EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ALOE VERA IN DRUG INDUCED HEPATITIS

1Dr. Shaily Bhatt*, 2Dr. Harshvardhan Kumar, 3Dr. Monica Sharma, 4Dr. K.K. Saxena, 5Dr. Gaurav Garg and 6Dr. Ganesh Singh

1Lecturer, Department of Pharmacology L.L.R.M. Medical College, Meerut.
2EMO, L.L.R.M Medical College, Meerut.
3Asso. Prof & HOD, Deptt. of Pharmacology L.L.R.M. Medical College Meerut.
4Professor, Department of Pharmacology L.L.R.M. Medical College Meerut.
5Lecturer, Department of Medicine L.L.R.M. Medical College Meerut.
6Asst. Prof, Deptt. of S.P.M, L.L.R.M. Medical College, Meerut.

ABSTRACT
Drug-induced liver injury (DILI) is an emerging problem, and has been a long-standing concern in the treatment of various disease states. The liver has a central role in drug metabolism and detoxification, and is thus vulnerable to injury. Liver plays a vital role in elimination and metabolism of chemicals but some medicinal agents can damage the organ if given in therapeutic or high dose. Aloe Vera is one of the most popular plants in the world, and is has been used frequently in history for its medicinal properties.

Material & Methods: A total of 30 male and female patients of age group between 15- 65 years, diagnosed clinically and biochemically as the case of drug induced hepatitis and ready to give consent were recruited in the study from SVBP Hospital, LLRM Medical College Meerut (U.P), 250004. Subjects who fulfilled selection criteria were randomized into two groups. Fifteen patients belonging to control group received conventional treatment for drug induced hepatitis while fifteen patients enrolled in treated group were given conventional treatment for drug induced hepatitis supplemented with Aloe vera juice (Patanjali Ayurved ltd, Haridwar) in dose of 20 ml BD orally. Every patient was followed up for 6 weeks. Serum bilirubin, serum Alanine transaminase (ALT), serum levels were measured initially and at the end of 2, 4 and 6 weeks.
Results, Interpretation & Conclusions: Intragroup comparison using Wilcoxon rank sum test demonstrated a statistically significant (p<0.05) decrease in above mentioned parameters for both treated and control groups at all intervals of time. Intergroup comparison done by kruskal wallis test, revealed statistically significant (p<0.05) difference in both of the mentioned parameters between treated and control groups at all intervals of times.

INTRODUCTION

The pathogenesis and various types of DILI are described ranging from hepatic adaptation to hepatocellular injury. During treatment alanine aminotransferase (ALT) monitoring is recommended for those who chronically consume alcohol, take concomitant hepatotoxic drugs, have viral hepatitis, other preexisting liver disease, abnormal baseline ALT or have experienced prior hepatitis. During treatment of disease, in addition to these individuals, patients with HIV infection should have ALT monitoring. Some experts recommend biochemical monitoring for those older than 35 years.

Drug-induced liver injury (DILI) is usually a clinical diagnosis of exclusion. Histologic specimens of the liver are often not obtained for diagnostic purpose. Other causes of liver injury, such as acute viral hepatitis, should be methodically sought, and its their absence that makes the diagnosis plausible. Usually, the time of onset to acute injury is within months of initiating a drug. Rechallenge with the suspected offending agent with more than twofold serum alanine aminotransferase (ALT) elevation, and discontinuation leading to a fall in ALT, is the strongest confirmation of the diagnosis.[1] Rechallenge may, in some instances, endanger the patient and is usually restricted to essential drugs or used when multiple potentially hepatotoxic drugs have been administered concomitantly.[2]

Anti-Tubercular Drugs, Acetaminophen, Nimesulide, Diclofenac, Ibuprofen are Non-steroidal anti-inflammatory drugs (NSAIDs)[3] which are the centerpiece of pharmacotherapy for most rheumatological disorders, and are used in large numbers as analgesics and antipyretics, both as prescription drugs and over the counter purchases. These agents are most important cause of the drug induced toxic injury to several organ systems. Anti-Retroviral Drugs, Statins, fibrates, anaesthetics,[4] azathioprine,[5] methotrexate, antiepileptics,[6] antipsychotics,[7] MAO inhibitors, antihypertensives,[8,9,10] are few important ones.

