PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci.com. Email: pacjmedsci@gmail.com.

EFFICACY AND TOLERABILITY OF FIXED DOSE COMBINATION OF SILYMARIN, ALPHA LIPOIC ACID, N-ACETYL CYSTEINE AND SELENIUM IN THE MANAGEMENT OF SOME LIVER DISORDERS

*^ARIF A. FARUQUI, **SHASHIKANT PAWAR, ***SANJOY DASGUPTA

*Clinical Pharmacologist, Mumbai Maharashtra, India; **Pawar Nursing Home, Satara, Maharashtra, India

***Gangulibagan, Jadavpur, Kolkata-700047, West Bengal, India

^Corresponding Author: drfaruqui@gmail.com

EFFICACY AND TOLERABILITY OF FIXED DOSE COMBINATION OF SILYMARIN, ALPHA LIPOIC ACID, N-ACETYL CYSTEINE AND SELENIUM IN THE MANAGEMENT OF SOME LIVER DISORDERS

***^ARIF A. FARUQUI, **SHASHIKANT PAWAR, ***SANJOY DASGUPTA**

*Clinical Pharmacologist, Mumbai Maharashtra, India; **Pawar Nursing Home, Satara, Maharashtra, India
***Gangulibagan, Jadavpur, Kolkata-700047, West Bengal, India

^Corresponding Author: drfaruqui@gmail.com

ABSTRACT:
Globally liver disorders are major cause of illness death and death. Oxidative stress plays a critical role in the progression of alcoholic and nonalcoholic related diseases. The aim of the present study was to evaluate the efficacy and tolerability of fixed dose combination (FDC) of silymarin, alpha lipoic acid, N-acetyl cysteine and selenium in the management of liver disorders. This was an observational, non-randomized, open label, non-comparative, multi-centric post-marketing surveillance study. The above mentioned FDC was administered to 15 patients diagnosed with alcoholic or viral hepatitis for three months. Evaluation of liver function tests (LFT) were carried out at baseline and at the end of 3rd month of the treatment. Significant changes were observed in the LFT parameters at the end of three months of this study. aspartate aminotransferase (AST): (Mean ± SEM) 369.9 ± 128.0 to 97.00 ± 34.27 U/L, ($p < 0.0001$); alanine aminotransferase (ALT): 652.93 ± 214.57 to 194.40 ± 82.51 U/L, ($p < 0.03$); Alkaline phosphatase: 197.47 ± 25.57 to 151.60 ± 17.92 U/L, ($p < 0.0059$); Gamma glutamyl transferase: 156.67 ± 49.80 to 87.33 ± 22.94 U/L, ($p < 0.0490$); Total bilirubin: 3.44 ± 0.76 to 1.66 ± 0.57 mg/dL, ($p < 0.0192$) and bilirubin direct: 2.13 ± 0.58 to 1.00 ± 0.50 mg/dL, ($p < 0.0273$). Two patients reported mild gastrointestinal adverse events (nausea, bloating). This FDC was therapeutically effective under the circumstances of elevated oxidative stress and produces significant reduction in LFT parameters in alcoholic and viral hepatitis patients.

Keywords: Alcoholic liver disease, Oxidative Stress, Silymarin, Alpha lipoic acid, N-acetyl cysteine, Selenium
Submitted November 2015, Accepted December 2015

INTRODUCTION:

Liver disorders are the major cause of illness and death worldwide [1]. Although liver disease is stereotypically linked to alcohol or drugs, the truth is that there are more than 100 known forms of liver disease caused by various factors and affecting all age groups [2]. The major factors thus include viruses, alcohol, obesity and drugs. Overall half of global population is exposed to different forms of hepatotrophic viruses, suggesting viral hepatitis as a commonest cause of acute and chronic liver disorders [3]. In India, 40 million people are infected with hepatitis B,

constituting approximately 11 per cent of the estimated global burden [4].

Alcoholic liver disease, another major liver disorder involves liver damage due to alcohol abuse and usually occurs after several years of excessive alcohol consumption. Pathological steps in deterioration of liver function involve steatosis, steato-hepatitis to fibrosis and cirrhosis [5]. Furthermore in liver cirrhosis, it was established that chronic alcohol consumption is a risk factor for the development of hepatocellular carcinoma [6]. Moreover in the past 30 years, deaths due to liver cirrhosis have steadily increased with values more than 1 million in the year 2010 making it 2% of all deaths in that year. In Asia, hepatitis B and hepatitis C accounts for more than half of the liver cirrhosis burden [7]. Liver disease related to ethanol is a common problem and is one of the major medical complications of alcohol abuse. Daily consumption is usual for causation of liver disease. The World Health Organization (WHO) estimates that 140 million people worldwide suffer from alcohol dependency that produces damage to lives and economies [8].

