Original Article

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Induction of an Animal Model of Non-Alcoholic Fatty Liver Disease Using a Formulated High-Fat Diet

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ABSTRACT

BACKGROUND AND OBJECTIVE: Non-alcoholic fatty liver disease (NAFLD) is a reversible disease that is mainly a result of high-fat diets in humans. This study aims to induce fatty liver by formulating a high-fat diet in rats to provide a simple and accessible model for investigating various aspects of this disease.

METHODS: This experimental study was conducted using 18 male Wistar rats weighing 180±20 g, randomly divided into two groups (n=9). One group was fed with standard diet whereas the other group was fed with high-fat diet (based on animal fat and cholesterol) for 10 weeks. After this period, variables of weight change, glucose, liver enzymes and serum lipid profile were measured and histopathological changes in the liver tissue were investigated and compared between the two groups.

FINDINGS: At the end of the tenth week, the mean triglycerides and serum cholesterol were 53.71±9.1 mg/dl and 56.42±5.7 mg/dl, respectively in control group, revealing a significant difference compared with the high-fat group (90.85±13.4 mg/dl and 94.28±9.9 mg/dl, respectively) ($p<0.05$). The level of aspartate aminotransferase increased from 89.85±12.7 IU/L in the control group to 147.84±17.8 IU/L in the high-fat group. Moreover, the level of alanine aminotransferase increased from 46.28±7.2 IU/L in the control group to 86.85±9.2 IU/L in the high-fat group, which was statically significant ($p<0.01$). In addition, histopathological changes in liver including fat vacuole and hepatocyte swelling were observed in the high-fat group.

CONCLUSION: According to the results of this study, a formulated high-fat diet can well induce a non-alcoholic fatty liver in rats.

KEY WORDS: *Non-Alcoholic Fatty Liver, High-Fat Diet, Wistar Rat.*

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Introduction

Fatty liver is a reversible disease that is caused by accumulation of large amounts of triglyceride in liver cells. More than 5% of liver weight consists of fat in patients with this disease (1). Nonalcoholic fatty liver disease (NAFLD), which occurs in the absence of alcohol consumption, is known as a major health problem. In fact, nonalcoholic fatty liver disease is a chronic liver disease, which includes a wide range of clinical symptoms (from asymptomatic fatty liver to severe inflammation of the liver along with fibrosis and sometimes cirrhosis). Insulin resistance and cardiovascular diseases are highly prevalent among these patients (2).

Fatty liver disease was identified and introduced in 1980 for the first time. At that time, damage to liver cells was observed in a group of patients, which was similar to alcoholic patients. However, these patients had no history of alcohol consumption. There was also no sign of other liver cell diseases in these patients. Instead, 90% of them were obese, 25% of them had high blood lipids and 25% of them suffered from diabetes (3). Obesity, hyperglycemia, type 2 diabetes and hyperlipidemia are among the most important causes of nonalcoholic fatty liver disease (4).

Studies have shown that more than 75% of obese people suffer from this disease (5). Aging, history of fatty liver in the family, malnutrition, severe weight loss, medications (such as glucocorticoids and methotrexate) and some diseases (such as inflammatory bowel disease) are among other risk factors of this disease (6). In some studies, a significant relationship was observed between the incidence of this disease and excessive intake of saturated fats or carbohydrates (7).

A large number of patients with fatty liver have a normal weight, though they might have abdominal obesity and insulin resistance. Studies have demonstrated that these groups of patients follow an unhealthy diet (8). Fatty liver is a common hepatic disease. The prevalence of this disease ranges from 2.8 to 30% in different societies and ranges from 20 to 30% in western countries (9). Studies conducted in Iran show that 30% of Iranian people suffer from this disease, while most of them are 40 to 60 years old (10). There are several animal models that can be used to study fatty liver. A methionine and choline deficient (MCD) diet is one such model (11).

Another diet is the one designed by Zou et al. (12). In another study by Asgharpour et al. in University of Virginia conducted using a high-fat diet and a specified amount of glucose and fructose on rats, they were able to induce fatty liver after 8 weeks. They also induced diseases caused by high-fat diet including fibrosis and hepatocellular carcinoma during the 16th to 24th week by continuing the diet into more advanced stages (13). Animals have always played a key role in medical advancements and since some animals are appropriate human-like models for experiments and researches, many medical studies were conducted using animals for the first time (14). The advantages of using animals in clinical researches include modeling the diseases in animals, using animals in medical researches, similarities between humans and animals, short life span of animals and assessment of studied factors within a reasonable timeframe as well as the possibility of controlling the situation.

