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ORIGINAL ARTICLE

Antiviral Efficacy of Qust (*Saussurea lappa*) and Afsanteen (*Artemisia absinthium*) for Chronic Hepatitis B: A Prospective Single-Arm Pilot Clinical Trial

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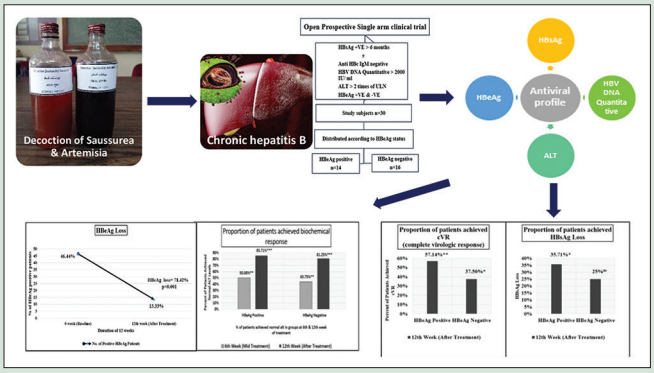
ABSTRACT

Background/Aim: Chronic hepatitis B (CHB) is a major health concern in terms of high prevalence, as well as restricted and costly health-care resources in India. The objective was to evaluate the antiviral effect of herbal drugs, *Saussurea lappa* and *Artemisia absinthium* against hepatitis B virus (HBV) in the management of CHB and to collect data to warrant further clinical trials. **Materials and Methods:** In an open prospective single-arm study, we assigned thirty patients with HBeAg-negative or positive CHB to receive decoction of *S. lappa*, 15 mL in the morning, and decoction of *A. absinthium*, 15 mL in the evening once daily empty stomach for 12 weeks. Test drug was evaluated for its efficacy in loss of HBsAg and HBeAg, plasma HBV DNA level of <200 IU/mL (complete virologic response [cVR]), mean reduction in HBV DNA, and normalization of alanine aminotransferase levels (ALT) at week 12 (after treatment). **Results:** HBsAg loss was observed in 35.71% (n = 14) and 25% (n = 16) of patients in HBeAg-positive (P < 0.05) and negative group (P = 0.10), respectively, at week 12 (after treatment). HBeAg loss was observed in 71.42% of patients at week 12 (P < 0.001). cVR was achieved in 57.14% (P < 0.01) and 37.5% (P < 0.05) and ALT normalization in 85.71% and 81.25% of patients (P < 0.001) in HBeAg-positive and negative group, respectively, at week 12. **Conclusions:** Our study has substantiated that *S. lappa* and *A. absinthium* have antiviral effect against HBV in CHB besides being quite safe for a treatment duration of 12 weeks.

Key words: Antiviral, Artemisia, chronic hepatitis B, hepatitis B virus, Saussurea, Unani medicine

SUMMARY

- Antiviral efficacy and safety of decoction of Unani drugs, *Saussurea lappa* (qust) and *Artemisia absinthium* (afsanteen) were evaluated in 30 patients of HBeAg-negative or positive chronic hepatitis B for a treatment duration of 12 weeks in an open prospective single arm clinical trial. The results suggest that test drug possess potential antiviral effect against HBV as effectively suppressed HBV DNA, HBeAg and HBsAg, and normalized ALT significantly in study patients without any significant adverse effects.



Abbreviations Used: CHB: Chronic hepatitis B, HBV: Hepatitis B virus, *S. lappa*: Saussurea lappa, A. absinthium: Artemisia absinthium, HBsAg: Hepatitis B surface antigen, HBeAg: Hepatitis B e antigen, HBV DNA: Hepatitis B deoxyribonucleic acid, cVR: Complete virologic response, ALT: Alanine aminotransferase.

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INTRODUCTION

Chronic hepatitis B (CHB) remains a serious global public health concern affecting 350 million people worldwide.[1,2] India has highest hepatitis B virus (HBV)-infected population after China with over 40 million hepatitis B-infected patients and alone contributes 9% of the total CHB cases of the world.[3,4] HBV infection if contracted early in life may lead to chronic hepatitis, then to cirrhosis of liver, ascites, and finally to hepatocellular carcinoma, usually after a period of 30–50 years.[3] Every year over 600,000 Indians die due to illnesses related to HBV infection.[4]

Available conventional treatment consists of interferon, entecavir, and tenofovir which cause bone marrow suppression, potential nephropathy, myopathy, lactic acidosis, and gastrointestinal disturbances. Long duration of treatment is associated with risks of adverse reactions, drug resistance, nonadherence, and increased cost.[5,6]

Hence, the need of antiviral therapy from complementary and alternative medicine (CAM) came to limelight. This is also in media that very soon AYUSH (Department of Government of India for CAM) will be the mainstream health-care system in India.[7]

The Unani system of medicine has been treating hepatitis under the entity "Warm-e-Jigar" with herbal drugs *Saussurea lappa (S. lappa)* and *Artemisia absinthium (A. absinthium)* for centuries. These drugs have exhibited potential anti-hepatitis B, hepatoprotective, immunomodulatory, anti-inflammatory, and antioxidant activities in

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various animal models.[8-12] With this background, the present pilot study was tried to be designed according to the guidelines of American Association for the study of liver diseases (AASLD) with an objective to evaluate the antiviral effect of *S. lappa* and *A. absinthium* against HBV in the management of CHB and to collect data to warrant further clinical trials.

MATERIALS AND METHODS

Ethical consideration

The protocol was approved by the Institutional Ethics Committee of Jamia Hamdard (JH IEC/1530 HOURS/AUGUST 12/15) and was implemented in accordance with provisions of the Declaration of Helsinki, ICMR, and good clinical practice guidelines. All the participants provided their written informed consent to participate in this study. Clinical trial is registered in the clinical trial registry of India, Government of India with CTRI no. CTRI/2017/11/010386.