Oxidative stress is defined as "a disturbance in the pro-oxidant-antioxidant balance in favor of the former". Oxidative stress plays a critical role in the progression of alcohol related liver disease [9]. Alcohol and its immediate metabolite acetaldehyde increases liver oxidative stress through generation of highly reactive oxygen species (ROS), reactive nitrogen species (RNS) and adducts that can injure the hepatocytes and liver parenchyma [10]. Apart from generating these free radicals, chronic alcohol ingestion significantly affects hepatocytes by depleting important components of antioxidant defense system (enzymatic and non-enzymatic). This imbalance created by alcohol mediated elevated generation of pro-oxidants as well as depletion in the enzymatic and non-enzymatic antioxidant defense systems in liver may contribute to the progression and development of alcoholic liver disease [11].

Prevention of ROS mediated damage is accomplished with the help of enzymatic and nonenzymatic processes. Glutathione peroxidase, Catalase and Superoxide dismutase (SOD) are supposed to be the primary antioxidant enzymes, as their involvement leads to direct elimination of ROS. Glutathione peroxidase, a selenoprotein, is a cytoplasmic and mitochondrial enzyme, crucial for detoxification of hydrogen peroxide in almost all the cells. Glutathione peroxidase contains a seleno-cysteine amino acid at the active site instead of a normal cysteine. Another important component of antioxidant network is Reduced Glutathione (GSH) which protects the human body from free radicals. Glutathione exists in the metabolic system as either in Reduced (GSH) or oxidized (GSSG) states. In reduced state, glutathione can donate an electron and stabilize a free radical. During this donation the glutathione becomes highly reactive and reacts with another glutathione molecule to form oxidised glutathione (GSSG). The GSSG is then converted to Reduced Glutathione (GSH) by the enzyme glutathione reductase [11].

Critical biochemical manifestation of alcoholic and nonalcoholic liver diseases is thus sustained increased oxidative stress. Alcohol, its metabolites and viral infections tip this balance in favour of oxidative stress. It is therefore, worthwhile to estimate the extent of oxidative stress and the front line component of antioxidant defense system in these patients. Current research plays an important role in recognizing the role of oxidative stress with respect to disease severity to build up the antioxidant defense system in the supportive management of oxidative stress induced liver disorders. Administration of antioxidant is therefore a vital therapeutic strategy and advocates that routine screening of antioxidant levels should be done to find out the deficient status [11].

Medicinal agents with antioxidant potential include not only vitamin A, C, and E, but also complementary and alternative medicine agents such as silymarin, S-adenosylmethionine, alpha lipoic acid, N-acetyl cysteine (NAC) and trace elements, such as zinc and selenium. Although most of these agents have numerous effects, such as free radical scavenging, membrane-stabilizing and anti-inflammatory properties, most experimental data reinforces ability of these agents in lowering oxidative stress as a fundamental of their beneficial effect [12]. In India and across the world, patients with chronic liver disorders are known to use these complementary and alternative medications; one study in the United States of America revealed that 39% of patients in their study used these agents, and another such study in Germany reported 65% of patients. This number probably underestimates usage, because many surveys show that 31%-40% of patients do not disclose their use of above mentioned agents to their physicians [12].

Silymarin, conventionally known as 'milk thistle' is one of the oldest and intensively researched medicinal plant in the treatment of liver diseases. Traditionally, milk thistle has been used for alcoholic hepatitis, alcoholic fatty liver, liver poisoning, ischemic injury, liver cirrhosis and viral hepatitis. Silymarin has been proclaimed to safeguard the hepatocytes from wide range of toxins, including ethanol, acetaminophen, carbon tetrachloride, arsenic and radiation [13]. N-acetyl-cysteine (NAC) is another vital antioxidant and is quite popular for its capacity to minimize and downstream the negative effects associated with oxidative stress. NAC is largely acknowledged to minimize the lipid peroxidation of cellular membranes and other such targets that is known to occur with oxidative stress [14]. Alpha-lipoic acid is manufactured in almost all tissues and is competent in solubilising in both water and fats thus can be useful throughout the body. Physiologically as an antioxidant, alpha-lipoic acid directly terminates free radicals, chelates metal ions, increases levels of cytosolic glutathione and vitamin C [11].

