Ameliorative Effect of Alcoholic Fatty Liver in Mice by the Medicinal Value-Enhanced Porridge Made with Macrotyloma Uniflorum and Vigna Radiata

Thirunavukkarasu C*, Nithyananthan S, Subhiksha S, Guha S, Mir IM, and Jainsenee Behera
Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry, India

*Corresponding author:
Thirunavukkarasu C,
Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry, India, Tel: +91 413 2654972;
Fax: +91 413 2655255,
E-mail: tarasu.bbm@pondiuni.edu.in

1. Abstract
The excessive consumption of alcohol leads to energy imbalance, which encourages lipid biosynthesis and fat accumulation in the liver. The continued accumulation of fat with high oxidative stress enhances liver fibrosis. The nutrient porridge was made with the germinated Macrotyloma uniflorum and Vigna radiata to treat ALD. The nutritional value of the porridge was improved with practical food processing skills such as germination, dehulling, and milling. The addition of herbs such as Trigonella foenum graecum, Cuminum cyminum, Zingiber officinale, Piper nigrum, and Curcuma longa has improved the medicinal value of the porridge. The porridge was supplied to the BALB/C albino mice having the Lieber-Dercarli ethanol diet. The porridge supplementation has improved the albumin biosynthesis, antioxidants in the ALD mice, and minimized serum liver markers. The histological preparations revealed a reduction in fat accumulation and fibrosis. This study concludes that the nutrient herbal porridge has protected the liver from alcoholic steatosis and fibrosis.

1.1. Practical Application:
The nutrient herbal porridge was made with effective food processing skills to increase the nutritional value and decrease the anti-nutritional factors. The Medicinal worth of the porridge was enhanced with the herbs containing the various biological role. The herbs were selected by targeting ALD progression pathways such as inflammation, fatty acid and cholesterol biosynthesis, and oxidative stress. Since the porridge is made with edible foodstuff, it may skip the FDA approval and have good translational potential. The nutrient herbal porridge can be suggested to the alcoholic fatty liver prevalence zone to minimize the risk.

1.2. Highlights:
The prepared herbal nutrient porridge has good nutritional value, high antioxidant content, less anti-nutritional factor, and attractive sensory scores.
- The porridge supplementation to the alcoholic fatty liver mice effectively prevented the hepatomegaly and hepatocyte damage due to fat accumulation.
- The nutrient porridge supply has managed hepatic antioxidant content, reduced fat accumulation, and liver fibrosis.
- The prepared herbal nutrient porridge served as a preventive agent against alcoholic fatty liver.

2. Introduction
The liver is the primary metabolic organ lace a significant role in the processing of the digested food. Its unique anatomical location receives a massive blood supply from the gut through the portal vein. The portal vein carries the hypoxic blood rich in nutrients and reactive oxygen species. The flow of such portal blood into the hepatic parenchyma influences the physiology of hepatocytes, which is divided into three zones. Zone 1 is the area around the portal area where the hepatic artery delivers the oxygenated blood. Zone 3 (Pericentral area) is around the central vein where the oxygen level is less,
and the hepatocytes actively involved in lipogenesis and xenobiotic metabolism. The hepatocytes of zone 1 (Periportal zone) involves the oxidation of metabolites and gluconeogenesis. The hepatocytes located between zone 1 and 3 are termed intermediary zone or zone 2 [26]. The liver consists of predominantly parenchymal cells such as hepatocytes and minor non-parenchymal cells such as endothelial cells, Kupffer cells, and hepatic stellate cells. The massive portal supplementation to the liver carries the damage-prone molecules like xenobiotics and bacterial products to the liver from the gut. The liver is being affected by various gut-derived substances and caused the non-neoplastic liver diseases such as Alcoholic Liver Diseases (ALD), Non-Alcoholic Fatty Liver (NAFLD), Non-Alcoholic Steatohepatitis (NASH), which gets promoted to neoplastic liver diseases and accounts for 2 million death worldwide. Hepatocellular Carcinoma (HCC) is contributed by non-neoplastic liver diseases such as hepatitis B (40%), hepatitis C (40%), alcohol (11%), and other causes (11%) [3].

Globally, ~ 6.4 L is the average per capita alcohol consumption per year with a geographic variation with low rates in Northern and Middle East Africa and high rates in Europe and Russia. The meta-analysis revealed that the rate of liver cirrhosis is high in alcohol-consuming women. Alcohol consumption also increased the Disability-Adjusted Life-Years (DALYs) from 38.8 per 100,000 people and 1759 per 100,000 individuals. 10.1% for overall death in Europe is due to the excess consumption of alcohol [33]. ALD occurs due to excessive alcohol consumption associated with increased accumulation of triglycerides, known as hepatic steatosis. The early stages of liver diseases are characterized by the increased fat accumulation called alcoholic steatosis, which is then further promoted into alcoholic steatohepatitis, which is characterized by the inflammation of the liver and increased deposition of extracellular matrix in the hepatic parenchyma [43].

