WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 16, 640-653. Research Article ISSN 2277– 7105

A CLINICAL STUDY ON HEPATITIS B UNDER THE INFLUENCE OF VASADI KWATH

Anupam Srivastava*[1], Ajai Kumar Pandey[2], Narasimha Murthy[3] and Rajendra Prasad[4]

[1]Junior Resident Department of Kayachikitsa, Faculty of Ayurveda, IMS, BHU, Varanasi.

[2]Assistant Professor (Co-Supervisor) Department of Kayachikitsa, Faculty of Ayurveda, IMS, BHU, Varanasi.

[3]Associate Professor (Co-Supervisor) Department of Kayachikitsa, Faculty of Ayurveda, IMS, BHU, Varanasi.

[4]Professor (Supervisor) Department of Kayachikitsa, Faculty of Ayurveda, IMS, BHU, Varanasi.

Article Received on
14 October 2017,
Revised on 03 Nov. 2017,
Accepted on 24 Nov. 2017
DOI: 10.20959/wjpr201716-10180

***Corresponding Author**
Dr. Anupam Srivastava
Junior Resident
Department of
Kayachikitsa, Faculty of
Ayurveda, IMS, BHU,
Varanasi.

ABSTRACT

Background: Hepatitis B is emerging as a challenging health problem in the present era. It is caused by Hepatitis B virus and is emerged as an important risk factor for liver cirrhosis and Hepatocellular Carcinoma. The consequences associated with Hepatitis B are increasing many folds in the affected individual. The treatment modalities are still evolving in conventional system of medicine but antiviral and nucleotide analogues therapies are emerged as promising hope for its management. However, research scholars are inclined to the other remedial resources due to adverse effects and side effects of available modern drugs for its management. **Aims:** To evaluate the clinical efficacy and safety of Vasadi Kwath in the cases of Hepatitis B and to evaluate the impact of Vasadi Kwath on the viral load in the cases of Hepatitis B. **Materials and Methods:** A total of 50 patients of Hepatitis B were registered; in which 40 patients turned up for full follow-up and 10 patients were dropped out from the study. Patients were randomly allocated into two groups; Group-A was treated with capsule Liv 52 HB (2 cap twice a day) while Group-B was treated with Vasadi Kwath (40 ml twice a day) and assessment was done at monthly intervals in three consecutive follow ups. **Results:** The selected trial drug has shown significant response on subjective and objective parameters

along with significant reduction in Hepatitis B viral load. The patients treated with Vasadi Kwath; 66.7% patients have shown reduction of Hepatitis B viral load <20 IU/ML, while 45.5% were observed in patients treated with Liv-52 HB. **Conclusion:** The Trial drug Vasadi Kwath possess Chologogue, Choleretic, Laxative, Purgative, Antioxidant, Adaptogenic and Immunomodulatory properties along with antiviral effect.

KEYWORDS: Hepatitis B, Kamala, Interferons, Jaundice, Ayurvedic drug, Antiviral drug.

INTRODUCTION

Hepatitis B is an infectious disease caused by the Hepatitis B virus (HBV), which affects the liver. It can cause both acute and chronic infections and puts people at high risk of death from cirrhosis of the liver and liver cancer. It is a major global health problem and India is considered to have an intermediate level of HBV endemicity.[1] About one million Indians are at risk of acquiring Hepatitis B infection and about 10,000 die from the virus every year. Of the 26 million infants born annually in India, approximately one million run a life time risk of developing chronic HBV infections.[2,3] HBV is spread through contact with infected body fluids. Blood is the most important vehicle for transmission. There are 3 modes of HBV transmission; perinatal, sexual and parenteral/percutaneous transmission.[4-20]

The symptoms of acute viral hepatitis appear after an incubation period. Generally, the incubation period of Hepatitis B ranges from 30 to 180 days[21] (mean 8-12 weeks). Acute infection with hepatitis B virus is associated with acute viral hepatitis – an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, dark urine and then progresses to development of jaundice.[22] It may be associated with some extrahepatic symptoms like skin rashes like erythematous, macular, maculopapular, urticarial, nodular or petechial lesions, Polyarthralgia, polyarthritis, urticaria, purpura, oral lichen planus, etc.[23] Diagnosis of Hepatitis B includes Detection of HBsAg and HBeAg in the serum, Presence of IgM anti-HBc, Anti HBs, HBV DNA etc.[24-25]

