Ayurvedic Formulation of Liv-Pro-08 Reduces Nonalcoholic Fatty Liver Disease in Rats Fed with High-fat Diet

M. Suriyavathana Vedanarayanan †,*, Nandhini Krishnan †

Department of Biochemistry, Periyar University, Salem-11, Tamilnadu, India

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Abstract
Nonalcoholic fatty liver disease (NAFLD) has emerged as a serious obesity-related disorder, and it will continue to be a major liver health issue worldwide in the coming decades. We aimed to determine the effect of Liv-Pro-08 (Nigella sativa, Entada pursaetha, and Ficus glomerata) an oral ayurvedic formulation on rats fed with high-fat diet. Rats were given a high-fat diet for a period of 7 days. After this period, Liv-Pro-08 (250, 500, and 750 mg/kg/body weight was given orally for 7 days. We examined the effect of the high-fat diet on various parameters related to obesity and insulin resistance. In the experimental rats who received the extract of Liv-Pro-08, their lipoprotein profiles were significantly improved compared with those that are not receiving the extract. Also, a slight reduction was observed in serum aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase enzymes. Moreover, Liv-Pro-08 significantly decreased their fasting serum glucose and fasting insulin levels. This experimental study suggests that Liv-Pro-08 can act as a therapeutic tool in preventing NAFLD progression (i.e., reducing hepatic lipid accumulation). Although further investigations and large randomized trials should be conducted, ayurvedic Liv-Pro-08 oral formulation may be a potential natural drug for NAFLD in the future.

1. Introduction
Nonalcoholic fatty liver disease (NAFLD) is becoming a major public health problem due to the increasing incidence of diabetes, obesity, and insulin resistance in India for the last two decades [1,2]. NAFLD comprises a wide spectrum of liver diseases that develop in the absence of significant alcohol consumption (b20 g ethanol/day) or other known causes of steatosis (e.g., abuse of drugs or toxins). The earliest and most common type of this liver disease is simple steatosis, which has long been thought to be a relatively benign state of liver injury. However, results from human studies indicate that fatty livers are more vulnerable to injury from various causes [3], and they can progress to steatohepatitis, thereby increasing the probability of further liver-related morbidity and mortality [4].
A diet high in fat may also lead to fat accumulation in the liver. It progresses to increased mobilization of the fatty acids from peripheral adipose depots; decreased utilization of fatty acids by the liver; increased hepatic fatty acid synthesis; decreased secretion of fat from the liver; and increased esterification of fatty acids into triglycerides [5,6]. This condition may lead to net fat accumulation in hepatocytes.

NAFLD affects approximately 15%–40% of people living in Western countries and 9%–40% of those in Asian countries. Urbanization and associated lifestyle changes, such as sedentary lifestyle and a fat-rich diet, adversely affect the risk factors of diabetes, unmasking the high genetic tendency that already exists in the Indian population. It has also been shown that Asian Indians have a higher body fat percentage and an adverse body fat pattern, including abdominal adiposity even when the body mass index is within currently defined normal limits [7]. Attempts to control its ill effect with lifestyle measures, physical activity, and antiobesity drugs are currently in vogue. However, herbal medicines have been used to treat liver diseases for a long time. A number of herbal preparations are available on the market, but there is scarce information about the effect of medicinal plants on NAFLD.

Herbal medicines are considered "nature's pharmacy" and form a major component in all indigenous traditional medicines [8]. It is a common element in Ayurveda, homoeopathy, naturopathy, Unani, traditional oriental, and Native American Indian medicines [8]. The sophistication of herbal remedies used around the world varies with the technologic advancement of the countries that produce and use them [8]. The plants used for the present study were *Nigella sativa*, *Entada pursaetha*, and *Ficus glomerata* (Liv-Pro-08). We studied the possible protective role of Liv-Pro-08 with NAFLD rats that were fed a diet high in fat.

*Nigella sativa*, which is one of species of the *Ranunculaceae* family, is a seed that is frequently added to bread and pickles as a flavoring agent. The seeds have been used as a natural remedy for many ailments over many centuries [9,10]. *E. pursaetha* belongs to *Mimosaceae* family. The *Entada* species have two amorphous saponins. The seeds have various medicinal uses, e.g., as a topical application in an ointment for the treatment of jaundice [11]. *Ficus glomerata* (family: *Moraceae*) is more commonly known as an atthi in Tamil and a cluster fig in English. The fruits are effective against leprosy, diseases of the blood, fatigue, bleeding nose, cough, diabetes, and leucoderma [12]. Both the root and the bark are credited with hypoglycemic activity [13]. *N sativa*, *E pursaetha*, and the dry fruit *Ficus glomerata* have been part of a high nutritional diet in many parts of the world, especially *N sativa* [14] and *F glomerata*, which are used as spices in cooking and as a carminative and diuretic by Asians all over the world.