Since the liver is a regenerative organ and the minimum damage can be repaired quickly, it is challenging to identify liver damage. Liver diseases are diagnosable when there is maximum damage to the hepatocytes. Hence a preventive therapy could provide a better outcome in treating liver diseases. Many chronic liver diseases such as ALD, cirrhosis, and NASH lead to malnutrition due to the liver's reduced protein biosynthetic capacity and increased protein utilization by the body cells. Nutritional therapy could reduce liver diseases and associated malnutrition [32, 68].

In this study, we have formulated the herbal porridge consist of the milled powders of cereals and grains, and herbs. The porridge is a liquid diet that has easy digestibility, absorption, and elimination from the gut. The rice-based herbal porridge is known to reduce diabetes in rats, and oat porridge prevented obesity in humans and maintained liver function properly (Chang et al., 2013; Senadheera et al., 2014). In the previous study, we found the herbal porridge known to reduce CCl4 induced liver fibrosis, NAFLD, and NASH [37, 38]. In this current study, the porridge's herbal composition was increased by targeting various signaling pathways of ALD to study its preventive role.

3. Materials and Method

3.1. Materials

Vigna radiata (green gram), Macrotyloma uniflorum (horse gram), Trigonella foenum graecum (fenugreek), Cuminum cyminum (jeeraa), Zingiber officinale (ginger), Piper nigrum (black pepper), and Curcuma longa (turmeric) were purchased from the local market of Puducherry. All the chemicals used were of analytical grade and procured from HiMedia Pvt Limited, India. The plants were identified by Dr. N. Mathivanan, Director and Head, Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India.

3.2. Porridge Preparation

100 grams of clean seeds of Vigna radiata and Macrotyloma uniforum were weighed and rinsed in distilled water, surface-sterilized by soaking in 1.2% mercuric chloride solution for 10 minutes. Following this, seeds were rinsed 3-4 times in sterile distilled water to remove mercuric chloride. Surface sterilized pre-weighed Vigna radiata and Macrotyloma uniforum seeds were imbibed individually for 8 hours with distilled water. Seeds were germinated using a wet muslin cloth for 42 hours until the radicle length reached approximately 1 cm. After germination, seeds were de-hulled manually, allowed to dry for 10 days in a dry place under the shade at Room Temperature (RT). Succesively dried seeds were powdered then stored separately in sealed containers at 4°C [37, 38]. 100 grams of Trigonella foenum graecum was weighed, roasted under mild heat, cooled, and powdered. Cuminum cyminum and Piper nigrum, each weighing 100 grams, were powdered at home. Powdered Curcuma longa (Aschi brand Turmericic powder) and Zingiber officinale (sukku) were purchased from the local market of Puducherry, India.

Using the powdered seeds and turmeric powder, 0.5% percentages of porridges were prepared by suspending 0.5 g of Vigna radiata and Macrotyloma uniforum, 0.025 g of Trigonella foenum graecum, Cuminum cyminum, Zingiber officinale, Piper nigrum, and Curcuma longa. The dissolved mixture in 30 mL distilled water then added to 70 mL boiling distilled water and permitted to boil for 5 minutes with constant stirring. After boiling, the mixture’s volume was made up to 100 mL using hot distilled water, cooled to RT, and filtered through what-man no 1 filter paper. The filtrate collected was used for further study.

3.3. Nutritional Evaluation Of The Porridge

The total carbohydrate content of the porridge was determined by phenol sulfuric acid method, total protein by Bradford method, free amino acid by Ninhydrin method, total fat by Soxhlation method as described by [1, 27, 60]. The antioxidant activity of the porridge was determined by DPPH radical scavenging action. The composition of flavonoids and polyphenols was determined as described by [60]. The anti-nutritional factors such as oxalate and phytic acids were de-
terminated as explained by [1]. A sensory test of the porridge was done by a group of individuals (N= 30) to evaluate its aroma, appearance, taste, texture, and overall acceptance. The porridge was sensory assessed by a 9-point hedonic scale for liking and disliking preference [25].

### 3.4. Animal Experiment

24 male, BALB/C albino mice (weighing between 20-25 grams) were purchased from Biogene animal facility, Bangalore. Animals were housed 3 in a polypropylene cage and maintained at a temperature of 23°C ± 1°C and relative humidity of 60 ± 10% with 12 hours light and dark cycle in Central Animal House Facility, Pondicherry University. The animals were allowed free access to drinking water and feed ad libitum. After a week of quarantine period and acclimatization, the animals were randomly allocated into four groups (n=6). All the animal experiments were conducted as per the instructions and guidelines by the Institutional Animal Ethics Committee, Pondicherry University (PU/CAHF/22nd IAEC/2018/01, Dated 03.09.2018).

#### Group I: Control animals fed with Lieber-Dercarli regular diet for 8 weeks (n=6).

#### Group II: Animals were administered with freshly prepared porridge (8 ml/kg body weight orally) every morning between 9:30-10:30 am along with Lieber-Dercarli control diet for 8 weeks (n=6) [54].

#### Group III: Animals were supplied with Lieber-Dercarli ethanol diet for 8 weeks [14].

#### Group IV: Animals were supplied with Lieber-Dercarli ethanol diet and are treated with freshly prepared porridge as in Group II for 8 weeks.

