Hepatoprotective Effects of Betalain Supplementation among Patients with Non-Alcoholic Fatty Liver Disease

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Key Words: Beetroot, Betalain, LDL, C-Reactive Protein, TNF Alpha, NAFLD


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ABSTRACT

Pakistan has the lowest rate (13.5%) of “Non-alcoholic fatty liver disease” NAFLD meanwhile prevalence of NAFLD is 47% nationwide in Pakistan. However, betaine plays a considerable positive role in alleviating hepatic cell lipid accumulation by pacing the oxidation of fatty acid, the TCA cycle, and glycolytic processes in the liver. Objectives: To evaluate the comparative effect of conventional treatment with beetroot supplementation on inflammatory markers and the efficacy of betalain supplementation among patients with NAFLD. Methods: A total participant of 34 patients with NAFLD grade 1 older than 18 years were enrolled for the 12 weeks of the study, and participants were allocated into two groups, 17 each. In this study total of 45.6% of participants were male, and 54.4% were female. The groups G0 was considered as a control group with no supplementation, and group G1 where conventional treatment and supplementation were advised to participants. The 5g beetroot powder supplements in tablet form were given to the participants daily for 12 weeks. Results: The mean age group of NAFLD patients enrolled in the study was 43.04±3.79 years in G0, and in G1, it was 43.7±5.21. The mean BMI was 25.95 and 26 kg/m² in both groups. There was a substantial (P<0.05) fall in cholesterol and low-density lipoprotein (LDL) levels. High-density lipoprotein levels were also improved. On the other hand, C-reactive protein and TNF alpha levels were also. Conclusions: The current study concluded that beetroot supplementation could improve NAFLD patients' inflammation and lipid profile.

