CLINICAL STUDY

Randomized Clinical Trial of an Ayurvedic Formulation, BV-7310 in Alcoholic Liver Disease

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ABSTRACT

Alcoholic liver disease (ALD) is caused by excessive intake of alcohol for many years. The incidence is as high as 25% in the United States, India and several other countries. The disease spectrum varies from fatty liver in initial stages, to hepatitis and finally cirrhosis. Untreated ALD can be fatal. Yet the options for prescription drugs are limited, and not easily available or affordable to the masses worldwide. BV-7310 contains herbal extracts of *Phyllanthus niruri, Tephrosia purpurea, Boerhavia diffusa* and *Andrographis paniculata.* The individual plants are known hepatoprotective agents in Ayurveda. The objective of this study was to investigate the safety and efficacy of BV-7310, a proprietary combination standardized formulation, in subjects with ALD. A multi-centric, double-blind, placebo-controlled, randomized study of 61 subjects was conducted for a period of 12 weeks. Subjects on BV-7310 showed improvement in clinical features of ALD as compared to placebo, including reduction and normalization of transaminases. BV-7310 also reduced bilirubin levels to normal, showing improvement in the detoxifying and excretory capabilities of the liver. No significant adverse events were seen in the treatment group. Based on the data shown, BV-7310 shows promise as a safe and effective hepatoprotective in patients of ALD.

Keywords: Hepatoprotective, alcoholic liver disease, Ayurveda, alcoholic steatohepatitis, fatty liver disease, Periban®

Alcohol, its abuse and health effects of the same have been known to mankind since millennia. Alcoholic liver disease (ALD) spans a clinical and histological spectrum.[1] At the earliest and most common stage, it causes fatty liver or steatosis characterized by infiltration of fat within the liver cells. The next stage is that of alcoholic hepatitis or inflammation and destruction of the liver cells. This progresses to fibrosis and cirrhosis, which is replacement of normal liver cells with nonliving scar tissue. Untreated, this can be a precursor for hepatocellular carcinoma.[2,3] Nearly all heavy drinkers develop fatty liver. However, according to data from clinical and autopsy studies, only 20% to 30% develop alcoholic hepatitis or cirrhosis.[4,5]

Quantitatively, the liver is the major organ involved in the metabolic disposal of ethanol. The cytosolic alcohol dehydrogenase, microsomal ethanol-oxidizing system and peroxisomal catalase metabolize ethanol to acetaldehyde, which is a reactive metabolite that can produce injury in a variety of ways.[6] Acetaldehyde is further metabolized to acetate by acetaldehyde dehydrogenase localized in the mitochondria and abundantly found in the liver. The rate of acetaldehyde formation is also highest in the liver. Hence, the liver is an early target for alcohol-induced injury. Nonoxidative pathways of ethanol metabolism include formation of ethyl esters of long-chain fatty acids, which also affect mitochondrial function.[7] Several risk factors including genetic predisposition and malnutrition contribute to the cascade of events leading to sequential dysfunction of hepatic cells. This is manifested by cell membrane damage, hypermetabolic state in the hepatocytes most prominent in the perivenular area of the hepatic lobule, oxidative injury, steatosis, triggering of immune responses, precipitation of cytoskeletal elements and production of collagen. The cytokines, thus released can also induce apoptosis in the liver cells. With this understanding of the pathogenesis of ALD and in view of the relentless progression of the disease, there is a need for clinical evaluation of potential candidates for hepatoprotection.[8]

There are limited options for prevention and treatment of alcoholic liver damage. Several agents like S-adenosyl methionine (Sam-E),[9] dietary epigallocatechin-3-gallate (EGCG) in green tea,[10,11] propylthiouracil,[12,13] colchicine[14] and silymarin[15,16] have been tested in the management of

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ALD. Steroids, biologics,[17] N-acetylcysteine and pentoxifylline have also been targeted for liver disorders.[18,19] However, insignificant clinical and biochemical outcomes,[20] toxicity profiles and costs are limiting factors in the availability and use of these agents.[21,22] Overall, with the limitations of the tried agents, there is a definite need of effective and affordable therapies for prevention and management of ALDs. As conventional medicine pursues a more integrated approach to managing disease, select herbs that influence liver function are being revisited and evaluated for their overall health promoting effects.[23,24] In this respect, the herbal treasure chest of Ayurveda offers a host of new phytopharmaceutical products that can be used to manage a spectrum of liver-related imbalances.