In modern medicine, a synergistic approach of suppressing viral load and boosting the patient's immune response with immunotherapeutic interventions are required for better prognosis. The management of Hepatitis B includes short and long acting interferon and nucleotide analogues which boost up the immune system and improves the level of inflammation, but their optimal response rates and clinical outcomes have not been defined till today.[26]

Ayurveda stands first in providing the complete, reliable and successful outcome in the management of diseases and complications specially the area of Ayurvedic hepatology and virology. In Ayurveda, Bio-purificatory methods (Samshodhana) and Internal Medicine (Samshamana) are the modes of treatment indicated for the management of diseases and process of diathesis of disease.[27] In this clinical study Vasadi Kwath is selected as a trial drug by scanning various Ayurvedic lexicons, a large number of publications and reports and Liv-52 HB (which is proven anti-viral drug in patients of HBV) as control trial drug in patients of HBV.

ETHICAL CLEARANCE

The study was approved by "The Ethical Committee" of Institute of Medical Sciences, Banaras Hindu University with letter no. Dean/2014-15/EC/1166 Dated: 21.05.2015.

MATERIALS AND METHODS

A total of 50 diagnosed cases of positive HBV were registered from the OPD/IPD of Sir Sundar Lal Hospital, Banaras Hindu University, Varanasi.

Inclusion Criteria

1. Aged between 15-65 years including both male and female.
2. Diagnosed cases of Hepatitis B.
3. Patient willing to participate in the above trial and giving informed consent.

Exclusion Criteria

1. Hepatitis B associated with other systemic complications or associated diseases like DM, HTN, Asthma, CHF, Tuberculosis and AIDS.
2. Viral Hepatitis B complicated with Gross swelling of the limbs, Ascites, Portal Hypertension, Esophageal Varices, Bleeding disorders and Hepato-renal syndrome.
3. Pregnant and lactating women.
4. Patient undergoing other Panchakarma procedures.
5. Patients who took any other medication or discontinue the trial period without information of the investigator.
6. Patient infected with Hepatitis A, C, D & Hepatitis E.

The 50 registered cases were randomly divided into 2 groups having 25 patients in each group. Out of registered patients, 3 from Group I and 7 from Group II, (Total = 10 patients)

discontinued the treatment in between the study. So, only 40 patients completed the total duration of trial with all 3 regular monthly follow ups.

Group I: 22 patients were given the control drug Capsule Liv 52 HB 2 cap twice a day for the period of 3 months.

Group II: 18 patients of this group were given the trial drug Vasadi Kwath 40 ml twice a day for the period of 3 months.

Trial duration with follow up

All the cases were followed up at the interval of 1 month for total 3 months duration.

Selection of Control Drug

In this present study, the selected control drug was capsule Liv 52 HB. It is a US patent-pending phytopharmaceutical formulation, which is recommended for the treatment of Hepatitis B.[28] It protects the liver against various hepatotoxins, exerts antiviral activity and enhances antioxidant defense system.[29-33]

Selection of Trial Drug

In this present study, selected trial drug Vasadi Kwath has been taken from the book "Bhaishajya Ratnawali". It is clearly mentioned in Bhaishajya Ratnawali in the management of Kamala.[34] It is also used in the treatment of Pandu, Raktapitta and Halimaka etc.[35]

oklk·e`rk fuEcfdjkrdV~oh d"kk;¨·;a le/kqfuZihr% A

ldkeya ik.MqeFkklzfiRra gyheda gfUr dQkfnj¨xku~ AA

(ÒS"kT; jRukoyh 12@23)

Procedure for the Extraction of Decoction

Dried parts of Vasa (leaves), Guduchi (stem), Nimba (bark), Kirattikta (panchang) and Katuki (root) were procured from the local market. All the drugs were taken in equal amount. The drugs were cut into 1 inch long, cleaned dried and made (equal proportions) into coarse powder (Yavakuta churna) and packed into 400 gm polythene bags in the Pharmacy of Rasa Shastra, Banaras Hindu University. The raw material of the drug was given to the patients and the method of preparing decoction was advised. The method of preparation of decoction was guided according to the rule of Ayurvedic Formulary of India (AFI) which states that the ratio of drug and water should be 1:4 i.e. 40 gm drug in 160 ml water. (The equivalence of solid {raw material in gm} and liquid {water in ml} is same due to "1" gm/ml unit of density

of water). It should be boiled until the one fourth parts is left i.e. 40ml of decoction which should be used after sieving.