Nonmedicinal agents including industrial and environmental chemicals can also lead to hepatotoxicity and are called as hepatotoxins.[11] Liver transforms the xenobiotics by reducing
fat solubility and alters the biological activity after chemical transformation.\[12\] Some biochemical markers such as bilirubin and alanine transferase (ALT) indicates normal function of liver or liver damage.\[13\] Usually liver injury is suspected when bilirubin and ALT is more than three times of normal upper limit.\[14\] Normal function of liver can be changed by action of some toxins or due to infection.\[15\] Some agents such as CCL4 and PCM elevate the level of ALT which indicates damage to liver.\[16,17\] Traditional drugs have a great demand in developing countries because of their efficacy, low cost, lesser adverse effects and they are considered to be natural\[18\] and for biological and pharmacological assessment at least 20% of plants are used\[19\] but clinical trials are required to asses the role of medicinal herbs.\[20\] Aloe vera is green, fleshy, spiny and cactus like plant. Leaf filled with clear viscous gel \[21\] has high concentration of water and can survive in dry and harsh climate. It contains different nutrients including minerals, sugars, vitamins, saponins, enzymes (carboxypeptidase, amylase, lipase) phenolic compounds, amino acids, lignin and sterol.

Aloe vera contain many vitamins including thiamine, riboflavin and niacin and important antioxidants like vitamin A, C and E with traces of B\(_{12}\) folic acid\[22\] and choline. For reduction of inflammation aloe vitamin B and C play very important role. Lipase help in digestion of fat where as amylase hydrolyse starch. Zn present in aloe is an important anti-inflammatory agent by inactivation of bradykinin\[23\] through pancreatic carboxypeptidase and aloevera contain aspirin like compound as lupeol, campesterol and sitosterol possessing anti-inflammatory property.\[24\] Aloe vera contains monosaccharide and polysaccharide capsule responsible for potentiating immune response.

**MATERIAL AND METHODS**

The study presented here was a prospective, randomized, open, parallel group, interventional study carried out in a tertiary care hospital which was designed to evaluate the hepatoprotective activity of *Aloe vera in drug induced hepatitis*. Approval was obtained from Institutional ethics committee (approval no. STP/2011/14) and registration was done under CTRI (no. CTRI/2011/12/003064) .Subject recruitment was done from out and in patient department of Medicine LLRM Medical College Meerut, U.P, India.

A total of 30 male and female patients ranging between the age group 15- 65 years diagnosed clinically and biochemically as a case of acute drug induced hepatitis and ready to give consent were recruited in the study while pregnant/lactating females and patients of age
group <15 years or >65 years or having congenital liver disease, decompensated liver disease/chronic debilitating illness other than liver disease were excluded from the study.

Primary end point was set as normalisation of serum bilirubin levels while secondary end point was normalisation of serum alanine transaminase (S.ALT) levels, or development of any adverse effect. Routine blood investigations such as complete hemogram, blood glucose and serum creatinine were carried out at the time of recruitment for assessing general health status of the patient. On randomization of subjects who fulfilled selection criteria using coin toss method, 15 patients were allocated to control group and were given conventional treatment for drug induced hepatitis while 15 patients were allocated to treated group and were given conventional treatment for drug induced hepatitis supplemented with Aloe vera juice (patanjali ayurved ltd) in dose of 20 ml twice daily orally. Every patient was followed up for 6 weeks. Serum bilirubin & serum ALT levels were measured at the time of recruitment and then at the end of 2, 4 and 6 weeks. Levels of these biochemical parameters were compared between control and treated group at all intervals of times. Safety monitoring was done throughout the study period for development of any adverse effect.

The results were expressed as Mean ± SE. Wilcoxon rank sum test and kruskal wallis test were employed for comparison between the two means as a measure of significance. $P$ value of <0.05 was regarded as a statistically significant.

**OBSERVATIONS AND RESULTS**

Out of 50 subjects screened, 42 fulfilled the selection criteria and were randomized to control and treated groups, however 12 subjects were lost to follow up and did not attend the hospital after first visit. There was no statistically significant difference in baseline parameters of both the groups.

**Effect on serum bilirubin:** On intergroup comparison mean serum bilirubin level initially was 8.26±0.18mg/dl in control group and 8.00±0.25mg/dl in treated group and at the end of 2 weeks was 5.70±0.40mg/dl in control group and 4.20±0.18mg/dl in treated group. At the end of 4 weeks 3.41±0.10mg/dl in control group and 1.90±0.09mg/dl in treated group and at the end of 6 weeks 2.42±0.05 mg/dl in control group and 1.16±0.02mg/dl in treated group (table 1).