According to Ayurveda, the liver (*yakrut*) along with the spleen (*pleeha*) is the origin of the blood channels. Liver diseases such as *kamala* (jaundice) and its types are explained in ancient Ayurvedic texts. Consumption of excessive alcohol and its effects are described as "*madatyaya*". Its clinical diagnosis is similar to modern day ALD, and the management is well described. Ayurveda is an officially recognized healthcare system in India and countries such as Hungary, Switzerland, Brazil, United Arab Emirates, Malaysia and Romania among others. Ayurvedic science recognizes that balancing liver function is pivotal to ensuring overall health. In dealing with problems of the liver, the primary goal within the system of Ayurveda is to enhance the liver detoxification processes, reverse the injury and help protect against further damage to the liver. Based on traditional use, herbs are selected and combined for their ability to promote "balance" within the body and to nourish the liver and related functions, including digestion and bile acid secretion. These herbs act on multiple biochemical pathways to nourish the body as a whole and support various organ systems especially the liver.[25,26] BV-7310 (Periban®) has been formulated and developed using this same theory. The individual plant components of BV-7310 are known hepatoprotective agents singly for use in alcoholic and other chemically damaged liver subjects.[27-30]

However, there are limited validation studies for these individual herbs or in combination with others. Recently, we have shown the hepatoprotective effect of BV-7310 in different cellular and animal models.[31] The objective of this study was to investigate this proprietary and standardized formulation, BV-7310 (Periban®), for its safety, efficacy and hepatoprotective activity, in subjects with ALD.

MATERIAL AND METHODS

Composition of BV-7310

Each capsule of BV-7310 contained 480 mg of extracts of *Phyllanthus niruri (Bhuiamla), Andrographis paniculata (Kalmegh),* root extracts of *Tephrosia purpurea (Sharapunkha)* and *Boerhavia diffusa (Punarnava).* The dry powders were mixed in a weight/weight ratio of *P. niruri : T. purpurea : B. diffusa : A. paniculata ::* 2.5 : 1.75 : 1.5 : 1, respectively. Each of the specialized extracts were prepared by a proprietary process, and standardized using various methods such as high-pressure liquid chromatography (HPLC), thin layer chromatography (TLC) and ultraviolet visible spectrophotometry.

The plant extracts used in the formulation have been authenticated and standardized to ensure reproducibility of results. The plant materials under study were identified, authenticated and deposited in a Government of India Herbarium; namely the Central Council for Research in Ayurvedic Sciences (CCRAS) based in Pune, Maharashtra, India. The herbarium voucher numbers are *A. paniculata* (4401), *B. diffusa* (4402), *T. purpurea* (4403) and *P. niruri* (4404). Each of the 4 plants has been checked and is an accepted name by The Plant List www.theplantlist.org Version 1.1 of September, 2013. All plant extracts used in this study came from commercial crops. The use of resources and work has also been approved by the National Biodiversity Authority of India.

This was followed by qualitative standardization by TLC and quantitative standardization by HPLC of different batches of the final formulation, and stringent stability tests so that the efficacy and safety of the finished product is reproducible. The study formulation was tested for absence of residual solvents, pesticides and heavy metals. In addition, rigorous *in vitro* and *in vivo* testing was conducted to ensure safety of the formulation prior to entering into human clinical trials (data under publication).

The placebo capsules were identical in look and color, and contained inert excipients only.

Study Design

A multi-centric, double-blind, placebo-controlled, randomized clinical trial of 61 subjects was conducted over a period of 12 weeks. The study was conducted in two large fully accredited teaching hospitals and research centers namely, Topiwala National Medical Center/BYL Nair Hospital, Mumbai and King Edward Memorial Hospital, Pune, both in India. This study was

approved by the Ethics Committees of both institutions, as well as the in-house Ethics Committee of Bioved Pharmaceuticals Pvt. Ltd., India, which comprised of leading scholars, scientists, judicials and physicians. The study was conducted under guidelines of Good Clinical Practices (GCP) and the Declaration of Helsinki, and in conformance with International Council for Harmonization (ICH) guidelines, 1996.