Dose of medicine- 40 ml twice a day

Anupana- Madhu (as indicated in Bhaishajya Ratnawali)

Parameters of Assessment

Treatment response was assessed on improvement in the subjective and objective parameters.

A. Subjective Improvement: Reduction in the scores of symptoms and signs.

B. Objective Improvement:

(i) Liver function tests: attaining normal ranges and

(ii) Reduction in the titer of HBV viral load.

Scoring and Gradation Pattern of Complaints

Reddish yellow urination

Absent=0

Mild=1 Yellow

Moderate=2 Yellow orange

Severe=3 Orange

Very Severe=4 Reddish orange

Reddish yellow defecation

Absent=0

Mild=1 Yellow

Moderate=2 Yellow orange

Severe=3 Orange

Very Severe=4 Reddish orange

Indigestion

Absent=0 Normal digestion of >2 meals per day

Mild=1 Able to digest only two proper meal with no bloating

Moderate=2 Able to digest two meal with bloating

Severe=3 Unable to digest one meal with no bloating

Very Severe=4 Unable to digest even one meal and with bloating/vomiting

Generalized Weakness

Absent=0	No presence at all
Mild=1	Occasional Feeling of uneasiness
Moderate=2	Feeling of uneasiness after the daily usual work
Severe=3	Constant feeling of uneasiness in usual wok
Very Severe=4	Impairing all the normal activities

Anorexia

Absent=0	Normal appetite
Mild=1	Unable to take two meals
Moderate=2	Able to take 2 meals without interest
Severe=3	Unable to take one meal
Very Severe=4	Vomiting just after intake of meal

STATISTICAL METHODS

Qualitative variables were assessed by Chi-square ($\chi 2$) test for significant difference among the groups. To assess the effect of drug from base line to different follow ups in Quantitative and Qualitative variables respectively unpaired 'T'-test, 'Z'-test and Mann Whitney Test was applied. To assess the Quantitative and Qualitative variables within the group respectively Paired 'T'-test, 'Z'-test and Wilcoxon signed ranks test was applied.

OBSERVATIONS AND RESULTS

In the present study, maximum patients had symptoms like Indigestion (in 56% cases), Gen. Weakness (36% cases), Reddish yellow defecation (28% cases), Anorexia (26% cases) and Reddish yellow urination (24% cases) (Graph 1). A gradual and steady decline was observed in all the complaints during each follow up in both the study groups (Graph 2 and 3). The result in the subjective improvement was highly significant from base line to 3rd follow up (Table 1 and 2). The intergroup comparison was statistically not significant at before and after the treatment.

Sr. Bilirubin/D. Bilirubin - The mean score of Sr. Bilirubin/D. Bilirubin was 1.167/0.439 in Group I and 0.967/0.371 in Group II at before treatment. There was a progressive decline in the value of Sr. Bilirubin/ D. Bilirubin in each follow up. After the trial treatment the mean score was reduced up to 0.797/0.235 in Group I and 0.687/0.235 in Group II respectively. Mean decrease in Serum Bilirubin/ D. Bilirubin was statistically significant in Group I only.

The intergroup comparison was statistically not significant (p>0.05) at before and after the treatment.

T. Protein- The mean score of Total Protein was 7.279 in Group I and 7.211 in Group II at Before Treatment. There was mild improvement in the value of Total Protein after 3 month of follow up in both the Groups. After the trial treatment the mean score was increased up to 7.570 in Group I and 7.442 in Group II respectively. Mean increase in Total Protein was statistically significant in Group I only. The intergroup comparison was statistically not significant at before and after the treatment.