INTRODUCTION

The term “non-alcoholic fatty liver disease” (NAFLD) refers to a category of disorders where there is a buildup of extra fat in the liver [1]. The non-serious condition known as fatty liver is the most prevalent type of NAFLD [2]. Fat builds up in the liver cells in fatty liver. Although having fat in the liver is abnormal, it is unlikely to harm the liver [3]. Non-alcoholic steatohepatitis (NASH) is a more severe condition that a tiny subset of NAFLD patients may experience [4]. The prevalence rate of NAFLD is highest among South Asians and South Americans and lowest among Africans [5]. Children's NAFLD is also turning into a significant health issue. In South Asia, NAFLD prevalence is from 13 to 34%. Pakistan has the lowest rate (13.5%), while Bangladesh has the highest (34.34%). NAFLD is more
common in obese people (15–80%), people with dyslipidemia (25–60%), and people with diabetes (35–65%). In children, NAFLD occurs 8% of the time in the general population worldwide and 34% when obesity is present [6]. The prevalence of NAFLD is 47% nationwide in Pakistan [7]. Concerning the different provinces of Pakistan, Khyber Pakhtunkhwa recorded the most significant prevalence of NAFLD (58%), followed by Punjab (23%) and Sindh (12%) [8]. The nitrogen-containing water-soluble pigments known as “betalain” (reddish purple betacyanins and yellow xanthophylls) are abundant in beets. The shikimate pathway is necessary to produce betalain and the primary beetroot polyphenols [9]. In particular, the epidermal and sub-epidermal tissues of flowers, fruits, and plants where betalain is generated collect the substance. Along with beetroot, cactus pear, and purple-red dragon fruit are excellent sources of betalain [10]. Glycine and three aromatic rings combine to generate betaine, which works well as a methyl donor for methionine's homocysteine cycle. It has been suggested that betalains may have favorable impacts on human health. Since these substances can reduce oxidative stress by effectively eliminating ROS, their exceptional antioxidant activity is essential [11]. Betaine plays a considerable positive role in alleviating hepatic cell lipid accumulation by pacing the oxidation of fatty acid, the TCA cycle, and glycolytic processes in the liver [12]. There has been an increase in interest in the biological activity of red beets (Beta vulgaris rubra) and their potential utility as functional foods that support health and ward against disease. Nitric oxide (NO) accessibility may be raised in vivo naturally by consuming beetroot. Endothelial dysfunction, characterized by low NO bioavailability and hypertension, are two conditions it has shown promise in treating and preventing. Beetroot is another intriguing treatment possibility for various clinical conditions associated with oxidative stress and inflammation. Both in vitro and in vivo studies have shown that its constituent parts, especially the betalain pigments, have potent anti-inflammatory, antioxidant, and chemo preventive actions [13]. Anti-inflammatory and anti-lipogenic effects, ameliorating insulin resistance, and mitochondria function are the remarkable characteristics that make betaine a beneficial remedy in treating NAFLD [14]. Betain significantly reduced leukocyte migration to inflammatory areas, decreased superoxide anion levels, and increased IL-10 levels, which have anti-inflammatory properties. Tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1), two cytokines that stimulate the inflammatory response, were produced at lower amounts. Additionally, there was a significant rise in the levels of IL-10, a cytokine with anti-inflammatory properties that works by preventing the generation of pro-inflammatory cytokines. These findings support using betalals in treating inflammatory illnesses due to their significant anti-inflammatory capabilities [15]. The hepatotoxic chemical CCl4 caused liver damage. The study’s findings confirmed that betalain-containing extracts can protect the liver from damage caused by CCl4, hepatic lesions in rats. The juice of beetroot is known to exhibit hepatoprotective properties. In the rat model of oxidative liver damage induced by N-nitrosodiethyamine (NDEA), the intake of beetroot juice for an extended period exhibits a safeguarding effect of Beta vulgaris. It has been demonstrated that beetroot may be implicated in the prevention as well as treatment of alcoholic liver disease, which is considered the main pathological factor that causes aggregation of fat in the liver cells [16]. The study also found that betaine may be helpful in treating NAFLD due to its beneficial effects on fatty tissue functions. The study suggests that betaine may be helpful in treating NAFLD due to its insulin-sensitizing effect [1]. The study suggests that beetroot supplements could be a potential treatment for NAFLD and non-alcoholic steatohepatitis [17]. Thus, study concluded that betaine supplementation increased the export of lipid and fatty acid oxidation in rats fed a high-fat diet, effectively reducing fat accumulation in the liver [6].

**METHODS**

We conducted randomized control trial (RCT) at Bahria International Hospital with the target population of liver disease grade 1 at the age of 18 or older for the duration of 12 weeks. The sample size was calculated using the formula of 2 independent mean-taking mean bilirubin levels among the treatment and control groups. Hence 14 patients were calculated through a formula taking a 20% dropout rate. 17 patients were taken in each group. The sample size was 34. The statistical package for social sciences version 25.0 has been utilized to test the study hypothesis. Patients suffering from NAFLD were included while alcohol-consuming individuals were excluded. Individuals suffering from known allergies, malabsorptive disease, end-stage liver disease, or other chronic conditions such as CKS, AKI, and CAD were also excluded, along with pregnant and lactating women. Thirty-four patients met the criteria of our research, which were further divided into two groups: the treatment group (n=14) and the control group (n=14). Patient baseline characteristics, including anthropometrics and demographics measurements, were taken along with ALT, AST, ALT, LDL, HDL, TG, TNF-α, and BMI. The beetroot supplementation provided to patients was prepared in the lab following a standard procedure to ensure uniformity in supplemental intervention as shown in Table 1 [18].
First, the beetroots were washed with tap water; after removing physical contaminants like dirt, dust and foreign grains, cut the beetroot into small slices and placed the pieces in a hot air-circulated oven at 60°C for about 11-12 hours for drying after ground it in electric grinder mill and pass through a 60-mesh sieve to produce fine powder. Then, the powder was shifted into an airtight container of food grade (29-30°C) as shown in Figure 1.