Study design

This was a single-arm prospective study of 30 CHB patients, conducted in the Majeedia Unani Hospital, Jamia Hamdard, India, from August 2015 to February 2017. The inclusion criteria's were (1) clinically stable patients, (2) patients of both sexes, (3) patients in age groups of 18–60 years, (4) HBsAg-positive cases with a clinical history of more than 6 months, (5) patients with HBV DNA quantitative >2000 IU/mL, 6) alanine aminotransferase (ALT) >2 times of upper limit of normal (ULN), and (7) both HBeAg-positive and negative patients. Patients were diagnosed for their CHB status by positive HBsAg of >6 months and negative anti-HBc IgM status. The exclusion criteria's were (1) patient below 18 years; (2) patient above 60 years; (3) pregnant women and lactating mothers; (4) mentally retarded person; (5) patients who fail to give informed consent; (6) patient with cirrhosis, portal hypertension/ascites, and obstructive jaundice; (7) patients of diabetes and hypertension; (8) patients with kidney and heart disease; and (9) patients with neurological disorder.

Study treatment

Identification

Roots of qust (*S. lappa*) and plant of afsanteen (*A. absinthium*) were purchased from the local market at Khari Baoli, Old Delhi, India. Voucher specimens were deposited in the Herbarium of the Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India, and were identified and authenticated by Prof.(Dr.) M. P Sharma as qust (*S. lappa*) and afsanteen (*A. absinthium*), respectively. Physiochemical standardization of herbs was also done for quality control.

Dose

Decoction of the crude root of qust (*S. lappa*), 15 ml (containing approximately 1 g of dried extract), advised in the morning empty stomach daily, for 12 weeks. Decoction of the crude whole plant of afsanteen (*A. absinthium*), 15 ml (containing approximately 1 g of dried extract), advised in the evening empty stomach daily for 12 weeks [Figure 1].

Preparation of decoction

Decoction of each herb was prepared in the similar manner. *S. lappa* and *A. absinthium* were purchased in bulk to prevent batch-to-batch variation in the quality of herbs.

Plant materials were dried in the shade at temperatures between 21°C and 30°C for 15–30 days, after which these parts of plants were chopped and minced (ground) into small pieces of 0.5 cm or small. For setting the dose of decoction, drug (root of Saussurea or plant of Artemisia) 7 g was dissolved in 16 times of w/v (approximately 115 ml of water in 500 ml rounds-bottom flask) and allowed to soak overnight. Thereafter, drug solution was heated at a constant temperature of 40°C over mental for 5 h till it reduced to one-fourth of the original volume of water. Drug solution was strained and filtered. Liquid solution (approximately 30 ml) thus obtained was concentrated on its half (15 ml) over water bath. A single dose of 15 ml/day of decoction of each herb was established.

Figure 1: Test drug

Fifteen milliliters of this decoction was further evaporated to dryness. The dried extract was afterward kept for 5 min in hot oven and constant weight of approximately 1 g was obtained. Decoction of each herb was prepared once in bulk in this clinical trial through the same above-mentioned procedure with the same proportion of herb and water at the same temperature for the same duration. Drug solution was reduced and concentrated in the same proportion and preservative (sodium benzoate at a concentration of 0.1%) was added.

Fifteen milliliters from this decoction, prepared in bulk was further evaporated and dry extract value was measured approximately 1 g. Thus, the quality of decoction was maintained. Decoction was packed in air-tight plastic bottles of 500 ml capacities and labeled Decoction (*Joshanda*) Saussurea or Decoction (*Joshanda*) Artemisia. The decoctions of both the drugs were stored at room temperature in the laboratory before use. Thin-layer chromatography and high-performance thin-layer chromatography of decoction of both the drugs were also done for the purpose of quality control [Figures 2-7].

Efficacy end-points

Test drug was evaluated for its efficacy in suppression and loss (negativation) of HBsAg at week 12 (after treatment). Similarly, loss (negativity) of HBeAg in HBeAg-positive patients after treatment was considered a serological response. These responses were compared with the efficacy of standard antivirals. Serum HBsAg assay was performed through mini VIDAS®, a compact automated immunoassay system based on the enzyme-linked fluorescent assay technology. Serum HBeAg assay was performed through fully automated bidirectionally interfaced chemiluminescent immunoassay (CLIA) technology.

In HBV DNA, efficacy end-point was (complete virologic response [cVR]) a plasma HBV DNA level of $<10^3$ copies/mL (200 IU/mL) with normalization of ALT at week 12 (after treatment). Other end-point was significant mean reduction in HBV DNA of >1 $\log_{10}$ copies/mL in HBeAg-positive and negative group was considered as partial virologic

response. Viral breakthrough is the term utilized to denote an increase in HBV DNA quantitative >1 $\log_{10}$ copies/mL after 12 weeks of treatment. Serum HBV DNA was quantified by real-time polymerase chain reaction assay, the Taq-Man HBV quantitative test, which had a lower limit of quantification of 10^3 copies/mL (200 IU/mL). 1 IU/mL was equivalent to 5.82 copies/mL.

Biochemical response was defined as recovery to normal ALT level. Serum ALT was measured with an enzymatic assay at basal, week 6, and week 12. ULN of ALT, which in healthy adults was taken 40 IU/L for males and 30 IU/L for females.

Safety analysis

For safety evaluation of the test drug, patients were assessed on clinical, hematological (hemogram and blood sugar fasting and postprandial), and biochemical parameters (kidney and liver function test) at baseline, 6th week, and 12th week. Adverse events, significant laboratory abnormalities, discontinuation of the test drug due to adverse events were evaluated. In this protocol, ALT flares were considered to be an adverse event. Flares were defined as an ALT level that was more than twice the baseline level and >10 times the upper limit of the normal range, with or without associated symptoms. There was an open form of possible adverse symptoms in the case record form. Patients were asked directly whether they had these symptoms (nausea, vomiting, skin rashes, epigastric discomfort, anorexia, hypoglycemia, flatulence, diarrhea, headache, any other etc.).

Statistical analysis

Analysis of results was performed through GraphPad Prism, version 7.00 for Windows created on March 31, 2016. Continuous variables were compared with Students *t*-test (unpaired and paired), Wilcoxon signed-rank test, and Tukey–Kramer multiple comparisons test. Categorical variables (proportions) were compared by Fisher's exact test.

Differences were considered statistically significant when the $P < 0.05$. Test results were ranked as: Ns – nonsignificant, $^*P < 0.05$ significant, $^{**}P < 0.01$ very significant, and $^{***}P < 0.001$ extremely significant.