ALT/AST- The mean score of ALT/AST was 56.76/50.392 in Group I and 45.286/49.408 in Group II at Before Treatment. After the trial treatment the mean score was reduced up to 38.53/43.528 in Group I and 30.961/31.338 in Group II respectively. Mean decrease in ALT/AST level was statistically significant in Group II only. The intergroup comparison was statistically significant at after the treatment.

ALP- The mean score of ALP was 105.49 in Group I and 111.920 in Group II at Before Treatment. There was mild improvement in the value of ALP (from the border line) after 3 month of follow up in both the Groups. After the trial treatment the mean score was reduced up to 99.295 in Group I and 81.800 in Group II respectively. Mean decrease in ALP level was statistically significant in Group 2 only. The Intergroup comparison was statistically significant at after treatment.

HBV DNA

In Group 1, reduction of viral load i.e. <20 IU/ML was observed in 45.5% of cases. While in Group 2, reduction of viral load i.e. <20 IU/ML was observed in 66.7% of cases. Mean decrease in HBV Viral Load was statistically highly significant (p=0.001) in Group II than Group I (p=0.003) (Table 3). The intergroup comparison was statistically not significant (p>0.05) at before and after treatment.

Table 1: Showing the Improvement in severity of Symptoms in Group 1.

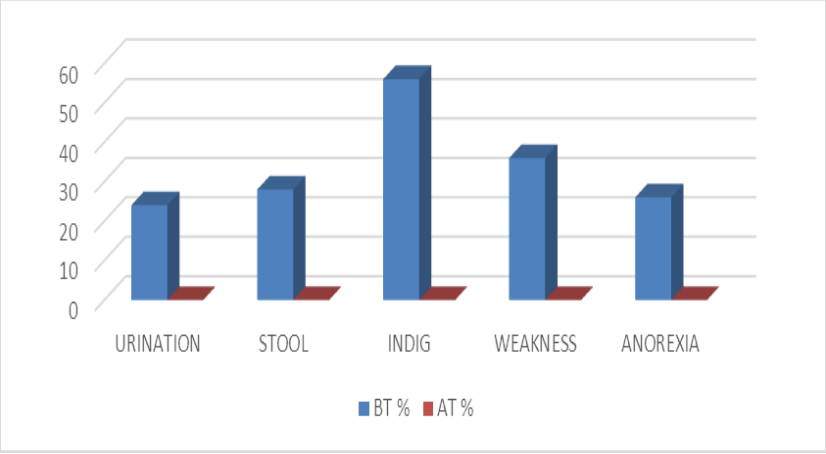
Symptoms	Group 1					
	BT	F1	F2	F3	$\chi 2$	p
Reddish yellow urination	24	13.6	9.1	0	11.842	0.008
Reddish yellow defecation	20	4.5	0	0	12.750	0.005
Indigestion	52	27.3	18.2	0	17.066	0.001
Generalised weakness	36	22.7	0	0	17.600	0.001
Anorexia	28	13.6	0	0	12.412	0.006

Table 2: Showing the Improvement in severity of Symptoms in Group 2.

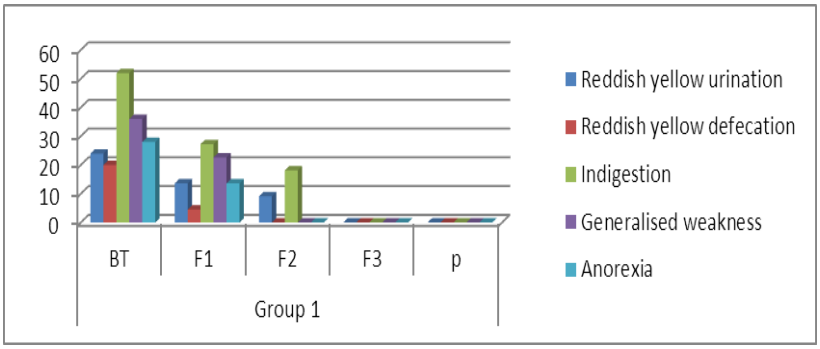
Symptoms	Group 2					
	BT	F1	F2	F3	$\chi 2$	p
Reddish yellow urination	24	10	0	0	14.400	0.002
Reddish yellow defecation	36	10	0	0	24.9	0.000
Indigestion	60	55	16.7	0	34.135	0.000
Generalised weakness	32	25	22.2	0	19.442	0.000
Anorexia	24	20	0	0	15.000	0.002

Table 3: Changes in HBV DNA Level in the cases of Hepatitis B.

