Beetroot Supplementation on Non-Alcoholic Fatty Liver Disease Patients

SHIKHA SRIVASTAVA,¹ ZEBA SIDDIQI,² TARUNA SINGH³ and LAKSHMI BALA*⁴

¹Departments of Food and Nutrition, Era’s Lucknow Medical College, Lucknow, Uttar Pradesh, India.
²Departments of Medicine, Era’s Lucknow Medical College, Lucknow, Uttar Pradesh, India.
³Departments of Radiology, Era’s Lucknow Medical College, Lucknow, Uttar Pradesh, India.
⁴Department of Biochemistry, Babu Banarasi Das University, Lucknow, Uttar Pradesh, India.

Abstract
Nonalcoholic fatty liver disease (NAFLD) has emerged as the one of the most common chronic liver disease worldwide. The pathogenesis of this disease is closely related to obesity and insulin resistance. Beetroot is proposed to have hepatoprotective and hypolipidemic effects due to presence of active compound betaine. The aim of this study was to evaluate the therapeutic effect of beetroot supplementation in patients with NAFLD. The present study was case control prospective study in which 40 cases and 40 controls with NAFLD were advised to follow a lifestyle modification along with prescribed treatment but in cases beetroot powder supplementation was also given orally for 12 weeks. The clinical, symptoms, biochemical parameters, and ultrasonography measured were recorded at baseline and after 12 weeks post beetroot supplementation. Beetroot powder supplementation improved clinical symptoms, significant reduction in liver enzymes and lipid profiles, as well as significant reduction in liver size as compared to controls. We did not find any significant effect of beetroot supplementation on fatty liver grade. The supplementation of beetroot powder for 12 weeks showed hepatoprotective effect in NAFLD subjects. Further long-term studies are recommended to assess beetroot powder supplementation effect on grade of fatty liver.

Introduction
In recent years Non-Alcoholic fatty liver disease (NAFLD) has emerged as the most prevalent chronic liver disease in the developed and developing nations because of the global obesity epidemic.¹ It has been estimated that NAFLD with epidemic obesity will become the major cause of liver associated morbidity and mortality by 2030.² The prevalence of NAFLD
is estimated to be about 9 - 32 % in general Indian population but the real prevalence of NAFLD is unknown due to under diagnosis.³ Prevalence of NAFLD in different populations are estimated to be as follows; United States 30 %, Middle East 32 %, South America 30 %, Asia 27 %, Europe 24 % and Africa 13 %.³ It is not surprising that the prevalence of NAFLD in increasing every year worldwide due to dietary irresponsibility and predominance of sedentary lifestyle. According one of the studies from United States it was found that the incidence of NAFLD was 10% higher in overweight individuals than in lean persons. As it currently stands, NAFLD has become the second most common cause for liver transplantation.⁴ Generally there is no effective treatment available for NAFLD.⁵ Beetroot is said to have a hepatoprotective effect and it effectively keeps away fat from depositing in the liver. This is probably due to presence of betaine in beetroot which is a methyl group donor in the liver transmethylation process.⁶ There is no single approved therapy for NAFLD patients. Several drug therapies have been tried in both research and clinical settings, yet the results have so far not been encouraging.

Keeping above background in view, present study was planned on beetroot powder with following objective. To assess the effect of short term supplementation of beetroot on NAFLD subjects with regards to Clinical, biochemical and radiological parameters.

Material and Methods
Procurement of Beetroot
The beetroot used in this study were organically grown in Ghaila Farm, Hardoi Bypass Road, Lucknow, U.P.

Beetroot Powder
The beetroot powder was prepared in Regional Food Research and Analysis Centre (r-frac), Lucknow, U.P. The procedure is given Fig.1.

Study Design and Site
This was a case control prospective study. In this study the beetroot powder supplementation was done in NAFLD subjects for three months of period at Era’s Lucknow Medical College and Hospital, Lucknow. The present study was done after approval from Institutional ethical committee and written informed consent for this study was taken from subjects enrolled who were then divided into cases and controls as follows:

1. Cases were NAFLD subjects who received beetroot supplement along with prescribed medication and lifestyle modification for the duration of 3 months.
2. Controls were NAFLD subjects who received only medication and lifestyle modification for the same duration.

Study Population
All known NAFLD subjects were included in this study according to following criteria.

Inclusion Criteria
Subjects older than 18 years with a diagnosis of fatty liver on ultrasonography were included.

Exclusion Criteria
Diabetic patients, those who consumed alcohol, patients of known liver disease apart from NAFLD, End stage disease or terminally ill patients, pregnant females and patients who could not take were excluded.

Sample Size
40 cases and 40 controls were included in this study.

Procedure
The organic beetroot powder was given to cases for three months of period along with prescribed medication and lifestyle modification. However, for controls only prescribed medication and lifestyle modification were given. In cases, 5 gm of beetroot powder twice a day were given after meal for 14 days. After that the dose was 5 gm per day for 3 months. The following parameters were evaluated in all subjects included in this study at baseline and after 12 weeks.