Selenium is a non-metal that exists in several oxidation states and is an essential component of the glutathione peroxidase enzyme system with a significant function of protecting the cell from the oxidative stress and free radical formation. The "rate limiting" substrate in the GSH-GSSG oxido-reduction is selenium and its deficiency affects

the generation of peroxidase enzyme leading to deterioration of the antioxidant protection by severely reducing the GSH-GSSG levels. Selenium-deficiency has been also shown to result in less robust immune responses to viruses, tumors, and allergens, in comparison to selenium-adequate control population. Selenium deficiency has been implicated as a cause of hepatic injury, possibly from accentuated lipoperoxidation due to diminished activity of the selenoenzyme, glutathione peroxidase, despite the absence of severe malnutrition [15]. Clinical studies have also confirmed that, in patients with alcoholic and nonalcoholic liver diseases, hepatic selenium levels are reduced compared to healthy population, suggesting the positive role of selenium supplementation in liver disorders [16]. Selenium is a potent nutritional antioxidant that carries out biological effects through its incorporation into seleno-proteins. Selenium is an active immune-modulator, much more potent anti-oxidant than vitamins E, C and A, beta-carotene [17, 18].

In light of the vital role of ROS and oxidative stress in the pathophysiology of liver diseases, antioxidants are understandably considered as a crucial therapeutic approach for the management of liver disorders [19]. Therefore, this study was designed to evaluate the efficacy and tolerability of fixed dose combination (FDC) of silymarin, alpha lipoic acid, N-acetyl cysteine and selenium in the management of patients suffering from alcoholic and nonalcoholic liver disorders.

METHODS:

This was an observational, non-randomized, open label, non-comparative, multi-centric post-marketing surveillance study. The FDC of silymarin, alpha lipoic acid, N-acetyl cysteine and selenium was orally administered as twice daily to the patients suffering from liver disorders including alcoholic and viral hepatitis for at least 3 months. Clinical diagnosis was performed based on patients clinical and biochemical investigations conducted at baseline. Biochemical investigations were carried out at Super Religare Laboratories (SRL) diagnostics across all study centers in India.

The investigational product i.e. Livrite marketed by Medley Pharmaceuticals Ltd, Mumbai and approved by regulatory authorities. Informed consent was obtained from the patients and the post marketing surveillance was done in accordance with the clinical principles laid down in declaration of Helsinki [20].

A total 15 patients with alcoholic (09 patients) or viral hepatitis (06 patients) were enrolled from 13 hepatology clinics in India. At the time of entry into the study, base-line demographics, clinical history, physical examination and biochemical evaluation were carried out. Patients were monitored for clinical signs and symptoms the end of 1st month of treatment, then subsequently 2nd and 3rd month of the treatment. Due to the ease and economic reasons of the patients, evaluation of liver function tests (LFT) was carried out at baseline and at the end of 3rd month of the treatment.

Inclusion Criteria: Both male and female patients over 15 years of age were included in this study.

Exclusion criteria: Patients with chronic active viral hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, liver cirrhosis, hepatocellular carcinoma were excluded from this trial. Patients were excluded if they had evidence of decompensated hepatic cirrhosis, a positive HIV antibody test result or positive result for HBsAg (surface antigen of the hepatitis B virus), or had used milk thistle products within the previous 30 days. Liver biopsy was not required for entry, although if obtained, the presence of moderate steatosis or steato-hepatitis were considered exclusions.

Intervention: Patients were treated with Livrite tablets (FDC of silymarin 210 mg, alpha lipoic acid 200 mg, N acetyl cysteine 200 mg and selenium 100 mcg) orally twice daily for three months.

Efficacy and Tolerability Evaluations: Primary efficacy variables includes change in LFT parameters such as, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), total bilirubin and bilirubin direct,total proteins & albumin. Safety outcomes included adverse events, which were recorded prospectively throughout the study. The patients were interviewed and asked for any type of adverse events throughout the study.

Statistical Analysis: The statistical analysis of LFT was carried out by using graph pad prism 5. Comparison between the baseline values and the values after 3rd month of treatment were made by paired T test. Values of $p < 0.05$ were considered statistically significant.

RESULTS:

A total of 15 patients made of 14 males and one female were enrolled and all of them completed the study. The age range of the patients was 15 to 50 years.