Subjects were screened in outpatient settings at both hospitals. Sixty-six male subjects aged 20 to 60 years, diagnosed with alcoholic hepatitis based on history of alcohol abuse (drinking >30 mL of alcohol per day for at least 5 years), presence of clinical features, biochemical evidence - specified by aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) more than 2 times of normal limits, increased serum gamma-glutamyl transferase (GGT), and absence of serological markers of hepatitis B (HbSAg) or hepatitis C (anti-HCV) were included in the study. A written informed consent was taken in all cases. Subjects with cirrhosis of liver or decompensated liver disease, any severe/known systemic disease, use of any other investigational drug within 4 weeks of trial, use of corticosteroids, silymarin, milk thistle or other therapies with hepatoprotective or immunomodulatory potential within 4 to 6 weeks of trial were excluded from the study.

All enrolled subjects received, in a randomized fashion, BV-7310 or placebo for 12 weeks. The treatment was administered in a double-blind manner as follows:

- Group 1 – BV-7310, 1 capsule 2 times per day after meals for 12 weeks.
- Group 2 – Placebo, 1 capsule 2 times per day after meals for 12 weeks.

Subjects were advised to stop alcohol but this was not mandatory. In case of subjects continuing alcohol consumption despite advice, details of alcohol consumption during the trial were recorded wherever possible. The serum enzymes and other liver function tests were tested at screening, and at the end of weeks 1, 2, 4, 8 and 12. Hepatoprotective activity of BV-7310 was assessed by comparison of change in serum enzymes between subjects on BV-7310 and placebo. Safety parameters were tested at screening, and at the end of weeks 4 and 12. Liver ultrasound was done at screening and at the end of week 12.

Statistical Analysis

Fisher exact test was used to determine significance between groups in all parameters including demographics, clinical findings, laboratory efficacy endpoints, ultrasound findings and safety. Chi-square analysis was used to compare the difference between subjects who abstained from alcohol and those who continued to consume alcohol during the duration of the study.

RESULTS

In total, 66 male subjects from Mumbai and Pune area in India were screened and qualified for the trial. Of these, 3 subjects dropped out from the study before completion of 4 weeks of medication. One subject died of encephalopathy immediately after the screening visit. All the above 4 subjects were on placebo treatment. One subject on BV-7310 died during the study – he had advanced portal hypertension, esophageal varices and hematemesis at enrollment. His death was determined due to advanced disease condition and not related to the study product. Among the 61 subjects who completed the study, 31 subjects were treated with BV-7310 and 30 on placebo.

Demographics

The average age of subjects was 44.8 ± 8.5 years in the treatment group taking BV-7310 and that of subjects in the placebo group was 42.9 ± 9.1 years. The age of subjects in both groups was thus comparable (p = 0.4). The body weights of subjects in both groups were also comparable with 60 ± 8 kg in the treatment group and 61 ± 14 kg in the placebo group (Table 1). The nature of alcohol consumption was noted in the subjects. The

Table 1. Demographic Characteristics at Baseline*

Characteristic (Unit)	BV-7310 (n = 31)	Placebo (n = 30)	P value
Age (in years)	44.8 ± 8.5	42.9 ± 9.1	P > 0.05
Body weight (in kg)	60 ± 8.0	61 ± 14	P > 0.05
Duration of consumption of alcohol (in years)	14.06 ± 7.6	13.6 ± 6.4	P > 0.05
Quantity of alcoholic drink (in mL)	238 ± 157	234 ± 153	P > 0.05

n = number of subjects in group.

*The values shown are based on available data.

most frequently used alcoholic drink of all subjects was "Country (hard) liquor" diluted with water. The Country liquor has up to 42.8% alcohol by volume. The average alcoholic drink consumption by subjects was 238 ± 157 mL per day in the subjects enrolled to take BV-7310 and 234 ± 153 mL per day in the subjects who took placebo. The subjects on BV-7310 had past history of consumption of alcohol for 14.06 years and those on placebo had consumed alcohol for 13.6 years. The quantity of alcoholic drink consumed (p = 0.9) and the duration of consumption (p = 0.8) were thus comparable in the two groups (Table 1).

Subjects were encouraged to abstain from alcohol during the course of the trial. However, a significant number of subjects continued to consume alcohol. Hence, analysis of confounding due to alcohol abstinence was carried out. Subjects treated with BV-7310 were analyzed for any difference in the liver function tests between those who continued to consume alcohol and those who supposedly stopped alcohol consumption during the study period.