At the end of the experimental period (8 weeks), animals were anesthetized with isofluorane inhalation. Blood samples were collected by retro-orbital puncturing. The animals were then euthanized by administering thiopental-sodium (50 mg/kg body weight, intraperitoneally). The liver was excised and washed with phosphate-buffered saline, and the wet weight was noted. A portion of the liver's median lobe was excised and fixed in 10% neutral buffered formalin. The rest of the liver tissue and separated sera were stored at -80°C until further analysis.

### 3.4.1. Serum Analysis: The serum samples were analyzed for albumin, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), and Alkaline Phosphatase (ALP) using commercials kits from Agappe Diagnostic Pvt Ltd and Beacon Diagnostic Pvt Ltd. The serum triglyceride (TG) was quantified using readymade kits (Beacon Diagnostic Pvt Ltd).

### 3.4.2. Evaluation of Hepatic Antioxidants:

The hepatic enzyme activity (U/mg of protein) [31, 56]. The Ellman method described, and the activity of enzymes are represented as specific activities.

### 3.4.3. Histological Evolution:

The neutral buffered 10% formalin-fixed liver samples were embedded in paraffin block and sectioned with a microtome (LeicaTM, Germany). The tissue sections were stained with hematoxylin and eosin (H&E) and Sirius red (0.1%) in saturated picric acid and observed under bright-field microscopy. The images were photographed using Olympus U-RFLT50 equipped with an imaging system. The Sirius red-stained liver sections were staged by the Ishak method for liver cirrhosis where 0-no fibrosis, 1-few portal fibrosis with or without septa, 2-moderate portal fibrosis with or without bridges, 3-moderate fibrosis with few bridges, 4-liver fibrosis with multiple bridges, 5-fibrosis with occasional nodule, and 6-cirrhosis [17].

### 3.4.4. Statistical Analysis: All the data were conveyed as the mean ± SEM. GraphPad Prism software was used for statistical evaluation. Statistical analysis was accomplished with one-way ANOVA followed by Tukey’s multiple tests. P<0.001, P<0.01, and P<0.05 were measured as statistically significant.

### 4. Results

#### 4.1. The Herbal Porridge Contains High Nutritional Composition and Fewer Anti-Nutritional Factors

(Table 1) is the representation of the composition of the herbal porridge. As displayed in the table, the herbal enriched germinated green gram and horse gram porridge contains the carbohydrate, amino acids, crude fats, and protein. The prepared porridge is rich in carbohydrates, protein, and amino acids and displayed reduced crude fat content. The anti-nutritional factors such as oxalate and phytic acid are reduced in the herbal porridge. The porridge also demonstrated the DPPH radical scavenging potential at a rate of 70%. The antioxidant property of the herbal porridge is due to the presence of flavonoids and polyphenols. The sensory studies provided an excellent overall acceptance score, as displayed in (Table 2). The prepared herbal enriched nutrient porridge showed an attractive aroma and taste as observed with scoring.

<table>
<thead>
<tr>
<th>Content</th>
<th>Amount range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional factor</td>
<td>283.57 ± 10.98</td>
</tr>
<tr>
<td>Carbohydrate (mg/dl)</td>
<td>86.7 ± 6.02</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>10 ± 1.15</td>
</tr>
<tr>
<td>Amino acid (mg/dl)</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Anti-nutritional factor</td>
<td>5.59 ± 0.46</td>
</tr>
<tr>
<td>Oxalate (mg/dl)</td>
<td>13.6 ± 0.4</td>
</tr>
<tr>
<td>Phytic acid (mg/dl)</td>
<td>70 ± 5.29</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>DPPH radical scavenging activity (%)</td>
<td>237.12 ± 4.99</td>
</tr>
</tbody>
</table>

Table 1: Representation of the nutritional, antioxidant, and anti-nutritional factor composition of the herbal porridge.
Table 2: Representation of the sensory characteristics of the nutrient herbal porridge

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Grade</th>
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<tbody>
<tr>
<td>Aroma</td>
<td>7.23 ± 0.25</td>
</tr>
<tr>
<td>Appearance</td>
<td>6.4 ± 0.23</td>
</tr>
<tr>
<td>Taste</td>
<td>6.27 ± 0.30</td>
</tr>
<tr>
<td>Texture</td>
<td>6.93 ± 0.20</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>6.87 ± 0.21</td>
</tr>
</tbody>
</table>

4.2. Nutrient Herbal Porridge Supplementation has Improved the Liver Index Of ALD Mice

The gross weight of body, liver, and liver index of porridge supplied healthy and steatotic mice were shown in Table 3. There were no significant changes in the initial body weight, while a decrease in body weight by 1.27-fold (P<0.05) is noticed in alcoholic steatotic mice (Group III) in comparison to the control (Group I). There is an increase in the bodyweight of porridge supplied steatotic animals (Group IV) by 1.16-fold (P<0.001) in comparison with alcoholic steatotic animals (Group III). There is an increase in the liver weight of alcoholic steatotic animals (Group III) by 1.4-fold (P<0.05) when related to the control animals (Group I). Porridge supplied steatotic animals (Group IV) showed a decrease in liver weight 1.62-fold (P<0.05) to the alcoholic steatotic animals (Group III). The liver index in the ALD animals (Group III) has a 1.7-fold (P<0.01) decrease than control animals (Group I). The liver index of the porridge supplied ALD (Group IV) is 1.83-fold (P<0.05), significantly higher than ALD animals (Group III), and is close to control animals (Group I). This data suggest that supplementation of nutrient porridge reduces the risk of ALD. Alcoholic fatty liver mice (Group III) have exhibited 1.81-fold decrease (P<0.01) in serum TG when related to the control mice (Group I). Porridge supplied alcoholic fatty liver animals (Group IV) showed 1.63-fold increase (P<0.001) in serum TG than alcoholic fatty liver animals (Group III).