Aspartate aminotransferase (AST): Treatment with Livrite resulted in significant reduction in AST from baseline to the end of 3rd month and this difference was observed to be statistically significant (Figure 1). (369.9 ± 128.0 to 97.00 ± 34.27U/L; $p < 0.0001$);

Alanine aminotransferase (ALT): Treatment with Livrite resulted in significant reduction in ALT from baseline to the end of 3rd month and this difference was observed to be statistically significant (Figure 2). (652.93 ± 214.57 to 194.40 ± 82.51U/L; $p < 0.03$);

Alkaline Phosphatase (ALP): Treatment with Livrite resulted in significant reduction in ALP from baseline to the end of 3rd month and this difference was observed to be statistically significant (Figure 3). (197.47 ± 25.57 to 151.60 ± 17.92U/L; $p < 0.0059$);

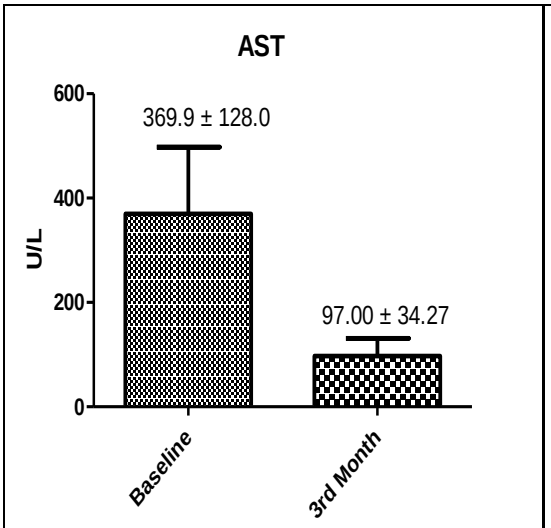

Figure 1: Change in AST from baseline

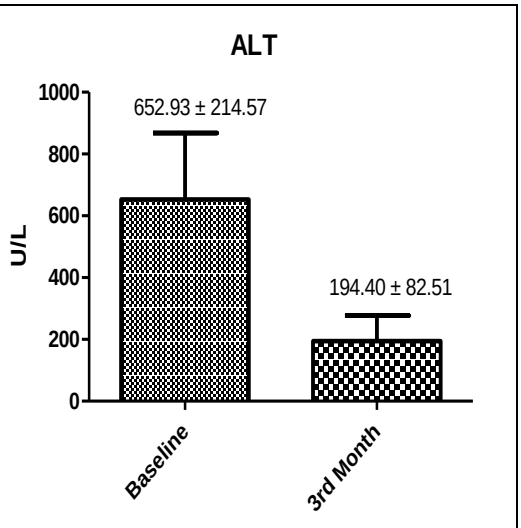

Figure 2: Change in ALT from baseline

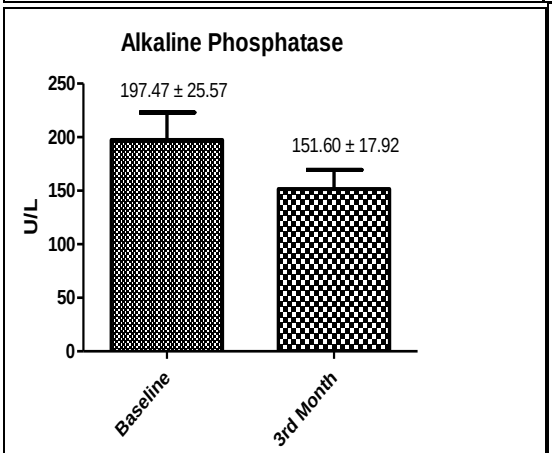

Figure 3: Change in Alkaline phosphatase from baseline

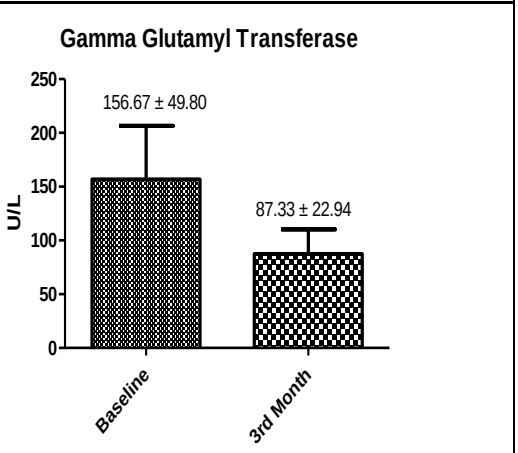

Figure 4: Change in G Glutamyl Transferase from baseline

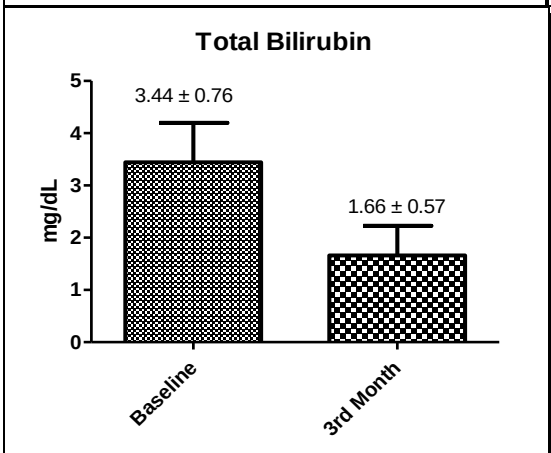

Figure 5: Change in total bilirubin from baseline

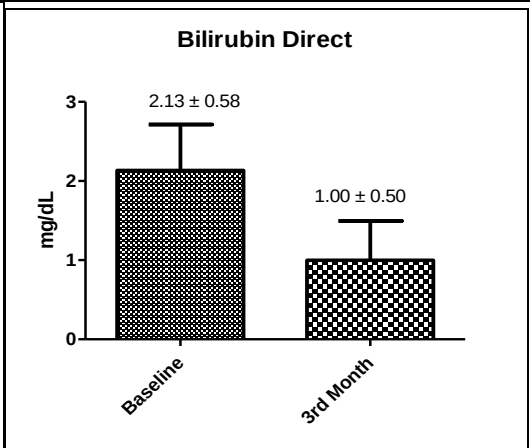

Figure 6: Change in Bilirubin direct from baseline

Gamma Glutamyl Transferase: Treatment with Livrite resulted in significant reduction in GGT from baseline to the end of 3rd month and this difference was observed to be statistically significant (Figure 4). (156.67 ± 49.80 to 87.33 ± 22.94U/L; $p < 0.0490$);

Total Bilirubin: Treatment with Livrite resulted in significant reduction in total bilirubin values from baseline to the end of 3 months; and this difference was observed to be statistically significant (Figure 5). (3.44 ± 0.76 to 1.66 ± 0.57 mg/dL; $p < 0.0192$)

Bilirubin Direct: Treatment with Livrite resulted in significant reduction in bilirubin direct values from baseline to the end of 3 months; and this difference was observed to be statistically significant (Figure 6). (2.13 ± 0.58 to 1.00 ± 0.50 mg/dL; $p < 0.0273$);

Total Protein & Albumin: The effect of Livrite on LFT parameters such as total protein and albumin was not significant. Total protein (baseline-7.29±0.54; after 3 months-7.32±0.67) g/dL Albumin: (at baseline 3.83±0.76 Vs. after 3 months: 4.11±1.03) g/dL.

