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# Effect of Liverubin™ on hepatic biochemical profile in patients of alcoholic liver disease: a retrospective study

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**Liverubin™ is an available drug in the Indian market that contains silymarin, the major active complex extracted from the medicinal plant milk thistle (*Silybum marianum* L.). The study retrospectively tracked and analyzed the data of 602 patients, out of which 230 were alcohol induced; 131 with alcohol-induced liver damage (ALD), 13 with liver cirrhosis, and 86 with fatty liver; to assess the effects of water soluble Silymarin (Liverubin™) on important hepatic biochemical parameters. The data was collected from 32 major cities treated by 72 physicians across India who were observed for the specified treatment duration of 11 months. Data was analyzed by using descriptive statistics. At the end of the treatment the hepatic biochemical profile was appreciably improved: the mean % of change in the levels of important hepatic biochemical parameters was observed as follows: total bilirubin 63.48% (direct bilirubin: 64.96%; indirect bilirubin: 61.63%). The serum SGOT and SGPT changed at a mean % of 65.43 and 69.31 respectively while serum alkaline phosphatase was changed at a mean % rate of 39.81. Liverubin™ proved to be safe & well-tolerated among the studied population and no significant treatment related adverse events were reported during the study. Liverubin™ treatment is found to bring about effective lowering of abnormally elevated hepatic biochemical parameters. Liverubin™, water soluble active Silymarin, in the popularly prescribed doses of 140-mg tid is observed to be a promising safe and effective drug in cases of alcoholic liver disease.**

**KEY WORDS:** Liver cirrhosis - Enzymes - Alcohol-induced disorders.

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Since the ancient Greek period, alcohol consumption has been known to be a cause of liver damage, and it is currently well-established in the social fabric of many adult populations causing liver disease. In the USA, alcohol induced liver cirrhosis is the fourth most common cause of death in the age groups 25-64.[1] In India, the number of persons dying from alcohol induced liver cirrhosis has increased over the last three years. Official statistics reveal that the number has jumped by more than 35% in just one year. From 183 deaths in 2008, the

casualties went up to 253 in 2009. In 2007, the number of deaths from liver cirrhosis was 168.[2] Alcohol consumption may also make a significant contribution to cardiovascular-related mortality.

The liver plays an important role in carbohydrate, protein, and fat metabolism, detoxification and synthesis of bile. Alcohol is metabolized in the liver into acetaldehyde (phase I) and acetic acid (phase II). Chronic consumption of alcohol results in a more rapid conversion of ethanol to acetaldehyde and acetaldehyde produces toxic effects on the liver cells by stopping the rate of Phase II metabolism. This causes an accumulation of acetaldehyde leading to the formation of firm adducts with cell protein, resulting in cellular death. When alcohol damages the liver, even after 75% of damage, it continues to function as normal, as the liver has a tremendous capacity to regenerate. Abnormal elevation of liver enzymes in the blood may be suggestive of inflammation (hepatitis) or hepatocellular injury due to alcohol abuse.[3]

An increasing number of people are drawing towards alternative, traditional or complementary medicines and other natural supplements primarily owing to problems of limited treatment options, adverse effects and cost, with the modern allopathy.

Treatment strategies for alcohol-induced liver damage (ALD) include lifestyle changes to reduce alcohol consumption, smoking and obesity; nutrition therapy; pharmacological therapy and possibly as the last resort – liver transplantation. Although ALD remains a major cause of morbidity and mortality in the United States, there is no FDA approved therapy for either alcoholic cirrhosis or alcoholic hepatitis. Some of the available popular for the treatment of ALD are *Curcuma. xanthorrhiza*, colchicines, pentoxifylline (PTX), corticosteroids, silymarin along with other complementary, alternative or traditional medicines.[4, 5]

Silymarin is an active complex extracted from the *Silybum marianum* L. Pharmacologic evidence from numerous studies supports potential therapeutic benefits of milk thistle (*Silybum marianum L*) and silymarin in hepatocyte proliferation.[6] Since the 1960s, milk thistle products have been prescribed by physicians for ALD as well as other liver-related conditions.[7-10]

Proper diagnosis and management of the complications of ALD are vital to decreasing the deterioration of the illness, improving quality of life, and possibly decreasing mortality.[4] Among the most sensitive and widely used liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are normally contained within liver cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the enzyme levels in the blood and signaling liver disease. In addition to AST and ALT, alkaline phosphatase, 5' nucleotidase, and gamma-glutamyl transpeptidase (GGT) are other enzymes located in the liver.[10]

The aim of the current study was to evaluate the effects of Liverubin™ (silymarin; a natural drug) on important hepatic biochemical parameters in Indian patients with ALD.

