammatory and antioxidant properties. Study found that many pharmaceutical drugs like nitrosamine, Adriamycin aflatoxin induce hepatotoxic effect[9]. Another study found that N3type protease of hepatitis virus is take part in viral replication and acting as RNA-helicase. This protease also plays a role in the pathogenesis and persistence of HCV. It is therefore thought that Ns3- protease is a good goal for the treatment of HCV. Due to its inspiring action, NS3 protease is an striking goal for HCV therapy[10,3]. A study experimentally proved that a compound 3-Deacetyl-3-azadirachtin of neem leaves have a property to bind NS3 protease of HCV and may inhibit the replication of HCV[1].

According to our study the levels of enzymes ALT and AST were significantly decreased after taking neem extract. A study demonstrated the extract of neem leaves or of Azadirachta indica may decreased the level of liver enzymes ALT and AST and proved the efficacy of herbal treatment[8]. An experimental study on rat also found the hepatoprotective effect of neem leaves and observed that by the use of these the levels of AST and ALT was significantly reduced. Besides, the necrosis of liver is also decreased as observed microscopically. It is proved that the constituent of neem leaves play an important role in treatment of HCV through modulation of cellular pathway of virus[6,11,12].

CONCLUSION

Extracted leaves of Azadirachta indica may serve as valuable regimen against hepatitis C virus as it has an ability to inhibit the protease responsible for replication of virus.

Ethical consideration: Permission was IRB.

Conflict of Interest: None.

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Contributions of authors: RK: Contribution : paper writing, TA: Data collection, HA: Data analysis, SA: Literature survey, M: Proof reading

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