4.3. Herbal Porridge Reduced the Hepatocyte Damage in ALD Mice

Alcoholic fatty liver causes damage to the hepatocyte, determined by serum liver biomarkers such as albumin, SGOT, and SGPT. The result is shown in figure 1. The fatty liver induced animals (Group III) have 2.4- & 2.2-fold elevated levels of SGOT (P<0.01) and SGPT (P<0.05) when compared to the control animals (Group I). The albumin biosynthetic potential of the liver was not altered significantly in the healthy animals supplied with porridge (Group II). Feeding of alcohol diet has decreased considerably (P<0.001) serum albumin level than control animals indicating ALD development. Nutrient herbal porridge supplementation has managed the serum albumin level close to control animals (Group I) and significantly lesser (P<0.01) than ALD mice (Group III). Supplementation of nutrient herbal porridge to healthy animals (Group II) has displayed negligible changes in hepatocyte damage markers in serum when related to control mice (Group I). Porridge-fed steatotic mice (Group IV) showed 1.42-fold SGOT and 1.48-fold SGPT levels negligible than control animals (Group I). When compared with alcoholic steatotic mice (Group III), there is a reduction in SGOT (1.7-fold, P<0.01) in addition to SGPT (1.5-fold, P<0.01) levels in the porridge fed alcoholic steatotic mice (Group IV). This report clarifies that the supplementation of nutrient herbal porridge reduced the hepatocyte damage in ALD mice.

4.4. Herbal Enriched Porridge has Increased in the Hepatic Antioxidant and Reduced Lipid Peroxidation Rate in ALD Mice

Hepatic antioxidant changes concerning steatosis and porridge supplementation are displayed in Table 4. The Lieber-Dercarli ethanol diet-induced alcoholic fatty liver (Group III) has reduced hepatic antioxidants such as Catalase (1.51-fold, P<0.05), SOD (1.55-fold, P<0.05), GPx (1.21-fold, P<0.05), GR (1.3-fold, P<0.05) and GSH (1.9-fold, P<0.05) significantly when related to control mice (Group I). Normal healthy animals administered with porridge (Group II) have displayed no significant changes in the hepatic enzymatic antioxidants when related to control (Group I). Porridge-fed steatotic mice (Group IV) showed minor hepatic antioxidant changes such as 1.15-fold for catalase, 1.13-fold forSOD, 1.08-fold for GPx, 1.05-fold for GR, and 1.17 fold for GSH when compared with control liver (Group I). In comparison with alcoholic steatotic animals (Group III), there is an increase in hepatic Catalase (1.32-fold, P<0.01), SOD (1.37-fold, P<0.05), GPx (1.12-fold, P<0.05), GR (1.2-fold, P<0.01) and GSH (1.63-fold, P<0.01) in porridge supplemented alcoholic fatty liver animals (Group IV). The supplementation of nutrient herbal porridge has managed the hepatic enzymatic and non-enzymatic antioxidants in ALD mice.

LPO rate in hepatic tissue of alcoholic fatty liver was determined as Thiobarbituric Acid Reactive Substance (TBARS) and displayed in (Figure 2). Fatty liver mice (Group III) have exhibited 2.08-fold (P<0.001) significantly enhanced the TBARS level than control (Group I). Porridge administered alcoholic fatty liver animals (Group IV) has established levels of TBARS, which is 1.47-fold lesser than alcoholic fatty liver mice (Group III) significantly, P<0.01. Whereas porridge-treated healthy animals showed a 1.03-fold reduction in lipid peroxidation compared to the control animal (Group I), indicating the porridge's antioxidant nature.

4.5. Nutrient Porridge Supplementation has Reduced Hepatic Fat Accumulation and Liver Fibrosis in ALD Mice

(Figure 3) and S1 is the representation of the H&E-stained liver sections (100× & 400× magnification) of the control (Group I), porridge supplied healthy animal (Group II), ALD (Group III), and porridge provided ALD mice (Group IV). The control and healthy animals displayed the normal histology of the liver. ALD mice showed the accumulation of fat as a microvesicle from the portal area to the central vein. Supplementation of the porridge to ALD mice has reduced fat accumulation and displayed significantly reduced macrovesicular changes. The ALD mice (Group III) revealed high macro-
sicular changes with reduced microvesicles. Still, the supplementation of nutrient herbal porridge has reduced the macrovesicular changes and displayed microvesicular changes supporting its preventive effect against ALD. (Figure 4) represents the Sirius red staining of the liver sections at 100× magnification. Control and porridge supplied animals displayed no deposition of extracellular matrix in the hepatic parenchyma. The Sirius red positivity is only noticed in the central and portal vein area due to the endothelial cells rich in the extracellular matrix. Hence the group 1 and 2 animals received the Ishak score of 0. ALD mice (Group III) displayed portal fibrosis and is extending and gains the Ishak score of 2, which is significantly higher than control. Administration of nutrient herbal porridge to the ALD mice (Group IV) displayed no fibrosis like control animals.