## Materials and methods

Liverubin™ is an available drug in the Indian market that contains Silymarin, the major active complex extracted from the medicinal plant milk thistle (*Silybum marianum* L.). Milk thistle is a popular herb traditionally in use for a variety of liver disorders. In Liverubin™, the active complex silymarin is claimed to be made water soluble by its manufacturers using the patented NDDS technology, resulting in its high bioavailability and commonly prescribed by physicians across India in liver disorders including the well-diagnosed cases of ALD. The active formulation of Liverubin is further claimed to contain highly water soluble silybins which are easy to dissolve and swallow to achieve better patient compliance (Figure 1).

The study retrospectively tracked the data to analyze the effects of water soluble silymarin (Liverubin™) on important

hepatic biochemical parameters in patients with liver disease. The data comprised 602 subjects, out of which 230 were alcohol induced (131 with ALD, 13 with liver cirrhosis, and 86 with fatty liver) from 32 major cities treated by 72 physicians across India, and who were observed for the specified treatment duration of 11 months (Tables I, II). Major inclusion criteria were as follows: adult Indian patients aged from 18 to 72 years; diagnosed with any liver disease such as fatty liver, hepatitis and/or liver cirrhosis; other than having undergone liver transplantation; with biochemical parameters altered twice the normal values. Exclusion criteria included age groups below 18 years and above 72 years of age; known cases of allergy or hypersensitivity to milk thistle, silymarin or its analogues/derivatives; patients taking metronidazole; pregnant and lactating women and patients unwilling to comply.

In majority of the patients, water-soluble Liverubin™ tablets containing 140mg active silymarin, was prescribed three times daily with a glassful of water. However, based on the physician's discretion the drug was also prescribed in the doses of 2, 4 or 5 tablets a day in a few patients.

Patients had taken the following biochemical tests and submitted the results to the physician before and after therapy. The SGOT and SGPT were estimated by UV kinetic method in which both were analyzed based on the enzyme coupled system.[12] The estimation of ALP involves hydrolysis of p-nitro phenyl phosphate by alkaline phosphatase to give p-nitro phenol, which gives yellow color in alkaline solution. Increase in the absorbance is directly proportional to the activity of ALP.[13] Estimation of total bilurubin involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm.[14] All the estimations were carried out by using standard kits.

### *Statistical analysis*

Statistical analyses of the observed and reported data were descriptive, and usually limited to mean±standard deviation (SD). The data were analyzed using the SPSS 16.0 software.

### *Observations*

This was a retrospective study. The data of 602 patients suffering liver diseases was collected from various hospitals across India. Tables III, IV summarize the relevant patients' demography characteristics and medical history respectively. Majority of the patients were male (77.57%) with a mean age of 41.64±11.96 years. Females accounted for 22.43% of the total population. The majority of the patients (411: 68.27%) were administered with 3 tabs/day. The mean duration of the treatment was 20.54+7.85 days.

In the biochemical profile illustrated in Table V, at the baseline the mean total Bilirubin was 6.58±4.23, the mean direct Bilirubin was 3.91±2.04, and the mean indirect Bilirubin was 2.69±2.35. The mean SGOT and SGPT levels were 396.98±224.46 and 148±124.36 respectively. The mean alkaline phosphatase levels were 247.01±79.58. At the end of the treatment the hepatic biochemical profile were appreciably improved as summarized below: the mean total bilirubin declined to 2.44 ± 2.04, where the mean direct bilirubin reduced to 1.40±1.27 while the mean indirect Bilirubin came down to 1.05±0.80. After the treatment, the mean SGOT and SGPT levels were found to be 131.09±71.04 and 56±46.18 respectively. The mean alkaline phosphatase was recorded as 154.84±60.65 (Table II).

As shown in the Figure 2, after the specified treatment duration the mean % of change in the levels of important hepatic biochemical parameters was observed as follows: total bilirubin 63.48% (direct bilirubin: 64.96%; indirect bilirubin: 61.63%). The serum SGOT and SGPT changed at a mean % of 65.43 and 69.31 respectively while serum alkaline phosphatase was changed at a mean % rate of 39.81.