This comparison was possible only with one of the two study sites where continued alcohol intake was recorded in 29 subjects. Data on continuing consumption of alcohol was not available with the other site. Six of 14 subjects on BV-7310 in that study site continued to take alcohol during the study. Ten of 15 subjects on placebo gave history of consumption of alcohol during the trial period. The difference in the two groups is however not statistically significant (Chi-square = 2.14). History of continuation of alcohol consumption could not be recorded in 32 subjects. All the subjects were reasonably compliant to the study methods. No history of concomitant medication could be elicited in any of the 61 subjects studied.

Efficacy Endpoints

The most common clinical features at screening were nausea, vomiting, loss of appetite, abdominal pain, dizziness and altered sensorium. Twenty-one subjects treated with BV-7310 were icteric at initiation of study compared to 20 subjects in the placebo group. Anorexia, nausea, vomiting and fatigue did not show significant difference in baseline values in the cases and the controls.

The improvement in these clinical findings over the duration of study was more prominent in the subjects on BV-7310 than in those on the placebo in terms of reduction in icterus, nausea, vomiting, fatigue and pain in abdomen (Table 2).

Table 2. Subjects with Improvement in Clinical Findings at the End of the Study

Parameter	BV-7310 (n = 31)	Placebo (n = 30)	P value
Icterus	11 (35%)	8 (27%)	P = 0.14
Anorexia	6 (19%)	8 (27%)	P = 0.20
Nausea, vomiting	4 (13%)	2 (6.7%)	P = 0.11
Fatigue	4 (13%)	3 (10%)	P = 0.01*
Pain in abdomen	2 (6.4%)	1 (3.3%)	P = 0.004*

n = number of subjects in specified group.

*Statistically significant.

Laboratory Findings

Liver function tests, especially AST and ALT were considered the primary efficacy endpoints. AST was greater than normal in all the subjects on BV-7310, at initiation of therapy and returned to normal in 8 of these 31 subjects. In the placebo group, 1 subject had normal AST at initiation of therapy and 4 had AST values in the normal range at the completion of trial. The mean reduction in AST was also analyzed. The improvement, i.e., reduction in the AST value by the end of study was significantly greater in the subjects on BV-7310 as compared to those receiving placebo (p = 0.04, Fisher exact test) (Table 3).

ALT was normal in 15 of 31 subjects on BV-7310 at initiation of therapy and in 27 subjects at end of treatment. In the placebo group, 17 subjects had normal values at initiation of therapy and 20 had ALT values in the normal range at the completion of trial. The number of subjects who achieved normal values of ALT at end of study is higher in subjects on BV-7310 than in those on placebo (p = 0.054, Fisher exact test). The differences at baseline were comparable. The number of subjects who showed improvement in the ALT values and return to normal range is 12/16 in the active group and 3/13 in the controls. This difference is highly significant (p = 0.01, Fisher exact test). The improvement, i.e., reduction in the ALT value was greater in the subjects on BV-7310 as compared to those on placebo (Table 3).

AST:ALT ratio was compared at initiation and at the end of the trial. Mean AST:ALT ratio at initiation of trial was 3.2 ± 1.2 in cases and 2.9 ± 1 in controls. The change in ratio at end of trial is however not statistically significant with a mean difference of 1.23 ± 0.93 in treatment group and 0.93 ± 0.96 in controls (p = 0.32). The mean serum GGT values were also analyzed. The baseline serum GGT values were comparable in

Table 3. Serologic Parameters in BV-7310 and Placebo-treated Groups

Serologic parameters	BV-7310 Mean ± SD (n = 31)		Placebo Mean ± SD (n = 30)		P value
	Initial	At end of treatment	Initial	At end of treatment	
ALT (U/L)	52.1 ± 31.8	38.8 ± 33.3	41.9 ± 18.3	34.9 ± 18.4	0.01*
AST (U/L)	144.1 ± 59.6	64.1 ± 40.0	113.8 ± 48.8	63.6 ± 36.7	0.04*
GGT (IU/L)	152 ± 121.0	79.3 ± 46.6	102.9 ± 74.2	62.7 ± 69.5	0.17
Bilirubin (mg/dL)	6.1 ± 7.2	2.8 ± 2.6	6.9 ± 8.7	3.5 ± 3.7	0.05*

n = number of subjects in specified group.

LFTs = Liver function tests; ALT = Alanine transaminase; AST = Aspartate transaminase; GGT = Gamma-glutamyl transferase.

Bilirubin values are expressed as mean ± SD.