Safety Evaluation: The patients were interviewed during each visit and at the end of the study for the presence of any adverse events. Adverse events were reported in 2 patients and clinicians recorded the severity of adverse events as of mild. The reported adverse events were bloating, dyspepsia, nausea and diarrhoea. None of the patient had history of allergy or hypersensitivity to any component of Livrite. However, as per clinicians decision, treatment with Livrite was neither withheld nor stopped, it was continued throughout the course of the trial, and adverse events were resolved completely.

Evaluation of Global efficacy and tolerability: As per clinicians assessment about efficacy and tolerability of FDC of silymarin, alpha lipoic acid, N-acetyl cysteine and selenium (Livrite), all patients tolerated the treatment well (except for 2 patients out of 15 who reported mild GI related adverse events) and were also benefitted in terms of improvement in LFT Parameters.

DISCUSSION:

Oxidative stress and associated damage represents the most common link between various forms of chronic liver injury and hepatic fibrosis. For example, oxidative stress leading to lipid peroxidation resulted due to oxidative stress is one of the crucial factors involved in the progression of nonalcoholic steatohepatitis (NASH) and liver cancer [21]. Lipid peroxidation is further triggered by viral infections or chronic alcohol abuse [21]. Alcohol abuse enhances the generation of ROS, decreases cellular antioxidant levels, and elevates oxidative stress in many tissues, prominently the liver. Primary mechanism by which ethanol produces hepatocyte injury is the oxidative stress induced by ethanol [22]. Since oxidative stress is a common pathogenetic mechanism contributing to progression of hepatic damage in inflammatory liver disorders, including acute and chronic hepatitis, antioxidants are understandably

considered as a crucial therapeutic approach for the management of these liver disorders.

Several natural compounds have the potential to scavenge the ROS molecule, thus reducing oxidative stress directly, or they may offer an indirect protection by activating endogenous defense systems [23]. Therefore, optimum doses of primary antioxidants should be the initial therapeutic strategy to restore and thereafter to maintain the serum and tissue concentrations of normal antioxidant values.