2. Materials and methods

2.1. Sample collection

The seeds (*N sativa* and *E pursaetha*) and dry fruit (*F glomerata*) were collected from Kolli Hills in the Namakkal District, Tamilnadu, India. Historically, plants have been used as a folk medicine against various types of diseases [15].

2.2. Preparing the extract

The seeds and the fruit were washed, shade dried, and then made into fine powder; 1 g of the fine ground sample was weighed into a test tube, 10 ml ethanol was added, and the sample was then extracted for 2 hours in a water bath at 65 °C. After extraction, the sample was cooled to room temperature and then centrifuged at 1500 rpm for 15 min. The sample residue was extracted repeatedly with 30 ml of ethanol. The extract was filtered with filter paper and evaporated to remove the ethanol. Then, the concentrated extract was stored at 4 °C until use.

2.3. Animals and experimental protocols

Five male albino rats of the Wistar strain that weighed 200–230 g were obtained from animal stock in the Institute of Veterinary Preventive Medicine (IVPM), Ranipet, Tamilnadu. The animals were housed in a well-ventilated experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. Feeding of animals was done *ad libitum*, along with drinking water. All experiments on the rats were carried out in absolute compliance, which also considers the ethical guide for the care and use of laboratory animals. Rats were given a normal diet for 1 week in order to adapt to the vivarium conditions, and then they were randomly divided into five groups (*n* = 1 per group). The five groups consisted of the normal controls, those fed a high-fat diet, and those fed a high-fat diet with water-soluble fraction of the ethanol extract of Liv-Pro-08 in various concentrations (250, 500, and 750 mg/kg.b.wt). The hepatic steatosis model was induced by a high-fat diet. Rats were given the high-fat diet (coconut oil, 1 ml; egg yolk, 1 g; ghee, 1 g, and vanaspathi, 1 g) by mouth every day for 1 week, with the exception of the control group that received equal volume of saline alone. In the treatment groups, 250, 500, and 750 mg/kg.b.wt of Liv-Pro-08 were given orally over a period of 7 days in rats given the high-fat diet. After 7 days the rats were sacrificed and blood samples were collected for biochemical analysis.

2.4. Biochemical analysis

The serum level of the lipoprotein profiles ([total cholesterol (CHOD/PAP method)], triglycerides (GPO/PAP method), Span diagnostics Ltd, G.I.D.C. Sachin 394230, SURAT, GUJARAT), high-density lipoprotein ([HDL], polyethylene glycol precipitation method) and low-density lipoprotein ([LDL]) were determined by using enzymatic kits. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assessed by kit method (ready-made reagent, it includes all the compositions) [16,17].

2.5. Insulin resistance status

Serum insulin was assayed using the kit method. The physiologic index of insulin resistance was the homeostatic
model assessment of insulin resistance (HOMA-IR), which was assessed from fasting glucose and fasting insulin concentrations using the following formula: HOMA-IR = [fasting insulin x fasting glucose] / 22.5.

2.6. Statistical analysis

All values are expressed as mean ± standard deviation (SD). The significance of the differences between the means of the tests and of the controls were calculated by a one-way analysis of variance, and p values less than 0.05 were considered significant.

3. Results

Ayurveda is a type of traditional medicine that deals not only with the treatment of some diseases, but it is a complete way of life [18]. This Indian system of medicine has laid down principles and methods of treatment for various diseases, including chronic illnesses, where there is no definite curative treatment [18]. Symptomatic relief is the only existing treatment option. Our qualitative phytochemical results showed the presence of alkaloids, phenols, flavonoids, and steroids. The Liv-Pro-08 was found to be safe up to 1000 mg/kg.b.wt with no sign of change in the behavioral patterns of the rats, which suggests that the plant extract is nontoxic and appears to be safe.

3.1. Effects of Liv-Pro-08 on insulin resistance

Fig. 1 represents the effects of Liv-Pro-08 at doses of 250, 500, and 750 mg/kg.b.wt, once a day for 7 days. It shows that Liv-Pro-08 reduced NAFLD in a dose-related manner. The oral administration of Liv-Pro-08 at 250, 500 and 750 mg/kg.b.wt decreased the progression of NAFLD. Rats fed a high-fat diet for 2 weeks showed no difference in body and liver weight in either of the groups.