RESULTS

Study population

Among 70 patients, 34 patients received the test drug dosage. Most patients who did not meet eligibility criteria had a low HBV DNA level, a low ALT level, or both or they had exclusionary findings such as hepatic decompensation, cirrhosis, ascites, shrunken coarse liver (confirmed through ultrasonography of abdomen), acute cases (anti-HBc IgM positive), and hypertension. A total of 30 out of 34 patients (88.23%) completed the whole study period with full dataset [Figure 8]. Baseline characteristics including age, sex, race, ALT, serum albumin, platelet counts, and HBV DNA levels are mentioned in Table 1.

Virologic response

- HBeAg-positive patients (mean HBV DNA 5.88 $\log_{10}$ copies/mL or 1095155 IU/mL): Among 14 HBeAg-positive patients, 8 (57.14%) patients achieved cVR at 12th week after treatment ($P < 0.01$). Mean reduction of serum HBV DNA level over 12 weeks was -2.64 ± 1.81 $\log_{10}$ copies/mL (1083525 ± 2321273 IU/mL) from baseline ($P < 0.0001$) [Table 2 and Figures 9-11]
- HBeAg-negative group (mean HBV DNA 4.68 $\log_{10}$ copies/mL or 15509 IU/mL): Among 16 HBeAg-negative patients, 6 (37.5%) patients achieved cVR at 12th week after treatment ($P < 0.05$).

Mean reduction of serum HBV DNA level over 12 weeks was -1.83 ± 1.6 $\log_{10}$ copies/mL (14381 ± 21081 IU/mL) from baseline ($P < 0.001$). Percentage change of mean HBV DNA (IU/mL) was 98.94% and 92.72% in HBeAg-positive and negative group, respectively, at the 12th week [Table 2 and Figures 9-11].

Serologic response

HBsAg loss

- HBeAg-positive group (mean HBV DNA 5.88 $\log_{10}$ copies/mL or 1095155 IU/mL): Among the 14 HBeAg-positive patients, 5 (35.71%) patients had HBsAg loss at the 12th week ($P < 0.05$) [Tables 2 and Figure 11]
- HBeAg-negative group (mean HBV DNA 4.68 $\log_{10}$ copies/mL or 15509 IU/mL): Among 16 HBeAg-negative patients, HBsAg loss was observed in 4 (25%) patients at 12th week of treatment ($P = 0.10$) [Table 2 and Figure 11].

After completion of treatment at 12th week, these 5 (31.71%) HBsAg-positive and 4 (25%) HBsAg-negative patients were followed and assessed for their HBsAg status again after 20–26th week, all nine patients (100%) maintained their negative HBsAg status. This showed sustained response of test drug on viral particles.