Anthropometric Measurement
Weight and Height were evaluated for calculating BMI.

Clinical Symptoms
Pain epigastrium, right hypochondrium pain and flatulence.
Biochemical and Radiological Parameters
Liver enzymes (Serum Bilirubin, ALT, AST, SALP), serum lipid profile (Fasting). The liver biopsy is currently the gold standard test for diagnosing NAFLD. But it has many drawbacks such as sampling error, high cost and risk of complications. However, ultrasonography is a non-invasive method for diagnosing NAFLD. So we have used ultrasonography method for diagnosing NAFLD (liver size and fatty liver grade) in all subjects.

Statistical Analysis
The results were analyzed using descriptive statistics and to make comparisons between the cases and controls with respect to various parameters. Catagorical data were summarized as in proportions and percentages (%) and continuous variables as Mean ± SD (standard deviation). A sample size of 40 in each group would give 90% power of study.

### Table 1: Baseline Characteristics of cases and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n = 40)</th>
<th>P - Value</th>
<th>Cases (n = 40)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>45 ± 2.3</td>
<td></td>
<td>42.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td></td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>BMI (kg / m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>29.5 ± 14</td>
<td>0.966</td>
<td>31.6 ± 1.9</td>
<td>0.377</td>
</tr>
<tr>
<td>After</td>
<td>29.5 ± 14</td>
<td></td>
<td>31.5 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean ± standard error (SE). Abbreviations: BMI, Body Mass Index. p -value shows the difference between baseline and 12 - weeks

### Table 2: Changes in biochemical parameters before and after beetroot supplementation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases(n = 40)</th>
<th>Controls(n = 40)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td><strong>LIVER ENZYMES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Bilirubin</td>
<td>1.4 ± 0.6</td>
<td>0.9 ± 0.3</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Serum AST</td>
<td>61.4 ± 14.2</td>
<td>57.7 ± 14.4</td>
<td>62.0 ± 14.2</td>
</tr>
<tr>
<td>Serum ALT</td>
<td>46.3 ± 11.0</td>
<td>43.1 ± 11.5</td>
<td>46.9 ± 11.3</td>
</tr>
<tr>
<td>Serum ALP</td>
<td>116.9 ± 10.8</td>
<td>118.6 ± 16.7</td>
<td>116.9 ± 10.8</td>
</tr>
<tr>
<td><strong>LIPID PROFILE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>229.5 ± 33.5</td>
<td>188.6 ± 7.5</td>
<td>229.5 ± 33.5</td>
</tr>
<tr>
<td>Serum LDL</td>
<td>140.9 ± 15.9</td>
<td>128.0 ± 11.7</td>
<td>144.6 ± 23.7</td>
</tr>
<tr>
<td>Serum HDL</td>
<td>54.5 ± 7.9</td>
<td>58.0 ± 6.7</td>
<td>54.1 ± 7.5</td>
</tr>
<tr>
<td>Serum TG</td>
<td>178.5 ± 24.1</td>
<td>152.9 ± 17.4</td>
<td>174.2 ± 23.8</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard error (SE). Abbreviations: Serum AST, Serum aspartate aminotransferase; Serum ALT, serum alanine aminotransferase; Serum ALP, Serum Alkaline phosphatase; Serum LDL, Serum low-density lipoproteins; Serum HDL, high-density lipoprotein; Serum TG , Serum triglyceride. p -value shows the difference between baseline and 12 - weeks.
Results
Total eighty subjects were included in this study out of which 40 were cases and 40 controls. Table 1 shows the baseline characteristics of cases and controls enrolled in this study.

According to the data present in this table there is no significant difference was observed in BMI after 12 weeks of follow-up in both cases and control. The change in clinical symptoms at baseline and after 12 weeks of follow-up was observed in both cases and controls. The symptoms of heaviness or pain in epigastrium and indigestion were observed in both cases and controls at 55% and 100% respectively on baseline. Although on baseline the flatulence was present in both cases and controls. It was 70% observed in cases and 30% was in controls. Post supplementation of beetroot powder for 12 weeks, changes in clinical symptoms were observed in both cases and controls. The symptoms like heaviness or pain in epigastrium, indigestion and flatulence were reduced to nil in cases and 2.5% in controls after 12 weeks of beetroot powder supplementation.

The changes in biochemical parameters (liver enzymes and lipid profile) characteristics of the study subjects are shown in Table 2. On comparing level of serum bilirubin from baseline to 12 weeks it was significantly (p < 0.05) decreased in cases than in controls. However, in serum AST there was insignificant difference between cases and controls from baseline to 12 weeks.