Figure 1: Representation of the serum liver markers such as albumin (A), SGOT (B), SGPT (C), and TG (D) in nutrient porridge supplied healthy and alcoholic fatty liver mice. The data are represented as mean with SEM. “a”-Vs. Control animals (Group I) & “b”-Vs. Alcoholic fatty liver animals (Group III). Observed differences with P<0.001 (*), P<0.01 (@), and P<0.05 (&) values were considered statistically significant. SGOT- Serum glutamate oxaloacetate transaminase, SGPT- serum glutamate pyruvate transaminase, TG- triglyceride, and Vs-versus

Figure 2: Representation of the hepatic TBARS content in healthy control & porridge supplied healthy and alcoholic fatty liver. The data are represented as mean with SEM. “a”-Vs. Control animals (Group I) & “b”-Vs. Alcoholic fatty liver animals (Group III). Observed differences with P<0.001 (*), P<0.01 (@), and P<0.05 (&) values were considered statistically significant. TBARS- Thiobarbituric acid reactive substance and Vs-versus
Figure 3: Representation of the histological changes of the liver upon nutrient porridge supplementation to healthy and alcoholic fatty liver mice under the magnification of 100×. Labeling such as CV represents the central vein.

Figure 4: Representation of the siris red staining for liver fibrosis in nutrient porridge supplied alcoholic fatty liver mice under 100× magnification. Labeling such as CV represents the central vein, PV- denotes portal vein area, and the arrow represents the fibrosis.

5. Discussion

Liver is the major metabolic organ involved in bile synthesis, bilirubin and nutrient metabolism, vascular and hematologic function, detoxification, minerals, and vitamin storage. The liver actively metabolizes the gut-derived metabolites, and the entry of toxins through the gut predominantly affects the liver [44]. The fungal toxins, alcohol, and bacterial derived lipopolysaccharide are known to induce liver maladies. Geographically, alcohol intake differs from beer (34%) of total alcohol intake frequent in Europe and America. However, overall, the spirit contributes 45% of the alcohol intake was highest in 2021, V6(6): 1-6.
Southwest Asia (88%), Western Pacific (59%) as well as Middle East Mediterranean (48%). Globally, the current percentage of drinkers is 54% males and 32% females, with a 3.8 male to female ratio [33]. The article about alcohol and health by World Health Organization (WHO) in 2018 states that Indians consumes 2.4 liters of liquor in 2005, which is 4.3 liters in 2010 and mounted up to 5.7 liters in 2016. As per the report, the highest alcohol intake is noted in South-East Asia, from 2005 to 2016 and responsible for more than 3 million people mortality, more than three-quarters of men’s were affected and accounts for 5% of worldwide disease load. The report emphasized that 51.1 males & 27.1 females per 100,000 population had liver cirrhosis (“WHO | Global status report on alcohol and health 2018” 2019). It is also observed that the prevalence and the incidence of ALD are high than other liver diseases and are predicted to increase up to 19.3/million from 16.6/ million by 2030 [30]. Excessive alcohol consumption is the major risk factor for ALD, and the other risk factors include age and gender. Women have a high risk for ALD development compared with men, even at lower alcohol consumption due to lower gastric alcohol dehydrogenase levels & synergistic oxidative stress by estrogen and body fat [12, 16]. Consumption of alcohol by obese individuals has a high risk of liver cirrhosis than the non-obese individuals due to alcohol mediated nitrosative stress, adiponectin resistance, macrophage activation, and mitochondrial stress (Jun Xu et al., 2011). The liver predominantly metabolizes ethanol. Alcohol is primarily processed by ADH, followed by CYP2E1 and mitochondrial catalase. Primarily ethanol is oxidized by ADH to acetaldehyde, a volatile toxic substance that later gets oxidized by acetaldehyde dehydrogenase (ALDH), forming acetate. In excess consumption of alcohol, the metabolism is taken up by CYP2E1, which leads to the free radicals mediated oxidative damage to the liver [63]. ALDH gene polymorphism influences alcohol sensitivity, whereas CYP2E1 gene polymorphism is related to the ability to metabolize alcohol [23]. Active conversion of acetaldehyde into acetate results in protein adducts damages nucleic acids, and leads to lipid peroxidation. It also hinders the biosynthesis of GSH, leading to oxidative stress to hepatocytes. The S-adenosyl methionine (SAM) is the methyl group donor in GSH biosynthesis, and ethanol depletes the hepatic SAM synthetase levels in the experimental liver cirrhosis (Anstee & Day, 2012). The microsomal ethanol-oxidizing system (MEOS) in Cytochrome P450 2E1 (CYP2E1) increases the ROS in hepatocytes. The enhanced ROS production by the ethanol leads to the reduction in the antioxidant. It activates cytokines such as tumor necrosis factor-α (TNF-α), and interleukin (IL-1β and IL-6) mediated inflammation and apoptosis [4]. HSCs are a subset of fibrogenic cells as a response to injury and repair mechanisms. Continued stimulation of HSCs secretes the excessive collagen, which is characterized as chronic liver diseases such as liver fibrosis, liver cirrhosis, NASH, and alcoholic hepatitis. Acetaldehyde also enhances type 3 collagen expression in the human hepatic stellate cells (HSCs) [52]. The oxidation process of ethanol causes an increased NADH/NAD ratio that interferes with gluconeogenesis. The acetyl Co-A from the citric acid cycle is diverted to fatty acid production and ketogenesis. Increased NADH/NAD proportion inhibits the β-oxidation process in the mitochondria, and an altered redox state contributes to the fatty liver formation [43, 21]. The alcohol ingestion increases the permeability of bacterial endotoxins such as lipopolysaccharides in the gut, which binds to receptor CD14 of the Kupffer cells, activates MyD88-independent pathway releasing pro-inflammatory cytokines that contribute to the inflammation of the hepatocyte [58]. Induction of TGF-β by the ethanol stimulates the HSCs to synthesize collagen, which leads to fibrosis (Lee & Friedman, 2011). Ethanol consumption elevates the mature SREBP-1 protein levels, which in turn upregulates the SREBP-1 regulated transcription. As a result, ethanol enhances the fatty acid production via SREBP-1 directly through its metabolite acetaldehyde or indirectly through the endoplasmic reticulum stress response, adenosine, as well as lipopolysaccharide (LPS) signaling. It similarly inhibits the negative regulators of SREBP-1 such as adiponectin, Adenosine Monophosphate-Activated Protein Kinase (AMPK), Signal Transducer And Activator Of Transcription 3 (STAT3), and Sirtuin 1 (SIRT1) (Lieber, 2004; Rasineni & Casey, 2012). Acetaldehyde in excess combines with PPAR-α transcription complex and form adducts, inhibits PPAR-α activity, and impairs fatty acid β-oxidation (Tuma & Casey, 2003). Ethanol inhibits phosphatidylcholine biosynthesis, an essential component of the very-low-density lipoprotein (VLDL), reducing triglyceride exportation from the liver to the peripheral organs, which leads to hepatosteatosis [8, 51].