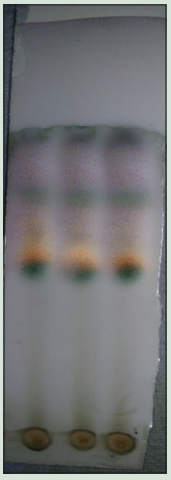

Figure 2: TLC Saussurea

Figure 3: TLC Artemisia

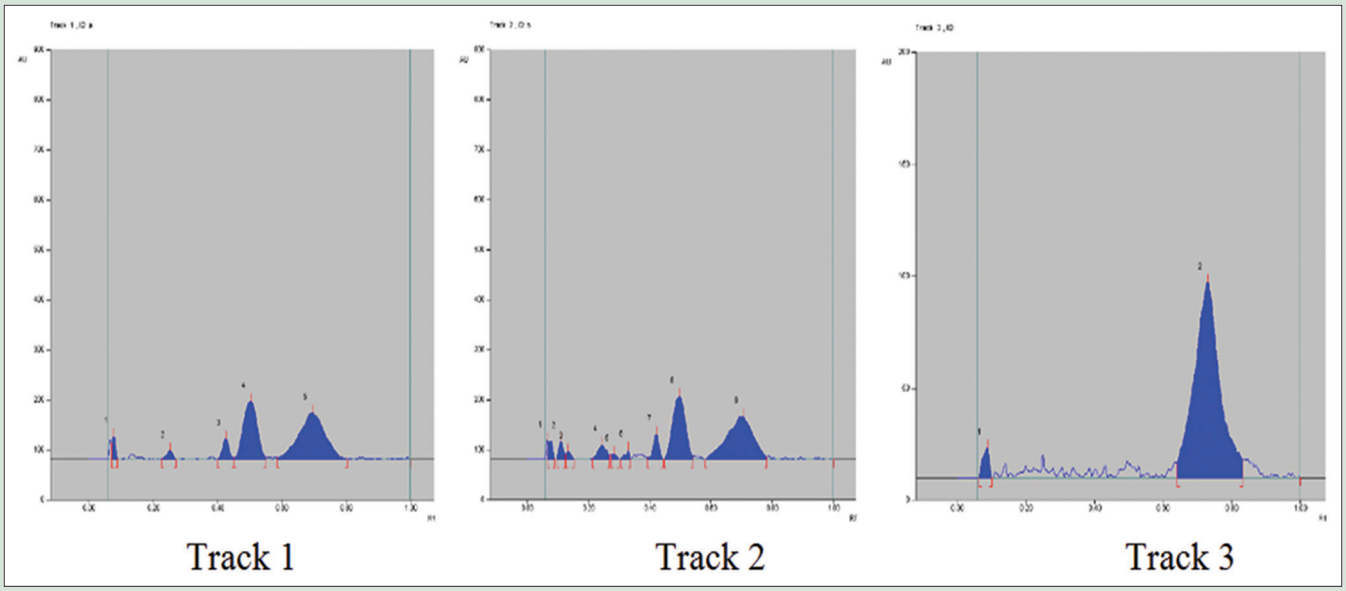

Figure 4: Decoction of Saussurea at 440 nm

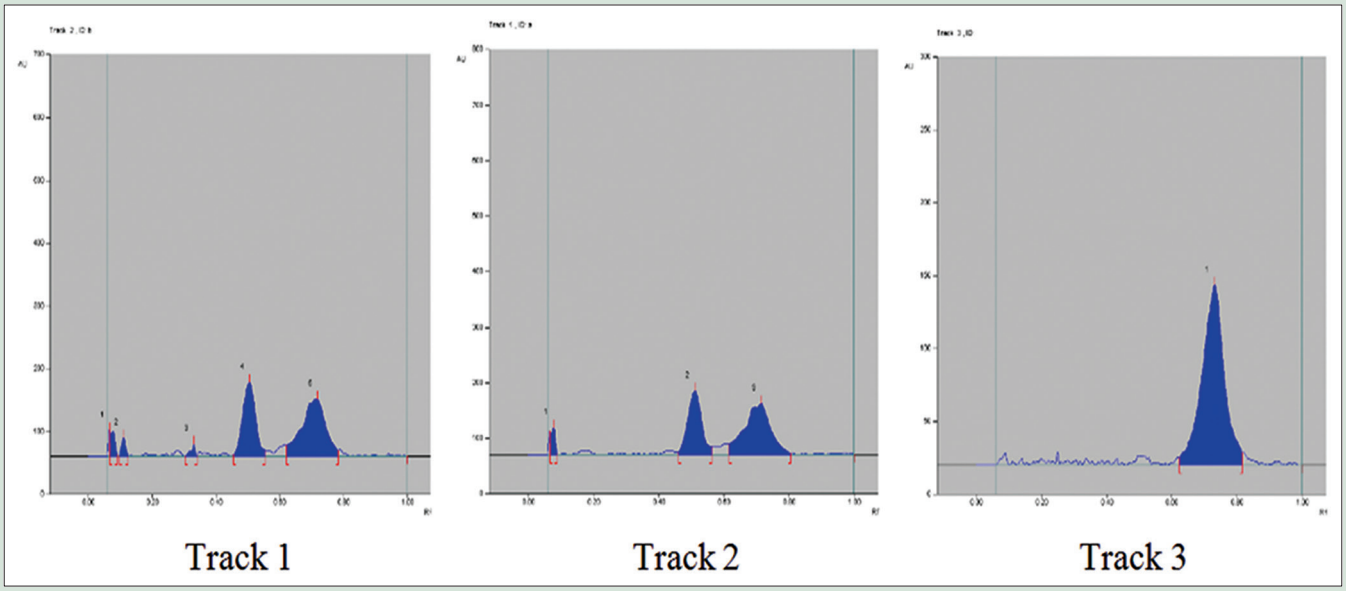

Figure 5: Decoction of Saussurea at 540 nm

HBeAg loss

Among 14 HBeAg positive patients, 10 (71.42%) patients became HBeAg negative after 12 weeks of treatment ($P < 0.001$) [Table 2 and Figure 12].

Biochemical response

- HBeAg-positive group: Mean ALT level in HBeAg-positive group was 350 ± standard deviation (SD) 576 IU/mL. Figure 13 shows that ALT normalization was observed in 7/14 (50%) patients ($P < 0.01$) at 6th week (mid-treatment) and 12/14 (85.71%) patients at the 12th week (after treatment) ($P < 0.001$)
- HBeAg-negative group: Mean ALT level in HBeAg-negative group was 204 ± SD 341 IU/mL. Figure 13 also shows that 7/16 (43.75%) patients at the 6th week ($P < 0.01$) and 13/16 (81.25%) patients at 12th week of study treatment, achieved normal ALT levels ($P < 0.001$).

Safety profile

No significant adverse signs or symptoms were observed during and after treatment. Mean baseline value of hemoglobin, total leukocyte count, serum albumin, platelet count, blood urea, serum creatinine, serum uric acid, and blood sugar fasting and postprandial had no significant difference from mean values of the 6th week and 12th week (after treatment) ($P > 0.05$). Mean packed cell volume of 40.91 ± SD 5.5 at baseline was increased to 43.16 ± SD 4.8 at 12th week but remained within normal limit (significant at $P < 0.05$). Similarly, ESR was significantly reduced toward normal value to 12.76 ± SD 6 at 12th week from 16.7 ± SD 8.8 at baseline ($P < 0.05$). Mean value of hemoglobin was 13.72 ± SD 1.84 at baseline. After treatment, mean hemoglobin was 14.08 ± SD 1.8, however statistically, it was nonsignificant ($P > 0.05$).