It is also interesting to note that with Liverubin™ therapy, the serum total bilirubin reduced to its normal limits in 36 patients. The average duration of this improvement

TABLE I.—*Patient disposition across the cities of India.*

| City HQ | Frequency | Percent |
|---|---|---|
| Ahmedabad | 53 | 8.8 |
| Ahmednagar | 10 | 1.7 |
| Ambala | 18 | 3 |
| Amritsar | 20 | 3.3 |
| Baroda | 10 | 1.7 |
| Bidar | 19 | 3.2 |
| Burdwan | 10 | 1.7 |
| Chandigarh | 15 | 2.5 |
| Chandrapur | 12 | 2 |
| Delhi | 58 | 9.6 |
| East Delhi | 15 | 2.5 |
| Guwahati | 19 | 3.2 |
| Hisar | 10 | 1.7 |
| Hissar | 10 | 1.7 |
| Hyderabad | 6 | 1 |
| Jalandhar | 20 | 3.3 |
| Jalgaon | 20 | 3.3 |
| Kolkata | 10 | 1.7 |
| Mumbai | 5 | 0.8 |
| Mysore | 17 | 2.8 |
| Nadiad | 10 | 1.7 |
| Nanded | 10 | 1.7 |
| North Delhi | 25 | 4.2 |
| Panajim | 12 | 2 |
| Patna | 43 | 7.1 |
| Pune | 20 | 3.3 |
| Sangli | 10 | 1.7 |
| Siliguri | 30 | 5 |
| Solapur | 10 | 1.7 |
| Suraat | 16 | 2.7 |
| Valsad | 39 | 6.5 |
| Vijayawada | 20 | 3.3 |
| Total | 602 | 100 |

was found to be 17 days, where the minimum and maximum time as recorded was 9 days and 32 days respectively. Similarly, the serum SGOT was corrected to its normal values among 40 patients within an impressive average treatment period of 18 days. The maximum and minimum time taken was 35 days and 9 days respectively. With Liverubin™ treatment 39 patients were able to reduce their serum SGPT level to absolute normal range within an average span of 18 days. The recorded minimum time being 9 days, while the maximum was 35 days. Remarkably another important hepatic enzyme serum alkaline phosphatase also declined to its normal values with an average duration of 18 days where the minimum and maximum time taken to achieve this improvement was 13 days and 35 days respectively.

TABLE II.—*Patient disposition across the states of India.*

| State | Frequency | Percent |
|---|---|---|
| AP | 26 | 4.3 |
| Assam | 19 | 3.2 |
| Bihar | 43 | 7.1 |
| Delhi | 98 | 16.3 |
| Gujarat | 128 | 21.3 |
| Karnataka | 36 | 6 |
| Maharashtra | 104 | 17.3 |
| Mumbai | 5 | 0.8 |
| Northeast | 20 | 3.3 |
| NorthEast | 30 | 5 |
| Punjab | 93 | 15.4 |
| Total | 602 | 100 |

TABLE III.—*Demographic characteristics of patients.*

| Demography (N.=602) | |
|---|---|
| | N. (%) |
| Age (years) | |
| Mean (SD) | 41.64 (+11.96) |
| Median | 41 |
| Min | 18 |
| Max | 90 |
| Sex | |
| Male | 467 (77.57) |
| Female | 135 (22.43) |
| Dosage | |
| 2 tabs/day | 9 (1.50) |
| 3 tabs/day | 411 (68.27) |
| 4 tabs/day | 21 (3.49) |
| 5 tabs/day | 2 (3.33) |
| Duration (days) | |
| Mean (SD) | 20.54 (+7.85) |
| Min | 2 |
| Max | 54 |
| Median | 18 |

TABLE IV.—*Medical history of patients.*

| Indication | Frequency | Percent |
|---|---|---|
| Alcohol induced | 230 | 38.20 |
| Alcohol induced liver damage | 131 | 21.76 |
| Fatty liver | 86 | 14.28 |
| Cirrhosis | 13 | 2.2 |
| Diabetes | 8 | 1.3 |
| Drug Induced liver damage | 30 | 5 |
| Jaundice/hepatitis | 169 | 28.07 |
| Liver fibrosis | 5 | 0.8 |
| Non alcoholic fatty liver disease | 22 | 3.65 |
| Viral hepatitis | 127 | 21.1 |
| Other | 7 | 1.16 |

Liverubin™ proved to be safe and well-tolerated among the studied population and no significant treatment-related adverse events were reported during the study.