The physiologic index of insulin resistance was assessed from fasting glucose and fasting insulin concentration using the HOMA-IR approach. Treatment with Liv-Pro-08 significantly decreased fasting serum glucose in group III (250 mg/kg.b.wt), IV (500 mg/kg.b.wt) and V (750 mg/kg.b.wt) (103.33 ± 0.76, 107.16 ± 1.04 and 109.13 ± 1.04, respectively) when compared with group II (119.80 ± 0.51), whereas group III was similar to group I (103.00 ± 0.50). Insulin levels and the HOMA index were higher in rats fed a high-fat diet (12.80 ± 0.10 and 68.15 ± 0.78) than in controls (12.10 ± 0.36 and 55.36 ± 1.75), and serum insulin and HOMA level were decreased significantly in the treatment groups who received 250, 500 and 750 mg/kg.b.wt (12.20 ± 0.20, 12.06 ± 0.20, 12.10 ± 0.36 and 56.02 ± 1.19, 58.36 ± 0.66, 58.70 ± 2.04, respectively). This was similar to that of the control group.

3.2. Effects of Liv-Pro-08 on the serum lipoprotein profile

To analyze the possible role of Liv-Pro-08 in lipid metabolism, which is the key factor in the formation of the fatty liver, Triglycerides (TG), Total cholesterol (TC), HDL, and LDL were investigated. The increased serum levels of TG, TC, as well as the serum LDL level, were significantly suppressed (Fig. 2), whereas the decreased serum HDL (high-fat diet group: 28.50 ± 0.30) level was obviously elevated by the Liv-Pro-08 treatment in the rats fed the high-fat diet compared with the control group (31.33 ± 1.52). These results suggest that the Liv-Pro-08 can prevent hepatoaestasis via the down regulation of lipid accumulation in serum.

3.3. Effects of Liv-Pro-03 on the serum liver marker enzymes

The level of liver marker enzymes is determined to assess liver function. Serum liver marker enzymes (ALP, ALT, and AST) in all treatment groups (250, 500 and 750 mg/kg.b.wt) were lower than the group II relative to the control rats. Treatment with Liv-Pro-08 in groups III, IV, and V had significantly reduced serum levels of the aforementioned enzymes (ALP, 113.50 ± 0.50 to 117.86 ± 1.10; ALT, 32.10 ± 0.10 to 32.53 ± 0.25; and AST, 22.13 ± 2.20 to 25.83 ± 0.60), whereas the 250 and 500 mg/kg.b.wt groups

![Figure 1](image-url)
Liv-Pro-08 reduces NAFLD in high-fat fed rats

Figure 2  Effect of the Liv-Pro-08 on serum lipid profile. Group I (control), group II (high-fat diet), group III (high-fat diet + 250 mg/kg.b.wt), group IV (high-fat diet + 500 mg/kg.b.wt), and group V (high-fat diet + 750 mg/kg.b.wt). Results shown as mean ± SD (n = 1) of triplicate measurements of each sample. HDL = high density lipoprotein; LDL = low density lipoprotein; TC = total cholesterol; TG = triglyceride. *p < 0.05 compared with a high-fat diet. †p < 0.05 compared with controls.

had a significant 5% level in transaminase release in the blood compared with the control group (Fig. 3). This result implies that the Liv-Pro-08 has a protective effect against high-fat-induced liver damage.

4. Discussion

NAFLD is a common disease characterized by excessive triglyceride accumulation in the liver. Nowadays, widely grown herbs and herbal formulations have been found to possess beneficial activities for treating hepatic disorders [19]. As with most human diseases that driven by diet, the fatty liver in rodents is also diet-inducible. A high-fat diet increases liver fat levels quite rapidly, e.g., within days, even before significant increases in peripheral fat deposition occur. High-fat diet could play a potential role in the pathogenesis of NAFLD. Our study results demonstrated that secondary metabolites, particularly alkaloids, flavonoids, phenols, and saponins from the Liv-Pro-08 possessed a significant hepatoprotective effect based on the determination of lipid profiles and hepatic enzymes (Figs. 2 and 3), suggesting that these secondary metabolites may be developed as a potential hepatoprotective drug. Phytochemical investigations of the ethanolic extract of Liv-Pro-08 possess secondary components, which may reduce NAFLD.