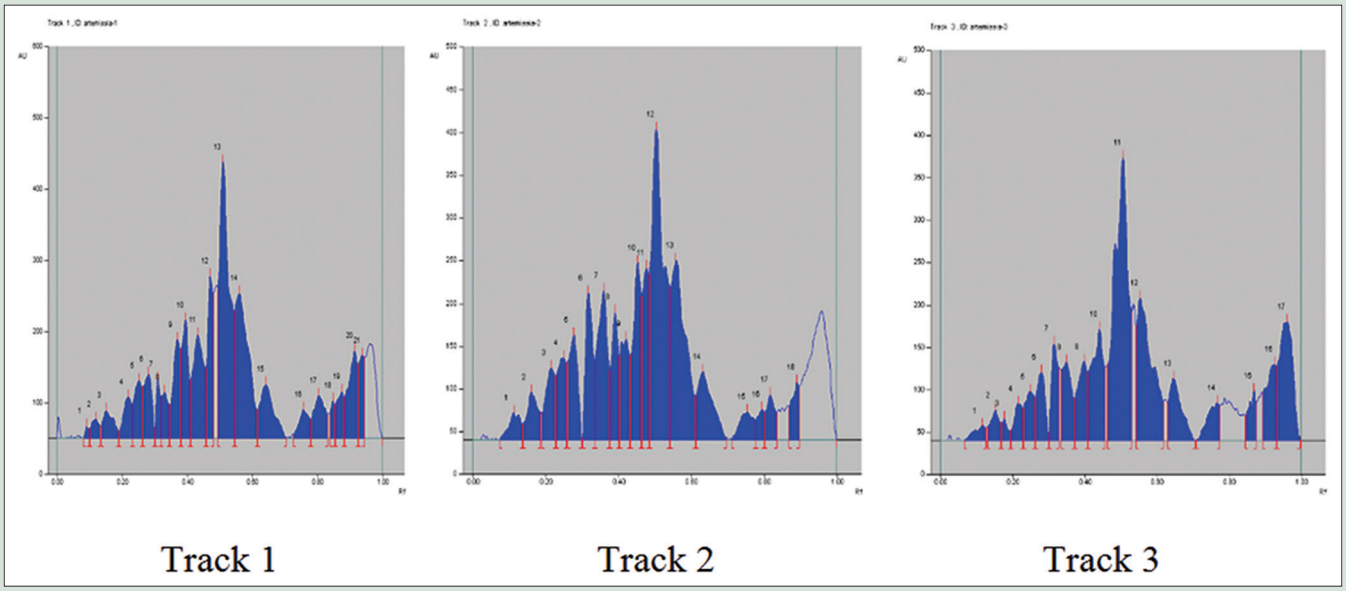

Figure 6: Decoction of Artemisia at 440 nm

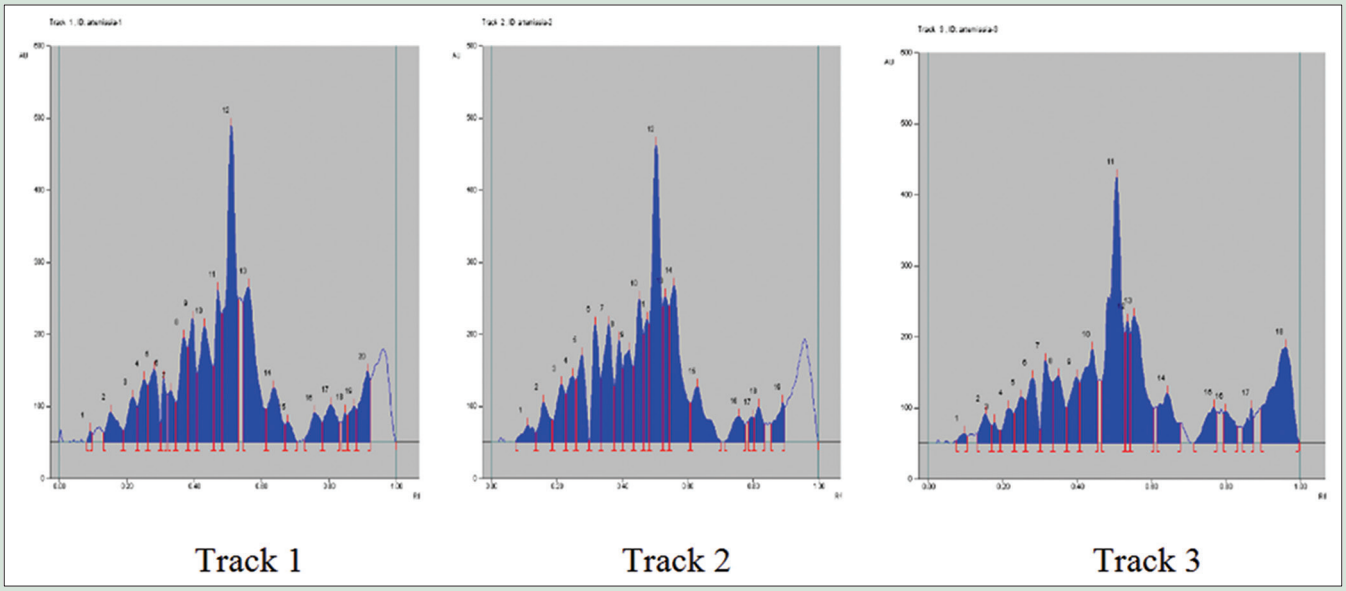

Figure 7: Decoction of Artemisia at 540 nm

DISCUSSION

Test drug, *S. lappa* and *A. absinthium*, have exhibited potent serologic, virologic, and biochemical responses, characterized by rapid and efficient HBsAg and HBeAg clearance, and significant reduction and suppression of HBV DNA levels along with normalization of inflammation and functions of the liver.

Terrault *et al.*, in AASLD guidelines (2015), reported HBsAg loss of 2%–7% after 6 months of pegylated interferon (pegIFN) therapy.[13] Similarly, treatments with tenofovir, entecavir, adefovir, and in untreated patients, HBsAg loss is observed in 3%, 2%, 0%, and 0%–2% of the HBeAg-positive patients, respectively, after 48 weeks of extensive treatment.[13,14] In our study, HBsAg loss was observed in 35.71% at 12th week of study treatment without any significant adverse events.

While in HBeAg-negative patients, after treatment with IFN and currently used entecavir, tenofovir, adefovir, and lamivudine for more than 48–52 weeks, HBsAg loss rarely occurred in HBeAg-negative patients.[14] While in our study, HBsAg loss was observed in 25% of HBeAg-negative patients after 12 weeks of treatment (n = 4/16). However, we had a small sample size of 16 patients, still 4 patients became HBsAg negative. If compare with small sample size study of Hou *et al.*, in which patients were treated with lamivudine for 48 weeks, 0% had HBsAg loss in HBeAg-negative group (n = 0/22).[15] These findings may preliminaries substantiate the efficacy of test drug.

Being a pilot study, high rate of HBsAg loss observed could be due to the inclusion of highly selected CHB patients who were clinically stable and were without liver fibrosis, compensated or decompensated cirrhosis of

liver, diabetes mellitus, hypertension, kidney, heart, and neurological diseases. On the other hand, most of the CHB studies for efficacy of conventional antivirals have included these variables. Anti-HBs was not done due to financial constraints.

Table 1: Demographic and baseline characteristics of the patients in the study (mean±standard deviation)

Characteristics	HBeAg positive (*n*=14)	HBeAg negative (*n*=16)
Age (years)	29.64±11.47	30.68±11.31
Sex (male:female)	12:2	9:7
Race: Asian	14	16
BMI (kg/m^2)	20.01±2.98	21.71±2.95
ALT (IU/mL)	350±576	204±341
Total bilirubin (mg/dL)	3.14±2.56	1.25±1.71
Serum albumin (mg/dL)	3.84±0.44	4.11±0.58
Platelet count (lacs/cumm)	2.32±0.93	2.27±0.99
HBV DNA level (Log$_{10}$ copies/mL)	5.88±1.07	4.68±0.5
Previous treatment with antiviral	0	6

HBV: Hepatitis B virus; BMI: Body mass index; ALT: Alanine aminotransferase

Table 2: Virologic and serologic response at week 12 in HBeAg positive and negative patients