Silymarin is one of the oldest and intensively researched medicinal plants in the management of liver diseases [24]. Silymarin is a free radical scavenger that interacts directly with the cell membrane components to prevent any abnormalities in the content of lipid fraction responsible for maintaining normal fluidity. One of the mechanisms that can explain the capacity of silymarin to stimulate liver tissue regeneration is the increase in protein synthesis in the injured liver [24].

Silymarin's hepatoprotective action is due to the ability to increase the cellular content of GSH, regulation of membrane permeability and to increase membrane stability; stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration, enhanced glucoronidation and protection from glutathione depletion, thus producing immunomodulatory effects 24].

A double-blind controlled study by Salmi and Sarna evaluated the effect of silymarin in 106 patients with alcoholic liver disease [25]. All patients had elevated serum transaminase levels (ALT and AST) and 90 had confirmed histological diagnosis. The patients were randomly allocated to either silymarin or placebo group and the duration of the trial was 4 week. A highly significant decrease in ALT and AST levels was observed with silymarin when compared with placebo [25].

In a Hungarian study of 36 patients, with chronic alcoholic liver disease, a dose of 420 mg/day of silymarin resulted in normalization of serum transaminases (AST, ALT and GGT), total bilirubin and an improvement in the histological examination of liver biopsies after 6 months of treatment [26].

Pares et al studied the effect of silymarin in alcoholics with liver cirrhosis with respect to their survival, clinical and laboratory changes [27]. This randomized double blind multicenter study compared 450 mg/day silymarin in three divided doses (n=103) with placebo (n=97), enrolled 200 alcoholics with histologically or laparoscopically proven liver cirrhosis. The primary outcome was time to death and the secondary outcome was progression of liver failure. Survival was similar in patients receiving silymarin or placebo and was not influenced by the gender, the persistence of alcohol intake, the severity of liver dysfunction or by the presence of alcoholic hepatitis in the liver biopsy [27].

Another component of the FDC used in our study is NAC which is frequently utilized where intracellular oxidant-antioxidant balance is concerned. A recent study has reported a significant decrease in liver steatosis and fibrosis

in patients with NASH receiving Metformin and NAC [28]. M Khoshbaten et al [28] performed another study in 30 patients with non-alcoholic fatty liver disease (NAFLD) and randomly assigned to receive either N-acetylcysteine or vitamin C. It was observed that a three-month supplement of NAC improved ALT and the size of the spleen in patients with NAFLD [28].

Selenium is essential trace element and a component of glutathione peroxidase and other peroxidases. Selenium is integrated into selenoproteins that have a broad range of pleiotropic effects, ranging from antioxidant to anti-inflammatory effects. Higher selenium status or selenium supplementation has also shown to have antiviral effects [29]. Moreover, selenium is an active immunomodulator, much more potent anti-oxidant than vitamins A, C, E and beta-carotene [17, 18].

Unlike other types of antioxidants, alpha lipoic acid is soluble in both fat and water, which allows the protection of cells throughout the body (both lipid and water components). It recycles other constitutional antioxidants (vitamin C, vitamin E, and glutathione). Furthermore, important coenzyme for the production of acetyl coenzyme A (important in liver cell metabolism) is alpha lipoic acid. Alpha lipoic acid reduces the levels of ethanol induced metabolic breakdown products, and thus may be an effective treatment for alcohol induced hepatitis & early cirrhosis [11].

Berkson BM et al [30] reported that the triple antioxidant combination of alpha lipoic acid, silymarin and selenium for a conservative treatment of hepatitis C because these substances protect the liver from free radical damage, increase the levels of other fundamental antioxidants, and interfere with viral proliferation. The 3 patients presented in their research followed the triple antioxidant program and recovered quickly and their laboratory values remarkably improved. Furthermore, liver transplantation was avoided and the patients were back at work, carrying out their normal activities, and feeling healthy [30].

Hepatotoxicity is a serious adverse drug reaction in tuberculosis (TB) patients receiving anti-TB drugs (isoniazid, pyrazinamide and rifampicin) and is one of the most challenging clinical problems worldwide [31]. Studies have shown that anti-TB-drug induced hepatotoxicity is primarily due to oxidative stress, caused by the anti-TB drugs and its metabolites. According to Funde et al. [32] oxidative stress due to free radical generation and subsequent lipid peroxidation of membrane play a crucial role in the pathogenesis of drug induced liver injury. Results obtained in the present study are comparable to the previous studies [25-28, 30]. With the use of silymarin, N-acetyl cysteine, alpha lipoic acid and selenium, this study showed significant improvement in the LFT parameters namely, AST, ALT, ALP, GGT, total and direct bilirubin at the end of 3 months. Based on the result of this trial, it can be concluded that, Livrite (FDC of silymarin, alpha lipoic acid, n-acetyl cysteine and selenium) demonstrates a hepatoprotective effect and thus has therapeutic usefulness in alcoholic and non-alcoholic disorders that involves oxidative stress.