On intragroup comparison of mean serum bilirubin levels, it was 8.26±.18mg/dl initially for control group and was seen to be decreasing on successive measurements and was
5.70±.40mg/dl, 3.41±.10 mg/dl and 2.42±.05 mg/dl at the end of 2, 4 and 6 weeks respectively.

While mean serum bilirubin levels in treated group was 8.00±.25 mg/dl initially and then it was found to be decreasing on repeated measurements and was 4.21±.18 mg/dl, 1.91±.09mg/dl and 1.16±.02 mg/dl at the end of 2, 4 and 6 weeks of follow up.

**Effect on serum Alanine aminotransferases:** On intergroup comparison mean serum ALT level initially was180.14±4.96 IU in control group and 192.51±4.40IU in treated group and at the end of 2 weeks was 155.04± 3.51IU in control group and 115.92±3.19IU in treated group. At the end of 4 weeks 120.96±2.01IU in control group and 86.83±2.16U in treated group and at the end of 6 weeks 59.94±1.15IU in control group and 45.87±1.41IU in treated group.

On intragroup comparison of mean serum ALT levels, it was 180.14±4.96IU/L initially for control group and was seen to be decreasing on successive measurements and was 155.04±3.51IU/L, 120±2.01 IU/L and 59.94±1.15 IU/L at the end of 2, 4 and 6 weeks respectively.

While mean serum ALT level in treated group was 192.51±4.40 IU/L initially and then it was found to be decreasing on repeated measurements and was 115.92±3.19 IU/L, 86.83±2.16 IU/L and 45.87±1.41 IU/L at the end of 2, 4 and 6 weeks of follow up.

**Table 1:** Comparison between control (N1=15) and Aloe vera treated group (N2=15) on serum bilirubin levels initially (0weeks) and at the end of 2, 4 and 6 weeks

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Control group S. bilirubin (mg/dl) (Mean ± SE)</th>
<th>Treated group S. bilirubin (mg/dl) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.26±0.18</td>
<td>8.00±0.25</td>
</tr>
<tr>
<td>2</td>
<td>5.70±0.40</td>
<td>4.21±0.18*</td>
</tr>
<tr>
<td>4</td>
<td>3.41±0.10</td>
<td>1.9±0.09*</td>
</tr>
<tr>
<td>6</td>
<td>2.42±0.05</td>
<td>1.16±0.02*</td>
</tr>
</tbody>
</table>

**Table 2:** Comparison between control (N1=15) and Aloe vera treated group (N2=15) on serum (ALT) levels initially (0weeks) and at the end of 2, 4 and 6 weeks

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Control group S. ALT(IU/L) (Mean ± SE)</th>
<th>Treated group S. ALT(IU/L) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>180.14±4.96</td>
<td>192.51±4.40</td>
</tr>
<tr>
<td>2</td>
<td>155.04±3.51</td>
<td>115.92±3.19*</td>
</tr>
<tr>
<td>4</td>
<td>120.96±2.01</td>
<td>86.83±2.16*</td>
</tr>
<tr>
<td>6</td>
<td>59.94±1.15</td>
<td>45.87±1.41*</td>
</tr>
</tbody>
</table>

δ: p <0.05 as compared to control group at the end of 2 weeks.
DISCUSSION AND CONCLUSION: DILI may result from direct toxicity of the primary compound, a metabolite, or from an immunologically mediated response, affecting hepatocytes, biliary epithelial cells, and/or liver vasculature. In many cases, the exact mechanism and factors contributing to liver toxicity remain poorly understood. Predictable DILI is generally characterized by certain dose-related injury in experimental animal models, has a higher attack rate, and tends to occur rapidly. Injurious free radicals cause hepatocyte necrosis in zones farthest from the hepatic arterioles, where metabolism is greatest and antioxidant detoxifying capacity is the least. Unpredictable or idiosyncratic reactions comprise of most types of DILI. These hypersensitivity or metabolic reactions occur largely independent of dose and relatively rarely for each drug, and may result in hepatocellular injury and/or cholestasis. Hepatocyte necrosis is often distributed throughout hepatic lobules rather than being zonal, as is often seen with predictable DILI. In hypersensitivity reactions, immunogenic drug or its metabolites may be free or covalently bound to hepatic proteins, forming haptons or “neoantigens.” Antibody-dependent cytotoxic, T-cell, and occasionally eosinophilic hypersensitivity responses may be evoked. Released tumor necrosis factor-α, interleukin (IL)-12, and IFN-γ promote hepatocellular programmed cell death (apoptosis), an effect opposed by IL-4, IL-10, IL-13, and monocyte chemotactic protein-1. Metabolic idiosyncratic reactions may result from genetic or acquired variations in drug biotransformation pathways, with synthesis or abnormally slow detoxification of a hepatotoxic metabolite.