*Statistically significant.

both groups. The improvement, i.e., reduction in the serum GGT value is greater in the subjects on BV-7310 (72.7 ± 117) as compared to placebo (40.3 ± 49.8); however, the difference is not statistically significant (p = 0.17) (Table 3).

Confounding Effect of Abstaining from Alcohol during the Trial

Subjects were encouraged to abstain from alcohol during the course of the trial. A significant number of subjects, however, continued to consume alcohol. Hence, analysis of confounding due to alcohol abstinence was carried out. The cases, i.e., the subjects on BV-7310 were analyzed for any difference in the liver function tests in the subjects who continued to consume alcohol during the course of treatment and those who supposedly stopped alcohol consumption in the study period. This comparison was possible only with one of the two centers (in 29 subjects) where continued consumption of alcohol was recorded. The difference in improvement in the efficacy criteria (AST, ALT and GGT) of subjects on BV-7310 (n = 14) is not significant in the two groups, i.e., those continuing and those who have discontinued alcohol in the study period (p > 0.05).

Other Laboratory Findings

Serum alkaline phosphatase (ALP) was not significantly affected by the course of BV-7310 therapy. Upon completion of therapy, serum ALP was increased in subjects on BV-7310 (as compared to initial values), the difference being 9.59 and decreased in subjects on placebo, the mean difference being 3.29. These changes are statistically insignificant (p = 0.1). Serum protein values increased in the subjects on BV-7310 by 1.37 ± 9.69. The values increased in the group on placebo by 0.77 ± 3.2. This finding is also statistically insignificant (p = 0.7) though showing a good trend for improving the liver functions to normal in subjects treated with BV-7310.

Serum bilirubin was within normal range in 8 subjects on BV-7310 at initiation of study, but was normal in 15 subjects by the end of study. In the placebo group, however, serum bilirubin was normal in 12 subjects at initiation of study and in 13 subjects by the end of study. Hence, a reduction in bilirubin to normal range was seen in 7/23 subjects with high baseline values on BV-7310 treatment and in only 1/18 subjects with high baseline values in subjects on placebo. Thus BV-7310 facilitated reduction in bilirubin values to normal values by the end of study (p = 0.05). The mean reduction in serum bilirubin is however comparable in both groups (Table 3). The INR (ratio of patient's prothrombin time with normal laboratory value) was $[1.5]^{ISI}$ ± 0.5 at screening in the cases and $[1.3]^{ISI}$ ± 0.5 at end of study. The values were $[1.5]^{ISI}$ ± 0.7 at screening in the controls and $[1.2]^{ISI}$ ± 0.3 at end of study. The results are comparable in the two groups.

The Maddrey's discriminant function, a fairly good indicator of prognosis in liver disease is derived from the prothrombin time and serum bilirubin. A score of >32 suggests poor prognosis and possible benefit from steroids. In this study, there were 12 patients in the active group with a score >32 at start and 7 at the end of the study. In the placebo group, there were 12 patients with a score >32 at start and 3 at end of study (p > 0.05) (data not shown). This suggests a better prognosis in subjects treated with BV-7310.

The mean corpuscular volume of erythrocytes did not show significant change in either group (p > 0.05). The mean reduction in blood glucose in the active treatment group was 18.7 and that in placebo or controls was 8.07; the difference was not statistically significant. This

parameter is not an indicator of activity under this study; however, it shows a general trend to normalize the liver metabolic functions with the study formulation and is a positive factor in this study.

Ultrasonography

Findings on ultrasonographic (USG) examination were recorded at initiation of therapy and at end of therapy in 28 of the 31 subjects on BV-7310 and in 26 of the 30 subjects on placebo. No subjects on BV-7310 showed any deterioration in USG findings over the course of study. Improvement and return to normal was seen in 13 subjects and no change in 15 subjects. In contrast, 5 of the 26 subjects on placebo had deterioration of USG findings at the last visit; improvement was seen in 3 subjects and no change in 18 subjects. The improvement in terms of size of liver, ascites and change in echo texture of the liver on USG examination was highly significant in the subjects on BV-7310 ($p < 0.05$) (Table 4).

Table 4. Number of Subjects with Improvement in Ultrasound Examination

Observation	Improvement	No change	Deterioration
Active (n = 28)	13	15	0
Placebo (n = 26)	3	18	5

n = number of subjects in specified group.