Alcohol abstinence is primarily crucial in the management of ALD. It gets achieved by rehabilitation and multidisciplinary approach such as psychological and pharmaceutical. By abstinence, the steatosis condition reverses, and its progression to the later stages gets halted. Alcohol can be avoided by cognitive behavioral therapy besides motivational enhancement therapy. The increased withdrawal rate is achieved by acamprose and naltrexone, which decreases the number of drinking days in a controlled trial (Menachec & Duseja, 2011). Many other pharmacological agents such as disulfiram, topiramate, baclofen, metadoxine, glucocorticoid, and antitumor necrosis factor-alpha are effectively used to treat chronic ALD [11]. The antioxidant vitamin E supplementation has reduced the ALD by stabilizing the membrane, reducing NFkB stimulation, and inhibiting TNF production, thereby preventing HSCs activation. Alcoholic hepatitis patients received a dose of N-acetylcysteine (150 mg/kg followed by 100 mg/kg/day for one week) and daily doses of vitamins E and A, biotin, zinc, selenium, copper, manganese, magnesium, coenzyme Q, and folic acid for 6 months displayed no significant outcome [54]. Corticosteroids with an anti-oxidant cocktail (vitamins C, β-carotene, vitamin E, selenium, allopurinol, methionine, N-acetylcysteine, and desferrioxamine) showed a low survival rate when compared with corticosteroids at 30 days on ALD [47].
Since liver diseases are poorly diagnosable in the early stage and much conventional therapy fails to treat the ALD, a preventive approach is needed. Several mechanisms contribute to malnutrition, such as decreased calorie intake, reduced intestinal absorption of nutrients, alteration in gut permeability, reduced intestinal enzymes, reduced bile secretion, liver dysfunction, and decreased processing & nutrient storage of the ALD. A high skeletal muscle and visceral protein breakdown contribute to protein-calorie malnutrition [17]. In this current study, the nutrient herbal porridge was made addressing the malnutrition in chronic liver diseases. The addition of herbs further improved the medicinal value of the porridge. Effective food processing strategies were applied to the preparation of nutrient herbal porridge. The horse gram and green gran were germinated to increase the nutritional value, and the dehulling enhanced the reduction of anti-nutritional factors in the porridge. Sprouted cereals have an enhanced amount of calcium, free amino acids, lysine, Vitamin C, methionine, zinc, iron, folate, and tryptophan when compared to the non-sprouted cereals [6]. Dehulling is a process of removing