Furthermore, hepatoprotective properties of Livrite can also be utilized to reduce the hepatotoxic effects attributable to anti-TB chemotherapy and to enhance the patient adherence to the treatment.

Clinicians would like to point out the three limitations of this present study: (1) the study was designed in an open-label fashion with no comparator arm; (2) the three month study duration may not have been long enough to assess long-term results; and (3) the sample size was relatively small. However, the positive results of the present study justify the need of larger double blind studies.

CONCLUSION:

From this study it could be postulated that antioxidants would be therapeutically effective under circumstances of elevated oxidative stress as in case of alcoholic liver disease, hepatitis & NASH or in cases exposed to a stressor that generates exacerbated oxidative injury like anti tuberculosis drugs. The FDC of silymarin, alpha lipoic acid, N-acetyl cysteine and selenium (Livrite) significantly decreased the ALT, AST, ALP, GGT, total and direct bilirubin level after 3 months of treatment from the baseline which shows the plausible benefits of anti-oxidant therapy in the management of hepatic disorders.

ACKNOWLEDGEMENTS:

Authors acknowledge the support of all the following doctors for providing their observations. These are, Dr. K.K. Tiwari, Dr. Rajiv Hambarde, Dr. Dilip Gogoi, Dr. Ch Arunkumar, Dr. S.K. Gupta, Dr. Samir Gupta, Dr. A.T.M. Saddiqui, Dr. Avik Roy Choudhury and Dr. M. Sanwarwalla. Authors are thankful for the immense help received from Dr. Mahesh Baviskar M.D (Pathology) for evaluating and interpreting the pathology investigational reports. The authors are also grateful to Mr. Shailesh K. Pallewar (M.Pharm-Pharmacology) for providing the statistical analysis.

Conflict of Interest: All authors had access to the data and vouch for the veracity and completeness of the data and the data analysis. The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES:

1. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China Hepatology. 2014 Dec; 60 (6):2099-108
2. http://www.liver.ca/liver-disease/ Cited on November 2015
3. Premashis Kar. World Hepatitis Day-new challenges; Indian J Med Res. 2011 Jul; 134(1): 1–3.
4. Viral hepatitis eradication in India by 2080 - gaps, challenges & targets. Indian J Med Res 140, July 2014, pp 1-4
5. Salvador Manzo-Avalos and Alfredo Saavedra-Molina. Cellular and Mitochondrial Effects of Alcohol Consumption Int J Environ Res Public Health. 2010 Dec; 7(12): 4281–4304.
6. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology. 2004 Nov; 127(5 Suppl 1):S87-96.
7. Ali A Mokdad, Alan D Lopez, Saied Shahraz, Rafael Lozano, Ali H

Mokdad, Jeff Stanaway, Christopher JL Murray and Mohsen Naghavi. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis BMC Medicine 2014, 12:145