Safety

No adverse events or side effects of treatment were seen during the study duration in both groups. Two subjects, 1 in each group, died during the study, their deaths were determined to be unrelated to the study or product under study. Pulse, respiratory rate, blood pressure and body temperature remained reasonably constant during the course of therapy in both subjects on BV-7310 and placebo.

The hemoglobin, red blood cell (RBC) count, total and differential white blood cell (WBC) count, platelets, erythrocyte sedimentation rate (ESR), blood glucose, blood urea nitrogen (BUN), serum creatinine and urine routine and microscopic examinations did not show any significant changes over the course of treatment in either group (Table 5).

Global Impression

The investigator/s and the patient/s rated the mode of treatment at the end of study. Ten subjects on BV-7310 and 8 on placebo rated the treatment as excellent. The outcome of treatment was rated poor by 1 patient on placebo.

The treatment outcome was rated as excellent by the investigators in 14 subjects on BV-7310 against 4 subjects on placebo. The outcome was poorly rated in 4 subjects, all on placebo.

Table 5. Blood Safety Parameters in the Two Groups and Significance of Difference

Parameter	BV-7310 (n = 31)		Placebo (n = 30)		Significance of difference of means
	1st visit	Last visit	1st visit	Last visit	
Hemoglobin (g%)	11.9 ± 2.8	11.67 ± 2.38	11.7 ± 3.2	11.5 ± 2.6	P > 0.05
RBC count (per mm^3)	4.5 ± 0.6	4.59 ± 0.4	4.67 ± 0.6	4.5 ± 0.6	P > 0.05
WBC count (per mm^3)	8,920 ± 3,450	8,071± 2,388	9,080 ± 3,540	7,642 ± 1,528	P > 0.05
Neutrophils (%)	71.8% ± 13.5	66.48% ± 8.67	71.4% ± 10.4	68.2% ± 9.2	P > 0.05
Lymphocytes (%)	25.3% ± 12.6	32.28% ± 8.35	25.3% ± 9.5	29.5% ± 9.6	P > 0.05
ESR (Westergren method mm/hr)	13.9 ± 10.6	11.4 ± 7.85	15.6 ± 8.6	13.2 ± 9.2	P > 0.05
Fasting blood glucose (mg/dL)	110 ± 42.7	93.71 ± 19.48	102.5 ± 25.6	93.2 ± 14.9	P > 0.05
Blood urea and nitrogen (mg%)	20.3 ± 14.7	15.36 ± 7.29	21.1 ± 14.9	14.8 ± 8.6	P > 0.05
Serum creatinine (mg%)	0.86 ± 0.39	0.764 ± 0.287	0.8 ± 0.3	0.8 ± 0.2	P > 0.05
Platelet count (per mm^3)	290,313 ± 2,84,970	237,538 ± 9,40,448	214,530 ± 1,10,030	211,615 ± 75,390	P > 0.05

n = number of subjects in specified group.

WBC = White blood cell; RBC = Red blood cell; ESR = Erythrocyte sedimentation rate.

DISCUSSION

BV-7310, commercially named Periban®, is a proprietary mix of 4 specialized herbal extracts, namely *P. niruri (Bhuiamla), A. paniculata (Kalmegh),* root extracts of *T. purpurea (Sharapunkha)* and *B. diffusa (Punarnava)*. While the 4 extracts have been studied individually for therapeutic applications, this unique combination was studied in a randomized clinical trial for the first time. Only males were included in the study because both alcohol abuse and dependence rates are higher in men than in women. However, that trend is changing rapidly in both developed and developing nations of the world. Of concern is that women's risk for developing severe ALD increases with daily intake of 20 to 40 g of alcohol, while the threshold for men appears to be 60 to 80 g.[32] Four subjects, all on placebo, dropped out before completion of 4 weeks of study and were not considered for any analysis. Demographics and average alcohol consumption was well-matched in the two groups.

There was significant improvement in nausea, vomiting, icterus, pain in abdomen and fatigue in the subjects on BV-7310 than those on the placebo. The reduction in the ALT and AST values is significantly greater in the subjects on BV-7310 as compared to placebo. This reduction and normalization of the transaminases indicates a role of the product in reduction of hepatocellular inflammation and necrosis. The mean AST:ALT ratio at initiation of trial was greater than 2 in cases and controls, which was highly suggestive of ALD. The change in ratio at end of trial is not statistically significant and suggests that a study of longer duration may be warranted or that a higher dose of BV-7310 would prove to be more efficacious.