Variables	HBeAg positive (*n*=14), *n* (%)	HBeAg negative (*n*=16), *n* (%)
HBV DNA<10^3 copies/mL	8/14 (57.14)**	6/16 (37.5)*
Mean reduction in HBV DNA (log$_{10}$ copies/mL)	2.64±1.81***	1.83±1.6***
Partial virologic response (HBV DNA reduction >1 log$_{10}$ copies/mL)	12/14 (85.71)***	10/16 (62.51)***
Non response	2/14 (14)	6/16 (37.5)
Viral breakthrough	0	0
HBeAg loss	10/14 (71.42)***	-
HBsAg loss	5/14 (35.71)*	4/16 (25) NS

*P<0.05 significant, **P<0.01 very significant, ***P<0.001 extremely significant. ALT: *Alanine aminotransferase*, NS: Nonsignificant

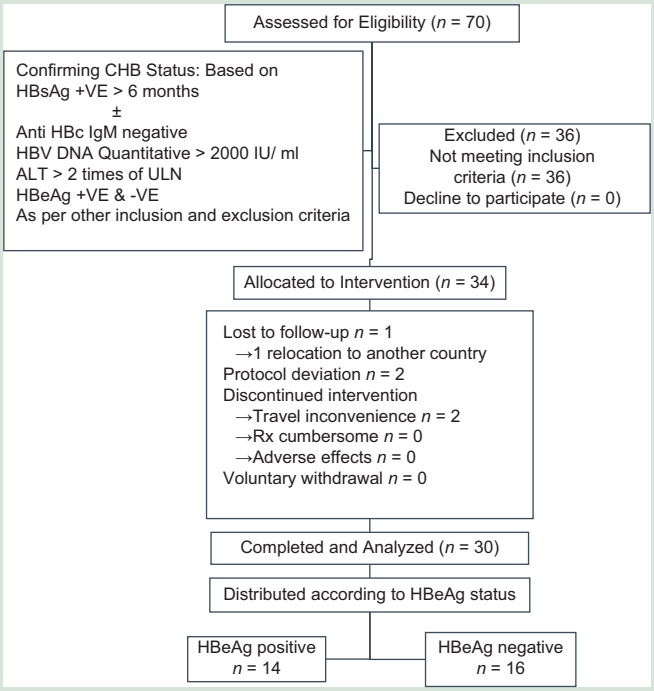

Figure 8: Enrollment and outcomes

In a comparative efficacy study between tenofovir and entecavir done by Pereira *et al.* which had almost similar baseline characteristics like our study has reported HBeAg loss of 79% in entecavir-treated group (n = 19/24) and 72% in tenofovir-treated group (n = 18/25) after more than 65 weeks of treatments.[16] In our study, 71.42% of the patients had HBeAg loss (n = 10/14) after 12 weeks of study treatment. High HBeAg clearance rate observed in our study in comparison of conventional antivirals may demonstrate the effectiveness of our test drug in clearing the cccDNA which is regarded as the hallmark of complete viral eradication or cure for the disease.[17]

Terrault *et al.* in AASLD guidelines (2015), Marcellin *et al.*, and Anna *et al.* have reported cVR of 66% with tenofovir, 60%–67% with entecavir, 21% with adefovir, and 10%–25% of patients with pegIFN after 48 weeks of treatment in HBeAg-positive patients. In HBeAg-negative patients, 71% of tenofovir-treated, 90% of entecavir-treated, 51% of adefovir-treated, and 63% of pegIFN-treated patients showed cVR after 48 weeks of treatment.[13,14,18] On the other hand, in our study, 57.14% of HBeAg-positive and 37.5% of HBeAg-negative patients achieved cVR at 12th week of the study treatment. The reason for less percentage of cVR patients in HBeAg negative could be due to those 6 (20%) patients who had treatment failure with conventional antivirals and joined our study. All these 20% antiviral-treated patients belonged to HBeAg-negative group. The chances of mutated strains of virus in these patients remain high. However, test drug significantly controlled HBV DNA in these HBeAg-negative patients despite previous antiviral failure.

This antiviral effect of test drug on HBV DNA, HBsAg, and HBeAg can be attributed to the constituents of the test drug. *S. lappa* which was used as decoction in the morning has shown to possess anti-hepatitis B activity in an animal model. Chen *et al.* reported that constituents costunolide and dehydrocostus lactone of *S. lappa* root had a strong suppressive effect on the expression of HBsAg and HBV DNA of Human hepatoma Hep3B cells. They have also observed suppressive effect of *S. lappa* on mRNA of HBsAg in HepA2 cells.[12] This could be a reason that the test drug effectively suppressed HBsAg in the study patients.

It is clear that the immune system plays an important role in HBV infection. The balance between the virus and the host immune system defines the outcomes from the disease, viral resolution, and extent of liver injury in response to HBV.[19] T-cells are the root soldier of the cell-mediated immunity in HBV infection. During HBV infection, B-cells secrete neutralizing antibodies which limit viral spread and promote the resolution of infection. Keeping this mechanism in mind, we found that hydroalcoholic extract of Saussurea root has increased the leukocyte