But in cases the level of AST were significantly decreased from baseline to 12 weeks. Similar results were observed in serum AST. But the differences in serum alkaline phosphatase between cases and controls after 12 weeks of beetroot powder supplementation was insignificant (p < 0.05). The changes in lipid profiles before and after supplementation of beetroot powder were also given in Table 2. After 12 weeks of beetroot powder supplementation the serum cholesterol levels was significantly decreased from baseline to 12 weeks in cases than controls. However in controls there was insignificant difference from baseline to 12 weeks. It was observed that serum LDL was significantly decreased from baseline to 12 weeks.

---

**Fig. 1: Flow chart of the procedure for preparation of beetroot powder**

- Fresh Beetroot
- Washed with tap water
- Cut into small Slices.
- Dried in hot air circulated oven at 60 °C for 11–12 hours
- Grinding in Electric Grinder
- Passed through 65 mesh sieve
- Beetroot Powder
- Packed in airtight containers of food grade (29-30 °C)
decreased in cases from baseline to 12 weeks but this was not seen in controls. Serum HDL was not changed significantly between controls and cases from baseline to 12 weeks.

But the level of HDL was significantly increased in cases from baseline to 12 weeks. The serum triglyceride was significantly decreased in cases as compared to controls after 12 weeks of beetroot powder supplementation.

The results of ultrasonographic parameters are given in Table 3. The liver size was significantly decreased in cases on comparing with controls after 12 weeks of beetroot powder supplementation and the significant differences were also observed in cases from baseline to 12 weeks.

Although the grade of fatty liver was not changed in both cases and controls from baseline to 12 weeks after supplementation of beetroot powder; in two cases it was found that the fatty liver grade improved i.e. in one subject; grade two was changed into grade one and in the other subject grade one was changed into no fatty liver.

**Discussion**

To our knowledge, this is the first case control prospective study that examined the effect of beetroot supplementation on NAFLD subjects. In this study, there is no significant change was observed in BMI of both case and control. It shows that consumption of only 10 to 5 gm beetroot powder for the period of 12 weeks is not sufficient for reduction of weight. However, the consumption of beetroot powder for 12 weeks decreased clinical symptoms i.e. heaviness pain in epigastrium, flatulence and indigestion more than controls. So our study has shown that beetroot is beneficial for relief of symptom of indigestion in subjects of NAFLD. Clifford et al. also reported in their review of potential benefits of beetroot that beetroot juice stimulates digestion.^{7} Amnah and Alushaibani (2013) reported in their study done on rats that consumption of biscuits prepared with beetroot powder significantly decreased liver enzymes, cholesterol and total lipids in cases than in controls.^{8} Nouri et al. (2017) found that with consumption of beetroot juice by male wistar rats the liver enzymes in were decreased in liver diseases rats.^{9}

Rabeh also reported that dried, fresh and of red beetroot significantly restored the liver enzymes to normal levels in against carbon tetrachloride induced rats.^{10} The study done in humans by Sigh et al. (2015) shown that the consumption of beetroot juice lowered the lipid profile i.e. LDL, total cholesterol, triglycerides levels and also significantly increased the levels of HDL in physically active individuals.^{11} Thus, our study confirmed these previous studies and showed that beetroot has a hepatoprotective apart from its lipid lowering effect. It may be due to the presence of active compound betaine in beetroot having antioxidant properties.

In this study, liver size was significantly decreased in cases than controls and the fatty liver grade did not changed significantly with the supplementation of beetroot powder for 3 months. The reason for less shows subjects an improvement in grade of fatty liver could be that in our study the supplementation of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases</th>
<th>Controls</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver size</td>
<td>14.4 ± 0.9</td>
<td>12.6 ± 1.0</td>
<td>14.4 ± 0.9</td>
</tr>
<tr>
<td>Fatty Liver Grade</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard error (SE). p -value shows the difference between baseline and 12 - weeks.
beetroot powder was done for short intervention of time. Another limitation of this study was the lack of liver biopsy as to confirm histological improvement.

However, we evaluated liver enzymes, lipid profile and ultrasonographic parameters which provided quantitative, noninvasive positive results. The novelty of our study is that to the best of our knowledge, it is the first study evaluating the hepatoprotective effect of beetroot supplementation on human subjects of NAFLD.

To conclude, this case control prospective study found some evidences that beetroot supplementation could improve clinical symptoms, decrease liver enzymes and improve lipid profiles when prescribed along with life style modification and pharmacological treatment in NAFLD subjects. However fatty liver grade showed improvement only in two subjects. This can be attributed to the short duration of supplementation. Thus further studies of longer duration and larger samples are required to validate and support our findings of beneficial effects of beetroot supplementation in NAFLD subjects.

Acknowledgment
We thank our institution Era’s Lucknow Medical College and Hospital for providing Ethical approval for this study and also providing infrastructure required for this study. The authors declare that there is no conflict of interests regarding the publication of this paper. The authors also declare that the funding required for this study was provided by Era’s Lucknow Medical College and Hospital.

Conflict of Interest
The author(s) declare no conflict of interest.

References