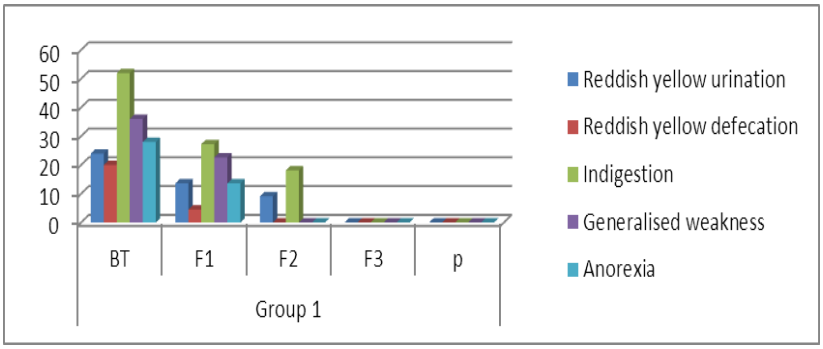
Group	Grade	Viral load	HBV DNA No. and %				Within the group comparison Wilcoxon signed ranks test
			BT		AT		
Group I	0	<20	0	0%	10	45.5%	z= 3.019 p= 0.003
	1	21-50	2	9.1%	1	4.5%	
	2	51-100	2	9.1%	0	0%	
	3	101-200	2	9.1%	3	13.6%	
	4	$201\text{-}10^3$	6	27.3%	2	9.1%	
	5	$10^3\text{-}10^4$	4	18.2%	1	4.5%	
	6	$10^4\text{-}10^5$	2	9.1%	2	9.1%	
	7	$10^5\text{-}5\times10^6$	2	9.1%	3	13.6%	
	8	$>5\times10^6$	2	9.1%	0	0%	
Group II	0	<20	0	0%	12	66.7%	z= 3.336 p= 0.001
	1	21-50	1	4%	0	0%	
	2	51-100	4	16%	0	0%	
	3	101-200	1	4%	1	5.6%	
	4	$201\text{-}10^3$	14	56%	3	16.7%	
	5	$10^3\text{-}10^4$	3	12%	1	5.6%	
	6	$10^4\text{-}10^5$	1	4%	1	5.6%	
	7	$10^5\text{-}5\times10^6$	1	4%	0	0%	
	8	$>5\times10^6$	0	0%	0	0%	
Between the group comparison Mann Whitney Test			z= 1.180 p= 0.238		z= 1.373 p= 0.170		



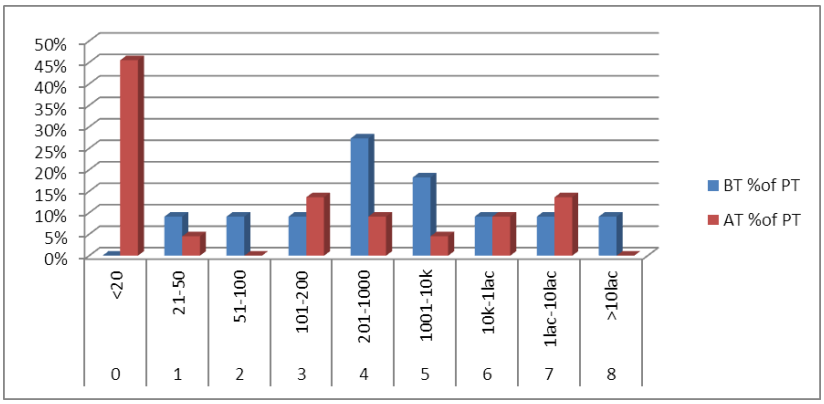
Graph 1: Showing the Incidence of Symptoms in the 50 cases of Hepatitis B.