Table 3: Representation of the changes in the body weight, liver weight, and liver index in nutrient porridge supplied healthy and alcoholic fatty liver mice. The data are represented as mean with SEM. “a”-Vs. Control animals (Group I) & “b”-Vs. Alcoholic fatty liver animals (Group III). Observed differences with P<0.001 (*), P<0.01 (@), and P<0.05 (&) values were considered statistically significant. Vs-versus

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bodyweight (g)</th>
<th>Liver weight (g)</th>
<th>Liver index</th>
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<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
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<tr>
<td>I</td>
<td>33.7 ± 1.04</td>
<td>33.6 ± 0.27</td>
<td>1.68 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>32.27 ± 1.27</td>
<td>34.08 ± 0.7</td>
<td>1.38 ± 0.1</td>
</tr>
<tr>
<td>III</td>
<td>29.32 ± 1.67</td>
<td>26.5 ± 0.77 a^k</td>
<td>2.3 ± 0.09 a^k</td>
</tr>
<tr>
<td>IV</td>
<td>30.1 ± 1.34</td>
<td>30.75 ± 1.54 b^k</td>
<td>1.43 ± 0.17 b^k</td>
</tr>
</tbody>
</table>

Table 4: Representation of the changes in the hepatic enzymatic and non-enzymatic antioxidants such as catalase, SOD, GPx, and GSH in nutrient porridge supplied healthy and alcoholic fatty liver mice. The data are represented as mean with SEM. “a”-Vs. Control animals (Group I) & “b”-Vs. Alcoholic fatty liver animals (Group III). Observed differences with P<0.001 (*), P<0.01 (@), and P<0.05 (&) values were considered statistically significant. SOD- superoxide dismutase, GPx- glutathione peroxidase, GSH-reduced glutathione and Vs-versus

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (U/mf of protein)</th>
<th>SOD (U/mg of protein)</th>
<th>GPx (U/mg of protein)</th>
<th>GSH (mg/g of the liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>161.56 ± 8.77</td>
<td>162.80 ± 5.56</td>
<td>279.89 ± 4.58</td>
<td>378.88 ± 17.22</td>
</tr>
<tr>
<td>II</td>
<td>159.26 ± 8.16</td>
<td>163.35 ± 4.38</td>
<td>251.47 ± 4.72</td>
<td>370.57 ± 28.37</td>
</tr>
<tr>
<td>III</td>
<td>106.75 ± 3.43 a^k</td>
<td>105.09 ± 2.29 a^k</td>
<td>189.12 ± 5.33 a^k</td>
<td>198.61 ± 3.75 a^k</td>
</tr>
<tr>
<td>IV</td>
<td>140.22 ± 3.19 b^k</td>
<td>143.57 ± 4.45 b^k</td>
<td>212.15 ± 4.40 b^k</td>
<td>323.37 ± 10.67 b^k</td>
</tr>
</tbody>
</table>