Processing of Beetroot Supplementation

**Figure 1:** Production of Betalain rich beetroot supplementation

Written informed consent (attached) was taken from all the participants, and the information and data collection were kept confidential. Participants remained anonymous throughout the study, and subjects were informed that they were free to withdraw at any time during the study process. The study period was around 9 months. The sample size was calculated using the formula of 2 independent mean-taking mean bilirubin levels among the treatment and control groups. Hence 14 patients were calculated through a formula taking 20% dropout rate. 17 patients were taken in each group on the basis of equal distribution of sample population. The sample size is 34.

We considered the probability of a type I error of 5% (α = 0.05). The following formula was used to calculate the sample size:

\[
n = (Z_\alpha/2 + Z_\beta) ^2 (S_1^2 + S_2^2) / (\mu_1 - \mu_2)^2
\]

It resulted in a minimum sample size in each group of 17 patients. We assumed a 20% dropout rate, leaving a final sample size of 17 in each group.

**R E S U L T S**

The mean age of G₀ (conventional group) participants was 43.04±2.19, and that of G₁ (treatment group) participants was 43.7±2.01. The mean weight of participants before treatment was 77.25±4.31kg in G₀ and 75.2±2.21in G₁. Total of 45.6% of participants were male, and 54.4% were female in this study. The mean BMI of participants before treatment was 26.07±1.43kg/m² in G₀ and G₁ was 26.98±1.93kg/m². At the start of the study the mean LDL level of participants was 164.34±5.67mg/dl in the G₀ group, and in the G₁ group mean LDL level of participants was 160.64±2.45mg/dl whereas the mean HDL level of participants before treatment in G₀ was 50.69±4.52mg/dl. The mean C-reactive protein level of participants before treatment in G₀ was 13.46±2.37mg/L and in G₁ 14.65±3.03mg/L (Table 2).

### Table 2: Characteristics of patients before intervention

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.04±2.19</td>
<td>43.7±2.01</td>
</tr>
<tr>
<td>BMI (Kgm⁻²)</td>
<td>26.07±1.43</td>
<td>26.98±1.93</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>164.34±5.67</td>
<td>160.64±2.45</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50.69±4.52</td>
<td>48.31±3.2</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>225.52±3.73</td>
<td>230.18±2.12</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>212.65±4.61</td>
<td>225.19±4.32</td>
</tr>
<tr>
<td>Alanine Transaminase (ALT) (U/L)</td>
<td>68.11±6.34</td>
<td>52.12±5.94</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST) (U/L)</td>
<td>92.07±7.8</td>
<td>73.95±7.67</td>
</tr>
<tr>
<td>C-reactive protein (CRP) (mg/L)</td>
<td>13.46±2.37</td>
<td>14.65±3.03</td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNF-α) (Pg/ml)</td>
<td>16.58±4.33</td>
<td>18.41±1.61</td>
</tr>
</tbody>
</table>

We observed change in the biomarkers of patients after the intervention of betalain in the form of beetroot extract supplementation as 5gm daily. Improvement was observed in ALT, AST as well as BMI which is described in detail in Table 3.

### Table 3: Characteristics of patients after and before supplementation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>Before Supplementation</td>
<td>Before Supplementation</td>
</tr>
<tr>
<td></td>
<td>After Supplementation</td>
<td>After Supplementation</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.25±4.3</td>
<td>75.2±2.21</td>
</tr>
<tr>
<td>BMI (Kgm/2)</td>
<td>26.07±1.43</td>
<td>25.82±1.23</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>164.34±5.67</td>
<td>152.56±4.43</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50.69±4.52</td>
<td>51.74±3.71</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>225.52±3.73</td>
<td>219.70±3.69</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>212.65±4.61</td>
<td>220.03±2.29</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>68.11±6.34</td>
<td>65.89±4.92</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>92.07±7.8</td>
<td>94.67±4.96</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>13.46±2.37</td>
<td>16.72±4.30</td>
</tr>
<tr>
<td>TNF-α (Pg/ml)</td>
<td>16.58±4.33</td>
<td>17.80±2.76</td>
</tr>
</tbody>
</table>