A recent trial studied a choline deficient and a high-fat diet to examine the effects of excess liver fat on the insulin resistance and glucose tolerance that accompanies diet-induced obesity [20]. The results suggested that the administration of Liv-Pro-08 protected against the development and progression of hepatic steatosis induced by a high-fat diet. As a causative factor of NAFLD and supported by reductions in serum lipids (TC, TG and LDL), the researchers observed Liv-Pro-08-administered in albino rats. Free fatty acids are esterified into TG and then exported as VLDL. Failure of synthesis and secretion of VLDL can further contribute to TG accumulation in the liver [21]. Al-Hader et al. reported that the N sativa seed decoction reduced blood glucose within 1 month, as well as plasma cholesterol and triglycerides thereafter. Indeed, Wang et al. demonstrated protective effects against hepatic steatosis in rats fed a high-fat diet. They showed that the flavonoids of Litsea coreaneleve also improved the release of adipokynes and increased the expression of the peroxisome proliferator activated receptor-α [22].

Dysregulation of triglycerides and free fatty acids, which result in the accumulation of lipids in the liver and the induction of oxidative and inflammatory stress, favor the progression of NAFLD. They were markedly reduced in rats in the high-fat diet group whose diet was also fortified with the Liv-Pro-08. Hepatic lipid accumulation generally occurs as a result of an increase in the supply of circulating free fatty acids to the liver, but it can also result from increased endogenous hepatic fatty acid synthesis [23]. Aqueous extract of N sativa normalized body weight in the obese diabetic models of Psammomys obesus [24] or Meriones shawi [25] sand rats. A significant reduction in blood glucose and cholesterol levels in humans was also reported by Bamosa [26].

The rat model used in this study mimics the typical Indian dietary habits which contains high-fat. The results confirm that a high-fat diet increases the serum levels of triglycerides, LDL cholesterol, as well as serum insulin, and results in an impaired HOMA index. In the case of high dietary intake of fats, animal studies have shown that fatty liver is the result of increased delivery of fatty acids through the portal circulation together with a 25% higher de novo lipogenesis [27]. Petroleum ether extract of the stem bark of N sativa plant reduced blood sugar level significantly [28], and this study supported our present work as very effective and
fruitful. Another study showed that *F glomerata* was found to be safe up to 2000 mg/kg with no sign of mortality or change in behavior. These results suggests that the plant extract is safe and nontoxic [29].

Increased concentrations of serum enzymes such as ALT, AST, and ALP have been observed in diets high in fat, which led to increased permeability, damage, steatosis, and inflammation in our rats [30]. In our work, the results demonstrated that phenols, flavonoids, and saponins from the Liv-Pro-08 possessed a significant hepatoprotective effect based on the determination of hepatic enzymes (Fig. 3). This suggests that the various concentrations of the Liv-Pro-08 treatment significantly reduced liver marker enzymes concentration in high-fat diet-induced groups.

In previous papers, some of the results showed that the lipid fraction of *N sativa* was rich in linoleic acid, which has a beneficial effect on blood lipids, and serum cholesterol [31]. Their unique fatty acid composition, relatively high polyphenol content and quality, and, hence, a high protection against oxidative stress, as well as a relatively good shelf life and other desirable physicochemical characteristics may lead to more diverse and novel applications of the *N sativa* seed in food, pharmaceuticals, cosmetics, and other nonfood industries [32].

*E pursaetha* can be used as a tonic for liver troubles, allaying body pains and arthritis [33]. Khanal and colleagues reported that saponins from the root of *Platycodon grandiflorum* supplementation may antagonize the development of oxidative liver injury by depressing the elevation of the CYP2E1 protein expression and through its ability to scavenge free radicals [34]. *E pursaetha* also has a saponin component that may avert the development of hepatic steatosis.

These results indicate that the Liv-Pro-08 attenuates the disorders of lipid metabolism in liver that resulted from a high-fat diet. The inhibitory effect of the Liv-Pro-08 on hepatic steatosis appeared to be related to the suppression of lipogenesis enzyme activity and the acceleration of fatty acid oxidation in the group that was fed a high-fat diet.

5. Conclusion

Our results suggest that the Liv-Pro-08 reduces hepatic steatosis and has lipid-lowering effects in Wister albino rats fed a high-fat diet. These effects are apparently mediated by the down regulation of lipid molecules involved in the lipogenesis and modulation of the hepatic lipid metabolism-related enzyme activity. These findings suggest that the Liv-Pro-08, which contains secondary plant metabolites, may be useful as a potential dietary intervention for those with hepatic steatosis and hyperlipidemia.

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References


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