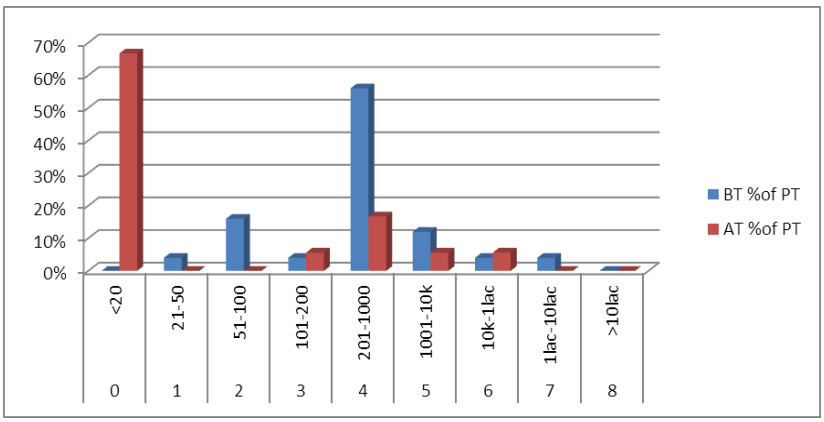
Graph 2: Showing the Improvement in severity of Symptoms in Group 1.



Graph 3: Showing the Improvement in severity of Symptoms in Group 2.



Graph 4: Showing the Antiviral Impact in Group 1.



Graph 5: Showing the Antiviral Impact in Group 2.

DISCUSSION

The demographic profile revealed that the majority of the cases 42% belonged to 26-35 years age group. This Adolescent and Young Adult aged Males (68%) were highly vulnerable to the exposure of HBV. In India, poverty and unemployment cause majority of the males to migrate and share a common housing facilities like hostels, lodgings in which history of sharing of razors, brushes, needles etc. is frequently reported. The barbers and unregistered medical professionals, who do not sterilize their instruments, contribute a great deal to the transmission of virus. Maximum incidence was observed in Hindus (92%) and only 8% was observed in Muslims. This is because of SS hospital, BHU which is situated in the area of Hindu dominant society and the lower incidence in Muslims, due to religious conflicts and lack of better quality education to come out their views in front of society. Out of registered patients, 58% belonged to urban areas that live in Varanasi or nearby districts followed by 42% in rural area. Because S.S. Hospital, BHU is a territory hospital in Purvanchal region, hence seeks attention from the patients of neighboring region of Varanasi. Majority of the cases i.e. (86%) were married against 14% unmarried patients. HBV virus infection is seen more commonly in Adolescents & Young adults and 26-35 year is the major age group of marriage. The higher incidence of the disease was found in the person having qualification up to Intermediate (46%) followed by Graduate person (34%). Most of them were of business class group who stay outside more or travel more for their business schedule including shopkeeper, welder, saloon, labor etc. had more incidences (30%) followed by 24% of housewives specially those of business class person who are ignorant of the disease and their route of transmission of virus. Maximum incidence was noted in upper middle class group 54% followed by lower middle class 46%. Since BHU Hospital is the major government hospital with free services in these geographical settings along with increased expenditure capacity of people, the incidence was observed more in upper middle class families. No incidence was observed in High Class society (>10 lac/anum) in this settings as they generally prefer to go for private hospitals or paid clinics.

Maximum patients had symptoms like Indigestion, Gen. Weakness, Reddish yellow defecation, Anorexia, Reddish yellow urination. All the symptoms were gradually relieved in both the groups during each follow up. After the trial treatment there were no any symptoms in any of the patients. In liver function test, the increased value of serum enzymes like ALT and AST was gradually came into their normal range. Rest others parameters like Sr. Bilirubin, Total Protein, Albumin, ALP and other investigation like CBC were normal before

and after treatment. There was no any adverse reaction observed during and after the treatment.

IMPACT OVER HBV DNA

In Group 1; maximum cases (27.3%) were having their viral load in the range of 201-1000 IU/ML followed by 18.2% having viral load in the range of 1000-10000 IU/ML at Before Treatment. After 3 month follow up in Group 1, a sharp reduction of viral load i.e. <20 IU/ML was observed in 45.5% of cases (Graph 4). In Group 2; maximum cases (56%) were having their viral load in the range of 201-1000 IU/ML followed by 16% having viral load in the range of 51-100 at Before Treatment. After 3 month follow up in Group 2, a sharp reduction of viral load i.e. <20 IU/ML was observed in 66.7% of cases (Graph 5).