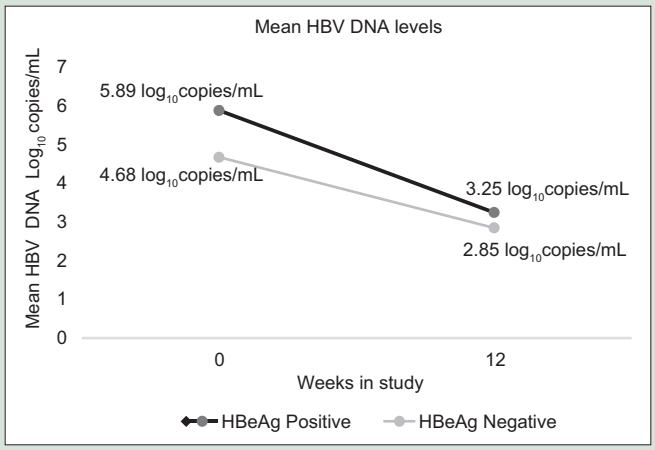

Figure 9: Mean hepatitis B virus DNA levels

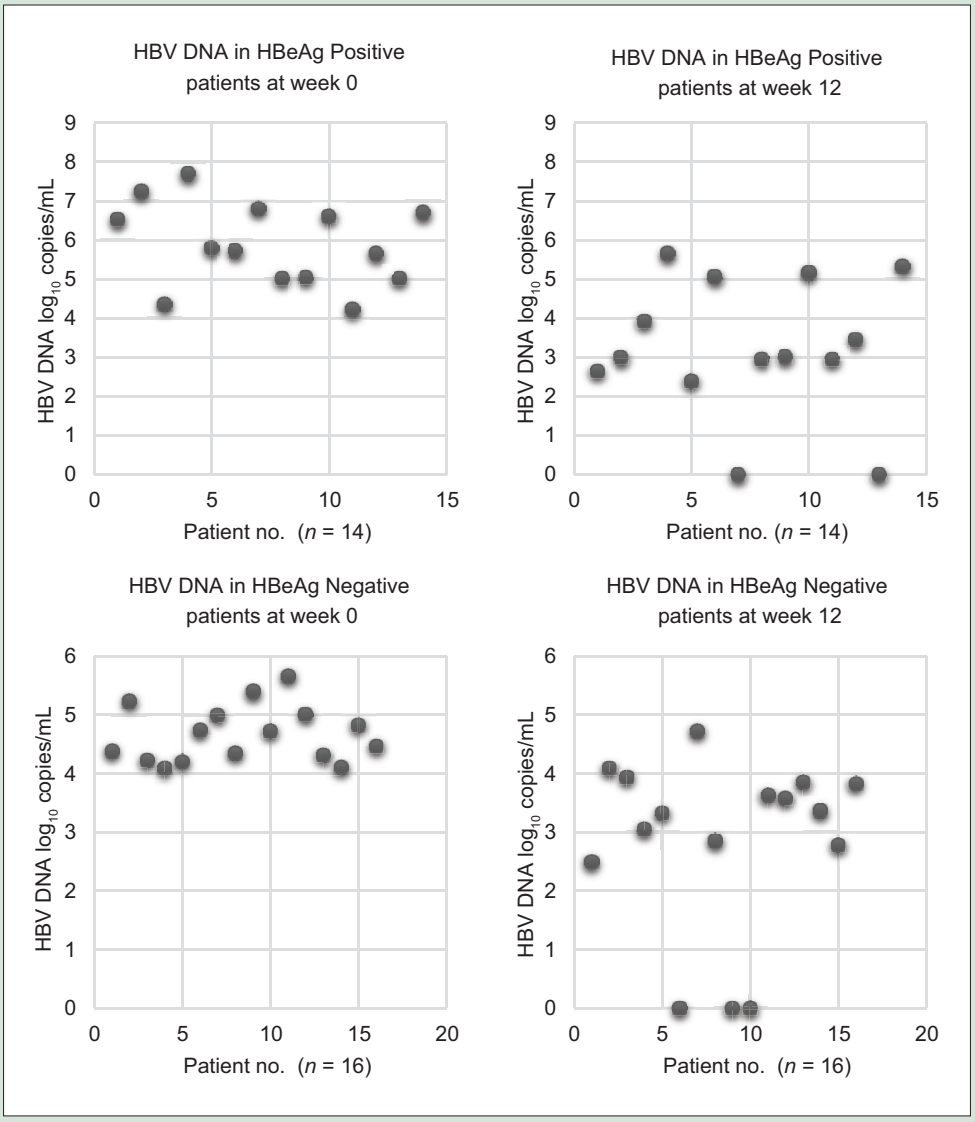

Figure 10: Hepatitis B virus DNA (log_{10} copies/mL) of patients at week 0 and 12

count, spleen weight, phagocytic index, and antibody-secreting cells in an animal study, observed by Pandey *et al*.[20] This could be the plausible mechanism of antiviral effect of the test drug by increasing T-cell phagocytic activity and activating antibody production which leads to viral suppression.

No patient (0%) had an increment of HBV DNA after 12 weeks of treatment. Patients who had mean reduction of HBV DNA <1 log_{10} copies/mL, could possibly be due to typical genotype or mutant variety of HBV which responds less effectively to treatment or test drug inefficiency in producing strong early virologic response [Table 2]. However, genotyping studies of HBV were not done in this study due to financial constraints.

Meta-analysis of studies for evaluation of antiviral efficacy in biochemical test has reported that 48 weeks of treatment with pegIFN, entecavir, and adefovir are associated with ALT normalization in 32%–41%, 68%, and 48% of patients in HBeAg positive, respectively, while 38%, 78%, and 72% of HBeAg-negative, respectively, have achieved normal ALT levels.[14] In our study, we have observed ALT normalization in 85.71% of patients in HBeAg positive and 81.25% in HBeAg negative group at 12th week which was not even reported with conventional antivirals after 48 weeks. These findings may convey efficacy of the test drug in improving liver condition better than antivirals.

Some degree of insignificant ALT fluctuation is common in CHB patients. Due to immunomodulatory aspect, drugs such as IFN causes benign or transient ALT rise which is associated with higher rate of HBeAg loss.[21] Hence, this could be a plausible cause of higher HBeAg loss of 71.42% at 12th week in our study due to immunomodulatory properties of test herbs.[5,8,20] It was normal at the inception of treatment.

Possible reason of normalization of deranged values of LFT after treatment could be due to hepatoprotective, anti-inflammatory and anti-oxidant properties of *S. lappa*,[8,11] and *A. absinthium* [9,10,22-26,30,31] have been observed in various animal and clinical studies. These drugs either prevented the further rise of ALT or resolved the inflammation of the liver through their mitigating effect by targeting inflammatory cascade or attenuating or scavenging effect on the generation of reactive oxygen species. Both the drugs have been used specifically in Unani medicine as liver tonic since centuries in the treatment of hepatitis, jaundice, and other pathological conditions of the liver.[27-29,32-35]

S. lappa, *Artemisia annua*, and *Artemisia capillaris* of traditional Chinese medicine (TCM) have been observed to possess anti-hepatitis B activity