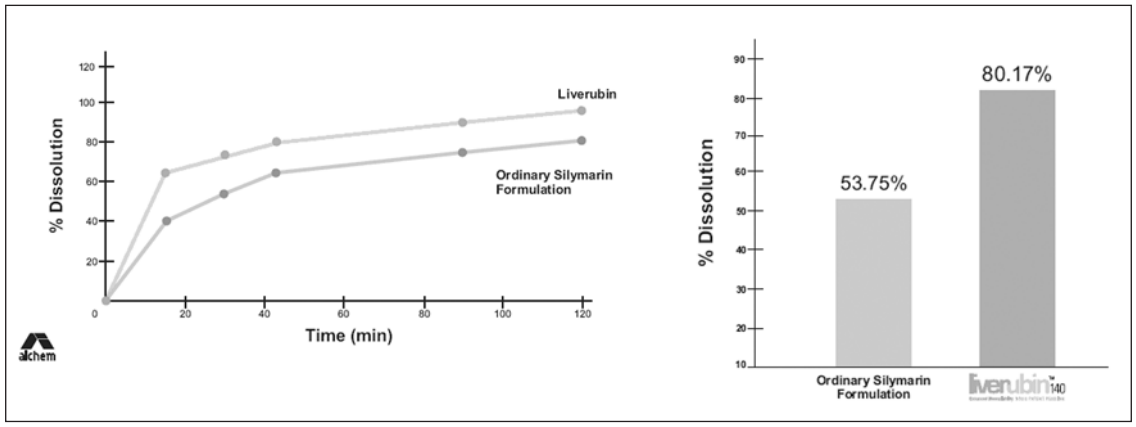


Figure 1.—Solubility of Liverubin™ formulation.

All important hematological, urinary and biochemical assays for renal function, serum lipids, and electrolytes were found to be within their respective normal limits during and after the treatment.

## Discussion

The current study of Liverubin™ may be well acknowledged as one of the most significant observations of its kind in Indian patients with ALD, in terms of both the number and geographic diversity of studied subjects. The present retrospective study focuses on the results as observed in 602 Indian patients suffering with liver disease. The study is therefore a momentous progress in this area of research on plant-derived drugs.

From the results (Table I), the baseline results reported here characterized a predominantly male patient population (467: 77.57%). By and large, the majority of patients with ALD are male; daily consumption of alcohol exceeding 40-80/day for a long duration can most likely cause ALD.[15-17] Administration of alcohol by healthy subjects as well as those with alcoholic fatty liver, results in mild elevation of the serum hepatic biochemical assay and alcoholic hyperlipemia.[18] Our study shows that the important hepatic biochemical parameters were considerably raised in ALD patients prior to the treatment. The findings, therefore, are concurrent with the earlier reports.[19] Liverubin™ therapy was observed to induce progressive and statistically significant improvements in the hepatic biochemical profile (Figure 1) among the studied population, within a mean duration of 20.54+7.85 days.

Liverubin™ as a single therapy may elicit a significant hepatocellular regenerative property with the added advantage of almost no toxicity, even when taken in large doses (as high as 700 mg / day) regularly for considerable duration (up to 8 weeks) (Table I).

Excessive alcohol would impair carbohydrate and lipid metabolism, causing diversion of metabolism to ketogenesis and fatty acid synthesis.[20] It is also associated with the enzymatic induction of Cytochrome P450 2E1 (CYP2E1) pathway of alcohol metabolism which perpetuates liver damage.[21, 22] After Liverubin™ treatment, the bilirubin levels (total, direct and indirect) were decreased significantly and the % change in the levels was considerable (Figure 1). In the current study, the serum alkaline phosphatase reduced by a mean 39.81% after treatment with Liverubin™.

In the last few decades, pharmacologic evidence from numerous studies has supported the potential therapeutic benefits of silymarin in liver necroinflammation and fibrogenesis properties, as well as its self-styled ability to selectively stimulate hepatocyte proliferation.[23] The results of the current study showed phenomenally decreased