8. Sabyasachi Ray; Alcoholic Liver Disease (ALD), Chapter 71, Association of Physician of India, Medicine update 2007
9. B. Poljsak and I. Milisav. The Neglected Significance of "Antioxidative Stress". Oxid Med Cell Longev. 2012; 2012: 480895.
10. Halina Cichoż-Lach and Agata Michalak. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol. 2014 Jul 7; 20(25): 8082–8091
11. Ms. Mamta Singh, Dr. Seema Gupta, Dr. Rajesh Pandey, Dr. H.K. Aggarwal, Dr. S. K. Aggarwal. Oxidative Stress and Chronic Alcohol Liver Disease: the Current Perspectives. IAJPR.2014:4 (03) Page 1428-1446
12. Lu Sc. Antioxidants in the Treatment of Chronic Liver Diseases: Why Is the Efficacy Evidence So Weak in Humans? Hepatology. 2008 Nov;48(5):1359-61
13. Luminita Pilat, Ciprian Mihali, Hildegard Herman, Cristina Popescu, Violeta Turcus, Aurel Ardelean, Ardelean Gavril, Teodora Mariasiu, Calin Popa, Anca Hermenean. Pharmacology of Silybum Marianum and its Active Constituents. Therapeutic Activity – Part 1; Jurnal Medical Aradean (Arad Medical Journal) Vol. XIV, Issue 2, 2011, Pp. 25-33
14. Chad Kerksick and Darryn Willoughby. The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress; J Int Soc Sports Nutr. 2005; 2(2): 38–44
15. Dworkin B, Rosenthal WS, Jankowski RH, Gordon GG, Haldea D. Low blood selenium levels in alcoholics with and without advanced liver disease. Correlations with clinical and nutritional status Dig Dis Sci. 1985 Sep;30 (9):838-44
16. Thuluvath PJ, Triger DR. Selenium in chronic liver disease. J Hepatol. 1992 Mar;14(2-3):176-82
17. Baraboĭ VA, Shestakova EN Selenium: the biological role and antioxidant activity. Ukr Biokhim Zh 2004 Jan-Feb; 76 (1):23-32.
18. Ozardali I, Bitiren M, Karakilçik AZ, Zerin M, Aksoy N, Musa D. Effects of selenium on histopathological and enzymatic changes in experimental liver injury of rats. Exp Toxicol Pathol. 2004 Oct; 56 (1-2): 59-64
19. Halina Cichoż-Lach, Agata Michalak. Oxidative stress as a crucial factor in liver diseases. World J Gastroenterol 2014 July 7; 20(25): 8082-8091
20. World Medical Association. Declaration Of Helsinki Ethical Principles for Medical Research Involving Human Subjects: www.wma.net/en/30publications/10policies/b3/17c.pdf assessed on November 2015
21. Hye-Lin Ha, Hye-Jun Shin, Mark A Feitelson and Dae-Yeul Yu. Oxidative stress and antioxidants in hepatic pathogenesis. World J Gastroenterol. 2010 Dec 28; 16(48): 6035–6043
22. Wu D, Cederbaum AI. Oxidative stress and alcoholic liver disease. Semin Liver Dis. 2009 May;29(2):141-54
23. Esrefoglu M. Oxidative Stress and Benefits of Antioxidant Agents in Acute and Chronic Hepatitis. Hepat Mon. 2012; 12(3):160-7.
24. Ghosh A, Ghosh T and Jain S. Silymarin-A Review on the Pharmacodynamics and Bioavailability Enhancement Approaches. Journal of Pharmaceutical Science and Technology Vol. 2 (10), 2010,348-355
25. Salmi HA, Sarna S. Effects of silymarin on chemical, functional and

morphological alterations of the liver. A double-blind controlled study. Scand J Gastroenterol 1982; 17: 517- 21.

26. Feher I, Deak G, Muzes G. Liver protective action of silymarin therapy in chronic alcoholic liver diseases. Orv Hetil 1989; 130 : 2723-7.
27. Parés A, Planas R, Torres M, Caballería J, Viver JM, Acero D, Panés J, Rigau J, Santos J, Rodés J. Effects of silymarin in alcoholic patients with cirrhosis of liver: results of a controlled doubleblind randomized and multicenter trial. J Hepatol 1998; 28 : 615-21.
28. Manouchehr Khoshbaten, Akbar Aliasgarzadeh, Koorosh Masnadi, Mohammad K Tarzamani, Sara Farhang, Hosain Babaei, Javad Kiani, Maryam Zaare, Farzad Najafipoor. N-Acetylcysteine Improves Liver Function in Patients with Non-Alcoholic Fatty Liver Disease. Hepatitis Monthly 2010; 10(1): 12-16
29. Rayman MP. Selenium and human health. Lancet. 2012 Mar 31;379(9822):1256-68
30. Berkson BM. A conservative triple antioxidant approach to the treatment of hepatitis C. Combination of alpha lipoic acid (thioctic acid), silymarin, and selenium: three case histories. Med Klin (Munich). 1999 Oct 15;94 Suppl 3:84-9
31. Khushboo Ambreen, Rolee Sharma, Kaleshwar Singh and Sudhir Kumar: Anti-Tuberculosis Drug-Induced Hepatotoxicity: A Review; International Journal of Advanced Biotechnology and Research(IJBR) Vol5, Issue3, 2014, pp423-437
32. Satyajeet K. Funde, Jugalkishore B. Jaju, Shrikant C. Dharmadhikari, Ganesh R. Pawar. Effect of Lagenaria siceraria fruit extract (Bottle gourd) on hepatotoxicity induced by antitubercular drugs in albino rats. Int J Basic Clin Pharmacol. 2013; 2(6): 728-734