Value represents as Mean ± SD; Conventional group (G₀) = Group without any supplementation; Treatment group (G₁) = Group with 5gm beetroot supplementation, Level of significance of p<0.05

### D I S C U S S I O N

The mean BMI of patients in G₀ was 26.07±1.43kg/m² as compared to 26.98±1.93kg/m² among patients in G₁ before...
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the treatment, whereas the mean BMI of group G2 and G1 after treatment was 25.82±1.23kg/m² and 25.02±1.23kg/m² respectively as mentioned in Table 3. An oral betalain supplement’s effect on the development of NAFLD in mice brought on by a Western-style diet (WSD) was researched. For this investigation, mice between the ages of 6 and 8 weeks were used. Each kilogramme of body weight received 2.5 g of betalain. At the start and completion of the experiment, glucose metabolism, liver damage, nitric oxide production, and intestinal function were assessed. According to statistical analysis, both groups’ body weights remained constant [19]. The mean LDL of patients in G1 was 164.34±5.67mg/dl as compared to 160.64±2.45mg/dl among patients in G, before the treatment, whereas the mean LDL of G1 and G, after treatment was 152.56±5.43mg/dl and 153.38±5.78c mg/dl respectively. There was statistically significant (p<0.05) difference in pre and posttreatment group of beetroot supplementation as described in Table 3. Our HDL, cholesterol and LDL change relates to previous study where the beetroot’s powerful biological activity, especially the betalains (betanin) and nitrates it contains, has recently garnered greater attention. It has been demonstrated that betalains can reduce oxidative and nitrative stress by scavenging DPPH, preventing DNA damage, raising HDL, and decreasing LDLc. It has also been demonstrated to have an anticancer impact by inhibiting angiogenesis and cell growth as well as by inducing cell death and autophagy. Nitrate is primarily responsible for lowering blood lipids, glucose, and pressure in various chronic conditions, although its function in treating hypertension and hyperglycemia is less obvious. In addition, eating nitrate–rich beetroot may improve athletic performance and lessen post-exercise muscular pain. This review aims to provide enough proof to explain the health advantages of beetroot, particularly in bio-oxidation, neoplastic disorders, certain chronic diseases, and energy augmentation [14]. The ALT and AST shift by beetroot supplementation provide a significant factor by acting as a hepatoprotective agent. According to certain research, betalains may have anti-inflammatory properties that lessen liver inflammation. Alanine transaminase (ALT) and elevated aspartate transaminase (AST) values might signify liver injury. A research on people with NAFLD discovered that drinking betalain–rich beetroot juice decreased ALT levels, pointing to a possible liver-protective impact. According to animal research, betalains may have hepatoprotective benefits, which means they might help shield the liver from harm brought on by numerous things like toxins or medicines. The antioxidant and anti-inflammatory characteristics of betalains may be responsible for these benefits. Beetroot pomace, a by-product of food processing, was examined for its free radical scavenging activity on stable DPPH radicals, and the reducing power of extracts from Kestrel, Cardeal-F1, Detroit, Bicor, and Egyptian beetroot pomace was evaluated spectrophotometrically. Detroit beetroot pomace extract showed the strongest antiradical action against DPPH radicals (DBPE; EC50 = 0.06 0.10 g/ml). Five beetroot pomace extracts all shown an increase in reducing power when the concentration was applied. The best reducing power was shown by DBPE (EC50 = 123.39 08.05 g/ml). By monitoring the activities of several enzymes (xanthine oxidase, catalase, peroxidase, glutathione peroxidase, and glutathione reductase) and measuring the levels of glutathione–GSH and thiobarbituric acid reactive substances (TBARS), in vivo antioxidant and potential hepatoprotective properties were assessed. DBPE at dosages of 2 and 3 ml/kg body weight had the greatest results in neutralising the oxidative stress caused by CCl4. When DBPE and CCl4 were applied simultaneously, GSHPx levels significantly decreased, but CAT and GSH levels increased, bringing them to physiological