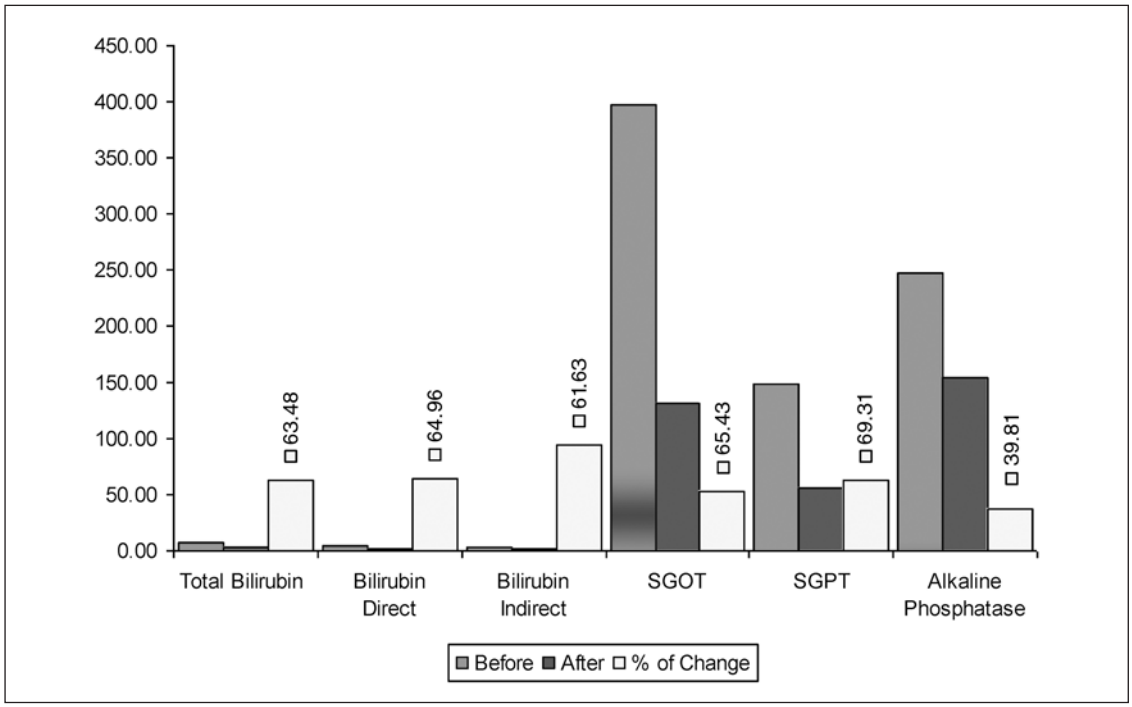


Figure 2.—Percentage of change in the level of liver enzymes: before and after treatment with Liverubin™ (N.=230).

TABLE V.—*Change in the level of liver enzymes after treatment with Liverubin™ (N.=230).*

| Liver enzyme | Before (Mean+SD) | After (Mean+SD) | % of change |
|---|---|---|---|
| Total bilirubin | 6.58 (4.23) | 2.44 (2.04) | 63.48 |
| Bilirubin direct | 3.91 (2.04) | 1.40 (1.27) | 64.96 |
| Bilirubin indirect | 2.69 (2.35) | 1.05(0.80) | 61.63 |
| SGOT | 396.98 (224.46) | 131.09 (71.04) | 65.43 |
| SGPT | 148±124.36 | 56 (46.18) | 69.31 |
| Alkaline phosphatase | 247.01±79.58 | 154.84 (60.65) | 39.81 |

levels of SGOT and SGPT (65.43% and 69.31%) after the treatment with Liverubin. Another published meta-analysis comprised five trials encompassing a total of 602 patients with liver cirrhosis and reported hepatic transaminase levels which were consistently lower in silymarin-treated groups.[6] Plasma levels of SGOT and hepatic total triglycerides were all significantly lower in the silymarin group, thus providing indirect evidence that silymarin is hepatoprotective and may potentially retard disease progression in ALD patients.[23] A double-blind controlled study by Salmi and Sarna [24] showed a highly significant decrease in ALT and AST levels with silymarin when compared with placebo. In a Hungarian study of 36 patients with chronic ALD, a dose of 420 mg/day of silymarin resulted in normalization of serum transaminases (AST, ALT and, GT), total bilirubin and an improvement in the histological examination of liver biopsies after 6 months of treatment.[25] Animal studies [26-29] and clinical trials [6, 23, 20-34] suggest that silymarin can be useful in the management of early or progressive liver damage when given for variable periods from 3-6 months. Human studies have shown that silymarin is generally nontoxic and without side effects when administered to adults in a dose range of 240-900 mg/day in two or three divided doses.[30]

The previous data on silymarin in ALD along with current evidence on the potential benefit of therapies in ALD are certainly encouraging to further plan comprehensive randomized, controlled clinical studies on silymarin drugs in well diagnosed cases and pathological variants ALD.[34] By and large, comparison with previous clinical studies on ALD, the results obtained from the current study are equivocal with regard to the potential clinical benefit of silymarin as preventive therapy in chronic alcohol use or ALD.

## Conclusions

Liverubin™ treatment is found to bring about effective lowering of abnormally-elevated hepatic biochemical parameters. Liverubin™, water soluble active Silymarin, in the popularly prescribed doses of 140-mg three times daily is observed to be a promising safe and effective drug in cases of alcoholic liver disease. It is therefore recommended that well-designed randomized; blinded clinical studies, comprising both modern allopathic drugs as well as other available silymarin drugs as controls, should be undertaken to further evaluate and establish the benefits and safety of high-bioavailability water-soluble silymarin formulations like Liverubin™ and also comprehend their advantage over the contemporary therapies.

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