The GGT values at baseline were similarly increased in both subjects on BV-7310 and placebo at initiation of therapy. GGT being an inducible enzyme is elevated in all forms of fatty liver, making it a sensitive indicator, though not specific of liver disease. The improvement, i.e., reduction in the GGT value is greater in the subjects on BV-7310 as compared to placebo though not significant. GGT has a long half-life of 26 days, making its role limited in evaluation of liver disease. This could be a reason for not obtaining a statistically significant difference in improvement though seen to be improved in the subjects on BV-7310 versus those on placebo.

Serum ALP was not significantly affected by the course of therapy. It is a marker of obstructive liver disease and therefore not a marker of ALD. Serum protein values have shown marginal increase in the subjects on BV-7310 as compared to the group on placebo. Serum proteins are good markers of hepatic function in terms of synthesis. However, a rise in total proteins may be seen even in the presence of liver disease in case of an acute phase response, the reactants of which are synthesized in the liver.

Serum bilirubin was raised in 23 subjects on BV-7310, and in 18 subjects on placebo, at initiation of therapy. This was however not manifested as icterus in some subjects as the rise in bilirubin was <3 mg/dL in these instances. BV-7310 facilitated significant reduction in bilirubin values to normal values by the end of study. This indicates that BV-7310 helps restore the detoxification and excretory functions of liver which play a pivotal role in progression of ALD. There is improvement in the blood sugar levels in BV-7310 group versus placebo. Though not statistically significant, this signifies a causative effect in improvement in overall metabolism and liver function with BV-7310. Blood counts, other chemistry and prothrombin time did not show statistical significance between the two subject groups. Of note is that leukocytosis and thrombocytopenia are common in ALD. These occur secondary to direct alcohol toxicity or to hypersplenism found in portal hypertension. However, both the leukocyte counts and the platelet counts were within normal limits in the subjects for the entire duration of study. The formulation, BV-7310, therefore had no obvious toxic effects on long-term consumption (for a period of 12 weeks in this study).

Most common findings on USG were bright echo texture of liver suggestive of fatty liver, free fluid in abdomen suggestive of ascites and hepatomegaly. The improvement on USG examination of liver is highly significant in the subjects on BV-7310 indicating improvement in overall liver function and recovery from injury. Subjective measures in investigation of therapeutic agents best reflect the influence on disease from the patient's and investigator's perspective, because a subjective response most likely integrates best the clinical efficacy and safety. The investigator's global impression here showed very good correlation with the nature of study formulation in the wake of the study being a double-blind trial. Treatment outcome was rated excellent in 14 subjects on BV-7310 as against 4 subjects on placebo. Poor treatment outcome was not seen in any patient on BV-7310 but was seen in 4 subjects on placebo.

CONCLUSION

Alcohol use disorders (AUD) and ALD are prevalent worldwide and increasing over time.[33] During the pandemic, there has been a further acceleration of

AUD and ALD numbers.[34] BV-7310 (Periban®) showed some improvement in clinical features of ALD as compared to placebo and significantly helped in reduction and normalization of the transaminases. This indicates a role of the product in improvement and reversal of hepatocellular inflammation and necrosis. These changes are observed despite continuation of use of alcohol during the course of therapy. BV-7310 aids reduction of elevated serum bilirubin levels to normal, thus playing a role in improving the detoxifying and excretory capabilities of the liver and facilitates correction of fatty deposits in hepatocytes, ascites and hepatomegaly. BV-7310 is safe for uninterrupted use for a prolonged period in the prescribed dose.

There is consistent improvement in parameters of alcoholic hepatitis in subjects in active group, even though some of the efficacy criteria may not be statistically significant in this study. The authors believe that being the first study of its kind, with a new formulation, the subjects were under-dosed in this study; and doubling the dose may show statistically significant improvement in the remaining parameters as well. Alcoholic hepatitis mimics the changes in liver structure and function seen in other chemically-induced liver damage such as with prescription medicines, e.g., antituberculosis drugs, cancer chemotherapy and biologic response modifiers; and with recreational drug abuse. Based on this clinical study, it is anticipated that BV-7310 may have a meaningful efficacy in these conditions as well.

Hence, BV-7310 (Periban®) shows promise as a safe and effective oral hepatoprotective and therapeutic agent for use in subjects of ALD. Further studies are warranted for use of BV-7310 (Periban®) both in ALD as well as other liver conditions.

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