values for the control group. HPLC was used to identify phenolics and betalains, which impacted the antioxidant and hepatoprotective status [20]. A clinical study, 50 patients were divided into two groups; one group got a placebo, while the other received daily supplements containing 2 g of betalain. Overall, the experiment lasted 12 weeks. Both the beginning and the end both had signs of inflammation. Anthropometric measurements and hepatic enzymes did not change substantially in either group. Our research showed that giving NAFLD patients 2 g of betalain daily for 12 weeks decreased inflammatory markers. More study using various betalain doses and a longer supplementation period is necessary [20]. According to certain research, betalains may have anti-inflammatory properties that lessen liver inflammation. Alanine transaminase (ALT) and elevated aspartate transaminase (AST) values might signify liver injury. A research on people with NAFLD (NAFLD) discovered that drinking betalain–rich beetroot juice decreased ALT levels, pointing to a possible liver-protective impact. According to animal research, betalains may have hepatoprotective benefits, which means they might help shield the liver from harm brought on by numerous things like toxins or medicines. The antioxidant and anti-inflammatory characteristics of betalains may be responsible for these benefits. In one of their studies, Darabi and his colleagues examined the effects of betalain supplementation on inflammatory markers in patients with NAFLD. 50 patients were divided into two groups for their clinical study; one group got a placebo, while the other received daily supplements containing 2 g of betalain. Overall, the experiment lasted 12 weeks. Both the beginning and the end both had signs of inflammation.
outcomes showed that the blood levels of nuclear factor kappa B (NF-B) and high-sensitivity C-reactive protein (hs-CRP) in the intervention group were considerably lower than those in the placebo group. Nuclear factor kappa B (NF-B) concentrations decreased from 15.1 to 4.74 to 14.6 to 2.02 nM. C-reactive protein (hsCRP) levels dropped from 5696-2.862 to 3901-2.760 with a p-value under 0.01. Additionally, betalain supplementation substantially decreased blood alanine aminotransferase (ALT) and hepatic steatosis (p=0.04). Anthropometric measurements and hepatic enzymes did not change substantially in either group (p=0.05). Our research showed that giving NAFLD patients 2 g of betalain daily for 12 weeks decreased inflammatory markers. More study using various betalain doses and a longer supplementation period is necessary [21]. Whereas, previous study results showed that the supplementation of betaine reduced the accumulation of TAG on the liver which was inducted by high fat-containing diet as compared to the rats that fed the high-fat containing diet. The results of liver histology also supported these findings. Furthermore, the activity of betaine– homocysteine methyl-transferase in the liver and also the abundance of its mRNA and level of lecithin also elevated by the supplements of betaine in rats that were fed high fat-containing diet and in the rats fed with control diet [6]. Further research is needed on the effect of betalain supplementation on a large population using different recipes. The cost-effectiveness and efficiency of treatment based on natural products can play a significant role in preventing NAFLD. There is a need to conduct this supplement intervention at a greater level as our sample size was minimal to prove its efficacy and exact mechanism.

**CONCLUSIONS**

The current study concluded that betalain supplementation effectively improves blood lipid profile among NAFLD. This research project showed a significant reduction in inflammatory markers. There is a significant change in C-reactive protein levels. There was no significant mean difference in change in Body Mass Index and weight among the study participants betalain supplementation has shown promising results with a combination conventional treatment. However, the exact mechanism to improve the non-alcoholic fatty liver is still unknown.

**Authors Contribution**

Conceptualization: Methodology: Formal analysis:
Writing-review and editing:
All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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