CONCLUSION

We finally conclude that patients in both the groups have shown significant improvement in clinical symptoms, liver function tests and reduction of HBV load. The trial drug Vasadi Kwath is clinically safe as no any side effects & adverse effects encountered and no any significant changes were observed in laboratorial findings such as CBC and RFT. The efficacy rate of trial drug is comparable to that of the current available treatment modalities. Besides anti-viral effect against HBV load, it is also presumed that Vasadi kwath exerts hepato-cellular regeneration, cholegogue and choleretic activity, membrane stabilizing effect, antioxidant effect, molecular nutrient effect and metabolic corrections. It helps in clearing the symptoms and elevated LFTs thereby restoring the functional status of liver and maintains its architecture. This study provides scientific validity of Ayurvedic formulation pertaining to the cases of HBV and provides a lead to work out Vasadi Kwath on larger sample size to reach the final conclusive remarks.

REFERENCES

1. Toukan A. Strategy for the control of hepatitis B virus infection in the Middle East and North Africa. Vaccine, 1990; 8: S117–S121.
2. Margolis HS, Alter MJ, Hadler SC. Hepatitis B: evolving epidemiology and implications for control. Semin Liver Dis., 1991; 11: 84-92.
3. Alter M. Epidemiology of hepatitis B in Europe and worldwide. J Hepatol, 2003; 39: S64-S69.
4. Scott RM, Snitbhan R, Bancroft WH, Alter HJ, Tingpalapong M. Experimental transmission of hepatitis B virus by semen and saliva. J Infect Dis., 1980; 142: 67-71.

5. Bancroft WH, Snitbhan R, Scott RM, Tingpalapong M, Watson WT, Tanticharoenyos P, et al. Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. J Infect Dis., 1977; 135: 79-85.
6. Sevens CE, Neurath RA, Beasly RP, Szmuness W. HBeAg and anti-HBe detection by radioimmunoassay. Correlation with vertical transmission of hepatitis B virus in Taiwan. J Med Virol, 1979; 3: 237-241.
7. Xu ZY, Liu CB, Francis DP, Purcell RH, Gun ZL, Duan SC, et al. Prevention of perinatal acquisition of hepatitis B virus carriage using vaccine: preliminary report of a random double-blind placebo-controlled and comparative trail. Pediatrics, 1985; 76: 713-718.
8. Xu D, Yan Y, Xu J, Wang W, Men K, Zhang J, Liu B, et al. A molecular epidemiology study on risk factors and mechanism of HBV intrauterine transmission. Natl J Med China, 1999; 79: 24-27.
9. Yan Y, Xu D, Wang W, Liu B, Liu Z, Men K, et al. The role of placenta in hepatitis B virus intrauterine transmission. Chin J Obstet Gynecol, 1999; 34: 392-395.
10. Xu D, Yan Y, Choi BC, Xu J, Men K, Zhang J, et al. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a casecontrol study. J Med Virol, 2002; 67: 20-26.
11. Xu D, Yan Y, Zou S, Choi BC, Wang S, Liu P, et al. Role of placental tissues in the intrauterine transmission of hepatitis B virus. Am J Obstet Gynecol, 2001; 185: 981-987.
12. Xu D, Yan Y, Xu J, Men K, Liu Z, Zhang J, et al. A molecular epidemiologic study on the mechanism of intrauterine transmission of hepatitis B virus. Chin J Epidemiol, 1998; 19: 131-133.
13. Wang S, Xu D, Yan Y, Shi M, Zhang J, Ma J, et al. An investigation on the risk factors of the intrauterine transmission of hepatitis B virus. Chin J Public Health, 1999; 18: 251-252.
14. Lin H, Lee T, Chen D, Sung JL, Ohto H, Etoh T, et al. Transplacental leakage of HBeAg positive maternal blood as the most likely route in causing intrauterine infection with hepatitis B virus. J Pediatr, 11987; 111: 877-881.
15. Wang Z, Zhang J, Yang H, Li X, Wen S, Guo Y, et al. Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies. J Med Virol, 2003; 71: 360-366.
16. Ohto H, Lin H, Kawana T, Etoh T, Tohysma H. Intrauterine transmission of hepatitis B virus is closely related to placental leakage. J Med Virol, 1987; 21: 1-6.

17. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clin Infect Dis., 1995; 20: 992–1000.
18. Alter MJ. Epidemiology and prevention of hepatitis B. Semin Liver Dis., 2003; 23: 39–46.
19. Alter M, Mast E. The epidemiology of viral hepatitis in the United States. Gastroenterol Clin North Am, 1994; 23: 437-440.
20. Luo KX, Liang ZS, Yang SC, Zhou R, Meng QH, Zhu YW, et al. Etiological investigation of acute post-transfusion non-A, non-B hepatitis in China. J Med Virol, 1993; 39: 219-223.
21. Hollinger FB, Liang TJ: Hepatitis B virus. In: Fields Virology, 4th ed. Knipe DM et al (editors). Lippincott Williams & Wilkins, 2001.
22. Weinbaum C, Lyerla R, Margolis HS: Prevention and control of infections with hepatitis viruses in correctional settings. MMWR Recomm Rep., 2003; 52(RR-1): 1.
23. Amarapurkar DN, Amarapurkar AD, Kriplani AL. Extrahepatic manifestations of viral hepatitis: hospital based study. J Gastroenterol Hepatol, 2000; 159: 30.
24. Pawlotsky J-M: Molecular diagnosis of viral hepatitis. Gastroenterology, 2002; 122: 1554.
25. Gish RG, Locarnini SA: Chronic hepatitis B: Current testing strategies. Clin Gastroenterol Hepatol, 2006; 4: 666.
26. Lavanchy D: Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepatitis, 2004; 11: 97. [PMID: 14996343]
27. Singh RH, The Holistic Principles of Ayurvedic Medicine, Chowkhamba Sanskrit Pratisthan.
28. Premashis Kar, Mohammed Asim, Manash Pratim Sarma, Pralhad. S. Patki; HD-03/ES: A Promising herbal drug for HBV antiviral therapy; Antiviral Research, 2009; 84: 249–253.
29. Arun Kumar Mahto, Narayanan Unnikrishnan; Double-blind clinical trial of HD-03/ES versus placebo in the management of Chronic Hepatitis B; Indian Journal of Clinical Practice, 2010; 21(5): 247-251.
30. A.K. Bhattacharya, P. Patki; A Preliminary study on the safety and efficacy of HD-03/ES therapy in patients with chronic Hepatitis B: A Prospective clinical study; Journal of Herbal Medicine and Toxicology, 2009; 3(2): 137-141.

31. Janardan Singh, Anupam Chakraborty, Sudhakaran C, Mukul Chandra Dhar Mitra SK; A Preliminary study on the safety and efficacy of HD-03/ES therapy in patients with chronic Hepatitis B; Indian Journal of Clinical Practice, 2006; 17(5): 39-43.
32. Kala Suhas Kulkarni, V.G. Bhagwat, U.V. Babu, S.K. Mitra, Mallikarjun N. Dixit, Ashwin Kumar Meru, Ekta Saxena; Reduction of HBsAg with decrease in viral load with herbal formulation, HD-03/ES: A case study; Medicine Update, 2002; (10)7: 61-63.
33. JS Rajkumar, MG Sekar, SK Mitra; Safety and efficacy of oral HD-03/ES given for six months in patients with chronic Hepatitis B virus infection; World Journal of Gastroenterology, 2007; 13(30): 4103-4107.
34. Ambika Datta Sastri, Pandu Roga Chikitsa Prakarana, Bhaishajya Ratnavali, Vidhyotani Hindi Commentary, Chowkhamba Sanskrit Series, 12(23).
35. Govindadasa. Bhashjyaratnavali, Shastri Ambika dutta, editor. 1st ed. Varanasi: Chaukhambha Sanskrti Sansthan; Panduroga, 2006; 1; 12/147-155. 737.