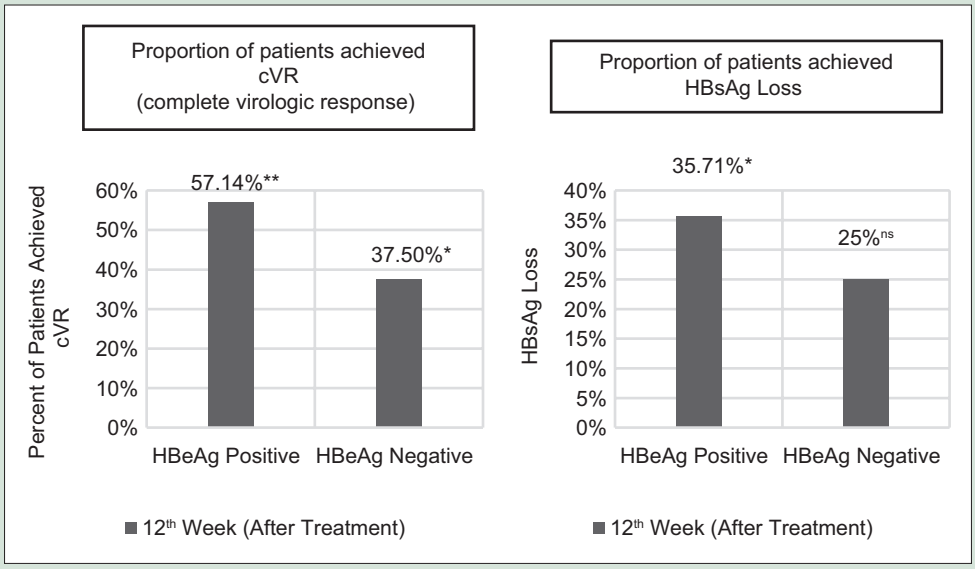

Figure 11: Complete virologic response and HBsAg Loss. $^{ns}P > 0.05$ nonsignificant, $^{*}P < 0.05$ significant, $^{**}P < 0.01$ very significant

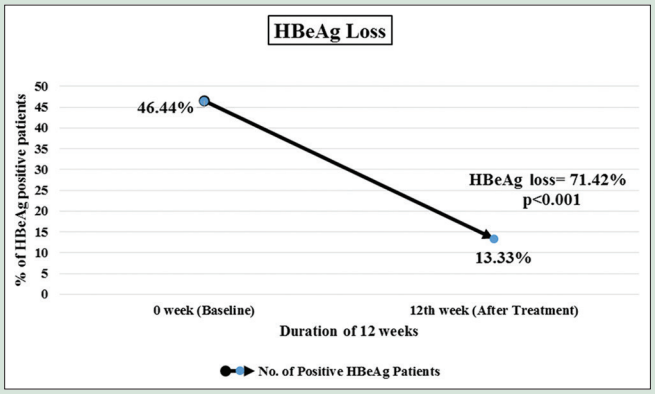

Figure 12: HBeAg loss

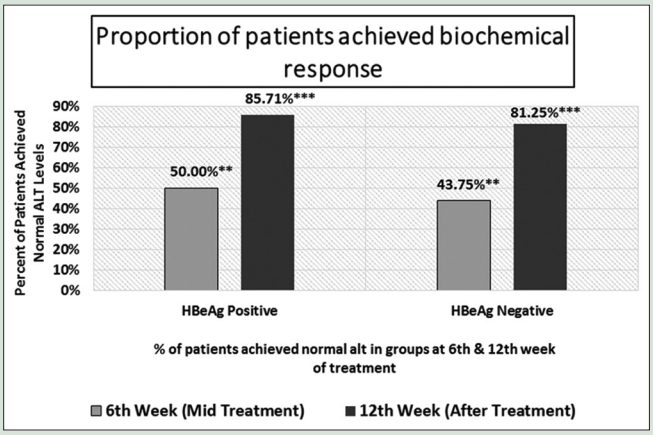

Figure 13: Biochemical response. $^{**}P < 0.01$ very significant, $^{***}P < 0.001$ extremely significant

in various cell lines studies. Various TCM compound formulations have been proven beneficial for their efficacy as an adjuvant with conventional medicines.[12,36,37] As per our knowledge, *S. lappa* and *A. absinthium* have never been clinically investigated in the present regime form and dosage. In our study, decoction of each drug was administered at a different time during the day as an independent test treatment which may substantiate their efficacy differ to TCM in the context of herb to herb and conventional drug interaction has been already demonstrated.[31]

Test drug may have better tolerated than IFN and NA's such as tenofovir, adefovir etc. which leads to gastrointestinal disturbances (anorexia, nausea, vomiting, diarrhea, dizziness, rashes, thrombocytopenia etc.), and ultimately nonadherence to drug and withdrawal.[6,18,21] This finding (no adverse effects) observed in our study could be a reason for compliance and adherence of the patients to the test drug. However, short duration of treatment and small sample size in our study may be inconclusive for probable adverse effects.

CONCLUSIONS

Our study has substantiated that *S. lappa* and *A. absinthium* have an antiviral effect against HBV in CHB. Test drug effectively suppressed HBV DNA, HBeAg, and HBsAg in study patients. Normalization of liver function test showed that the test drug can improve functions of the liver without any significant adverse effects for a treatment duration of 12 weeks, one of a reason for compliance of the patients in the study. Double-blind randomized clinical trial for comparing the efficacy with conventional antivirals with large sample size, longer duration, and including cirrhotic patients should be done. Further, cell lines studies should be done for elucidating the mechanism of viral suppression through the test drug.

Limitations

Limitation of the study includes small sample size and short duration of protocol therapy due to financial constraints. There was no placebo or active treatment for comparison in our pilot pre-, mid-, and post-treatment study. However, we feel it is important to have information regarding the HBsAg and HBeAg loss and mean reduction of HBV DNA following treatment with *S. lappa* and *A. absinthium* which can be used for sample size calculation in the subsequent randomized trial, comparing the effects between test drug and tenofovir or interferons. Although HBsAg was performed through MiniVIDAS technique, quantification was not

available in all the patients. Unlike other studies on CHB evaluating the effect of conventional antivirals, being a pilot study, we have enrolled only selected clinically stable CHB patients who were not diabetic and hypertensive, without liver fibrosis and compensated or decompensated cirrhosis of liver, and not have other neurological, kidney and heart disease. The other limitation was the lack of performing the investigation anti-HBs, liver biopsy or fibroscan in the study due to limited resources and funds. However, liver biopsy or fibroscan may not be needed as cirrhosis and liver fibrosis were exclusionary criteria's in this study.

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Conflicts of interest

There are no conflicts of interest.

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