Figure: Representation of the histological changes of the liver upon nutrient porridge supplementation to healthy and alcoholic fatty liver mice under 400× magnification. Labeling such as CV represents the central vein, the arrow represents the macrovesicular changes, and asters convey microvesicular changes.
the hull of the seed. It increases the fiber content of the product. It reduces anti-nutritional factors like trypsin inhibitors, phytic acid, and tannins, which increases the nutritional compounds, and makes protein digestion easy [9]. The medicinal value of the germinated cereal porridge was improved by the addition of Trigonella foenum graecum, Cuminum cyminum, Zingiber officinale, Piper nigrum, and Curcuma longa. The prepared herbal porridge displayed high nutritional value like high protein, amino acid, and total carbohydrate with less crude fat. Previous studies reported that the availability of protein and carbohydrate increases due to the soaking and germination of the millets. Germinated sprouts have enhanced the activity of proteinases and amylases, which breakdown the complex molecules into simple ones and improve the nutritional content [7]. Porridge prepared from sprouted green gram, horse gram, with other herbs has high nutritional and antioxidant potential. Previous reports prove that the germinated ones have enhanced antioxidant potential compared to the non-germinated ones. Increased redox-active compounds and oxygen radical absorbance capacity is prominent in fenugreek and turmeric [7]. The preventive, therapeutic approach is more reliable for ALD and ALD associate malnutrition treatment. Nutrient porridge could alleviate ALD malnutrition by giving nutritional support to ALD individuals. The addition of various herbal ingredients targeting various stages of ALD pathogenesis could effectively prevent ALD development, or its consumption may block the progression of ALD into more severe forms. 0.5% of the herbal porridge was prepared based on our previous study to scrutinize the preventive effect of medicinal value-enhanced porridge made from germinated cereals against ALD [37]. The supplementation of the herbal porridge to ALD mice has managed the liver index close to the control group indicating its protective effect against the ALD. The porridge supplementation prevented the hepatomegaly in ALD mice than the positive control. The reduction in the bodyweight of the ALD mice might be due to the liver's reduced protein biosynthetic capacity, as evidenced by reduced albumin biosynthetic capacity and the chronic liver diseases associated with malnutrition. The supplementation of the herbal porridge has improved the body weight in the ALD mice. The liver mass of the ALD mice is increased significantly with the reduction in the liver index as documented in many literatures indicating the onset of ALD pathogenesis [69]. As documented in other literature, the Liber-decarli ethanol diet supplementation increased serum liver markers such as SGOT and SGPT and reduced serum albumin. In contrast, the supplementation of the nutrient herbal porridge has reduced the serum marker level close to control and lesser than positive control indicating the protective effect [46]. The reduced serum TG in the ALD mice displayed the TG's poor transport, and porridge supply has managed the serum TG levels. The ALD generates the ROS in the hepatocyte, which hampers the liver's antioxidant enzymes and increases lipid peroxidation. The ALD mice administered with nutrient herbal porridge displayed reduced thiobarbituric acid reactive substance, displaying the enhanced antioxidant status of the liver and reduced lipid peroxidation as displayed in other literature [Jiesi Xu et al., 2016]. This reduction in lipid peroxidation could be due to the reduction in the activity of the CYP2E1 (L. Xu et al., 2018). The enzymatic and non-enzymatic antioxidants were significantly reduced in ALD mice, and the nutrient herbal porridge supplementation displayed better hepatic antioxidant content. The predominant component of the nutrient herbal porridge is the germinated green gram, and horse gram is known to have hepatic protectants like polyphenol and chlorogenic acid (Guan & He, 2015). The richness of antioxidants could alleviated the oxidative stress in ALD mice and maintained the hepatic antioxidant. The green gram, bean sprouts, dhal, and porridge is an essential grain legume in Southern Asia, especially in India. It serves as a source for protein (24-28%) as well as carbohydrates (59-65%) and provides an energy of about 3400 KJ/kg grain (Pataczek et al., 2018). The porridge supplementation has drastically reduced the hepatic steatosis and fibrosis development, whereas the ALD mice displayed macrovesicular changes with the extending portal fibrosis. The reduction in hepatic steatosis might be due to the germinated horse gram and green gram and the herbs. The green gram reduced the insulin resistance and lipogenesis, maintaine the blood glucose level and the plasma and liver fat content. Dietary munk bean proteins suppressed hepatic lipogenesis and also proved efficient against fat accumulation, diet-induced weight gain, hepatic inflammation, hepatic steatosis, and liver fibrosis (Watanabe et al., 2017). The horse gram containing protein inhibits the angiotensin-converting enzymes, has anti-oxidant and anti-carcinogenic property, reduced serum TG, reduces cholesterol levels, increased lean mass, regulates blood glucose levels, protects against pathogens, and contains the beneficial antinutritional factors which have the hypolipidemic effect (Prasad & Singh, 2015). The rich total polyphenol and tannins content of horse gram has reduced the lipid degeneration of the hepatocyte and bile stone formation in LG diet-induced liver damage. The prepared herbal porridge is also displayed a good amount of total phenolic content (Bigoniya et al., 2014). Fenugreek extract has dietary fibers, steroid, saponin, mucilages, flavonoids, and volatile oils, and the saponin is the major contributor to lowering the lipid and glucose in the blood (Kakani & Anwer, 2012). Fenugreek extract treatment to chang liver cells has neutralized the ethanol mediated oxidative stress, enhanced the antioxidant status, and inhibited the apoptotic involvement (Kaviarasan et al., 2006). Black pepper has a significant protective role against the oxidative damage brought by high-fat diet supplementation and maintains the levels of SOD, catalase, and GSH intensities in the liver (Vijayakumar et al., 2004). Zingiber officinale has reduced liver fibrosis by the induction of senescence in the activated HSCs and downregulates the collagen production, and predominantly might be due to the presence of antioxidant compounds such as 6-gingerol and shogaols and helps to overcome alcohol abuse and encourage alcohol abstinence (Motawi et al., 2011; Shati & Elsaid, 2009; Stoilova et al., 2007). Curcumin inhibits HSCs...
activation and and increase the MyD 88 mediated apoptotic induction of activated HSCs (He et al., 2017; Qin et al., 2018). The inhibition of nuclear translocation of NFkB in hepatocytes and downregulates the expression of the pro-inflammatory mediators (reduces pro-inflammatory cytokines like TNF-α, IL-6, IL-1β, INF-γ) which inturn prevents the liver inflammation. Curcumin also actively protects the hepatocyte by evoking the redox signalling system in ALD and also modulates the activity of CYP2E1 and AMPK expression (Farzaei et al., 2018). Cuminum cyminum and Piper nigrum reduces the activity of cholesterol biosynthesis regulatory enzyme such as 3-methylglutaryl-Co A-reductase and the secondary metabolites of cumin such as β-pinene, p-cymene, γ-terpinene serves as an antioxidant (Nalini et al., 2006).

6. Conclusion

The nutrient herbal porridge was made by applying effective food processing strategies such as seed germination, dehulling, and milling, and the porridge displayed high nutritional values and medicinal value. Supplementation of the nutrient herbal porridge to the ALD mice actively prevented ALD progression due to the formulation of the porridge with the herbs containing the various medicinal properties targeting the multiple pathways of ALD progression. The antioxidant status of the ALD liver supplied with nutrient herbal porridge was improved, and this study suggests that consumption of this diet could effectively prevent the ALD in the ALD prevalence zone.

References