Metabolic idiosyncratic reactions may have a widely variable latent period, but recur within days to weeks after re-exposure. Hepatic Enzyme Measurement an increase in serum ALT, formerly known as serum glutamate pyruvate transaminase (SGPT), is more specific for hepatocellular injury than an increase in aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase [SGOT], which can also signify abnormalities in muscle, heart, or kidney. Serum enzyme concentrations are measured by functional catalytic assays with normal values established from “healthy” populations. The normal range lies within 2 standard deviations of the mean of the distribution, with 2.5% of persons who are otherwise healthy having concentrations above and below the limits of normal on a single measurement. Populations used to set standard values in the past probably included individuals with occult liver disease, whose exclusion has led to decreases in the upper limit.
of normal (ULN). Interlaboratory variation in assay results can be substantial. Consequently, comparison of multiples of the ULN has become standard. In an individual, transaminases may vary as much as 45% on a single day, with the highest levels occurring in the afternoon, or 10 to 30% on successive days. ALT and AST elevation may occur after exercise, hemolysis, or muscle injury. A recent retrospective review of healthy volunteers participating in drug trials who received placebo found that 20% had at least one ALT value greater than the ULN, and 7% had one value at least two times the ULN. Serum hepatic transaminase concentration tends to be higher in men and in those with greater body mass index. Children and older adults tend to have lower transaminase concentrations. The National Academy of Clinical Biochemistry recommends that laboratories establish reference limits for enzymes adjusted for sex in adults, and for children and adults older than 60 years.

Increases in alkaline phosphatase and/or bilirubin with little or no increase in ALT indicates cholestasis. Alkaline phosphatase concentration may also increase because of processes in bone, placenta, or intestine.

An increased concentration of serum_-glutamyl transpeptidase, an inducible enzyme expressed in hepatic cholangioles, is useful in distinguishing liver-related from other organ-related alkaline phosphatase increases. Jaundice is usually detectable on the physical examination when serum bilirubin exceeds 3.0 mg/dl. Laboratory monitoring. A benefit of ALT and/or bilirubin monitoring in preventing or alleviating drug-induced hepatitis has not been rigorously tested. A recent small nonrandomized report suggested that monitoring may decrease the severity of pyrazinamide-induced liver injury. Disadvantages of laboratory monitoring include questionable cost-efficacy of frequent testing for rare adverse events, development and progression of injury between testing events, unclear enzyme thresholds for medication discontinuation, and confusion of hepatic adaptation with significant liver injury. The cost of obtaining AST with ALT is often marginal and may be useful in identifying alcohol related transaminase elevation, where the AST is characteristically higher than the ALT.

The diagnosis of a superimposed injury may be difficult with initially abnormal or fluctuating transaminases. Prior laboratory data may be of use in this regard. Monitoring and the use of a potentially less hepatotoxic regimen is generally recommended for those with preexisting liver disease in the hope that superimposed DILI may be detected preclinically and mitigated.
Transaminase elevation during the course of anti-TB therapy may in some instances actually represent coincidentally developed hepatitis A, B, or C.[35, 36] Aloe vera possess hepatoprotective activity and reduce the level of ALT which is a biomarker to evaluate liver disease. Reduction in ALT levels indicates restoration of normal function of liver. Aloe vera protects liver from oxidative stress and inhibits excessive free radical accumulation. In conclusion aloe vera has hepatoprotective and antioxidant activity through oxidative stress suppression.

Aloe vera has liver protective effect against hepatotoxic agents by restoration of glutathione, glucose 6 phosphatase, lipid peroxidation and microsomal aniline hydroxylase. Acute liver toxicity strongly bloked by the use. Aloe vera protects the liver from oxidative stress and inhibits the excessive free radical accumulation. In ayurvedic formulation aloe vera used for protection of hepatocytes and it possess many important constituents. Specific steroids and flavonoids are responsible to protect the liver from oxidative stress and play a key role as hepatoprotective agent.

REFERENCES


13. Mc clatchey, Kenneth D, clinical laboratory medicine, Lippincott Williams & Wilkins 2006; 288.


35. Turktas H, Unsal M, Tulek N, Oruc O. Hepatotoxicity of antituberculosis therapy (rifampicin, isoniazid and pyrazinamide) or viral hepatitis.