For creating a nonalcoholic fatty liver model, we currently need a pre-manufactured diet prepared from other countries, which is highly costly, yet hardly accessible. Therefore, this study aims to provide Iranian researchers with a model that includes accessible ingredients and is at the same time convenient and low-cost. Moreover, this model is highly similar to human models and is in accordance with Iranian diets regarding the type of fatty liver induction. For this purpose, we tried to induce nonalcoholic fatty liver disease using a diet based on animal fat and cholesterol to provide a simple and accessible model in our study.

Methods

Animals: This experimental study was conducted using 18 male Wistar rats with mean weight of 180±20 g, prepared from Animal Research Center of Baqiyatallah University of Medical Sciences. Animals were fed with standard laboratory feed and water ad libitum. They were housed in animal house and were exposed to standard conditions of temperature (23±2)°C, humidity of 50±5 %, and natural cycles of light and dark. All the rats were handled according to the ethical principles for animal experiments of International Council for Animal Protection. All the experimental procedures were approved by the research ethics committee of the university.

Design of experiment: Animals were randomly divided into two groups (n=9) and were separately kept in polystyrene animal cages. The first group (control group) was fed with standard rodent food (SD), and the

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other group was fed with (HFD) high-fat diet (based on animal fat and cholesterol) for 10 weeks. Considering the ingredients of standard rodent food, the high-fat diet used in this study included basic rodent food mixed with 15% animal fat, 4% cholesterol (Sigma-Aldrich Corporation, United States) and 1% cholic acid (Sigma-Aldrich Corporation, United States).

The calories and energy in this formulation were adequate to induce fatty liver (table 1). The rats were provided with unlimited amount of food in this period. At the end of each week, the rats were accurately weighed and their weight was recorded. At the end of the tenth week, the rats in both groups were anesthetized. Blood samples were taken and serum samples were prepared and kept at -20°C until the time of experiment. Liver tissue washed in normal saline and sliced into multiple parts and stored. For histopathological investigations, liver sample immerged in a 10% formalin.

Table 1. Ingredients of high-fat diet and standard diet

Group	Standard diet (%)	High-fat diet (%)
Fat	12	22
Carbohydrate	57	50
Protein	28	24
Other ingredients	3	4

Biochemical analysis: The concentration of biochemical parameters including glucose, lipid profile (triglycerides, total cholesterol, HDL-C, LDL-C), AST and ALT activities in serum were measured using Biochemical Kits (Bionics Corporation) and AutoAnalyzer Hitachi 912 (Japan).

Histologic study: Pathologic sections were prepared using common methods of passage in tissue culture, then blocking and incisions with 5 µ thickness and was stained by hematoxylin-eosin. The assessment of pathology sections was done using a semiquantitative and double-blinded scale and was graded based on the level of fat saved in cells, cell swelling and other histological characteristics. Histopathological changes regarding changes in hepatocytes were graded based on the severity of lesion according to the method of Wang et al. and Brunt et al. from zero to four (zero: without steatosis, one: less than 25%, two: 26 to 50%, three: 51 to 75% and four: 76 to 100% of hepatocytes have steatosis) (18).

Statistical analysis: The statistical analysis of data was done using SPSS 16. The quantitative data were analyzed as Mean±SD and the significant differences between groups were analyzed by independent t-test. $p<0.05$ was considered significant. Moreover, the assessment of pathology sections was done using a semiquantitative and double-blinded scale.

Results

The rats in both groups were weighed at the end of each week. The high-fat diet helped rats gain weight significantly in a 10-week period ($p<0.05$). This weight gain started to become significant from the sixth week, indicating the effect of diet on rats' weight gain (Fig 1).

The effect of high-fat diet on blood sugar level and serum lipid profile: At the end of the tenth week, a significant difference was observed between control group (53.71±9.1) and high-fat group (90.85±13.4) regarding mean serum triglyceride ($p<0.05$). Moreover, cholesterol level in control group (56.42±5.7) increased significantly compared with high-fat group (94.28±9.9) ($p<0.05$). But serum levels of HDL-C in high-fat group (39.28±3.1) significantly decreased as compared with control group (22.57±4.5) ($p<0.05$) (Fig 2). High-fat diet significantly increased serum levels of fasting blood sugar, triglyceride (TG), total cholesterol (TC) and LDL-C, compared with control group.

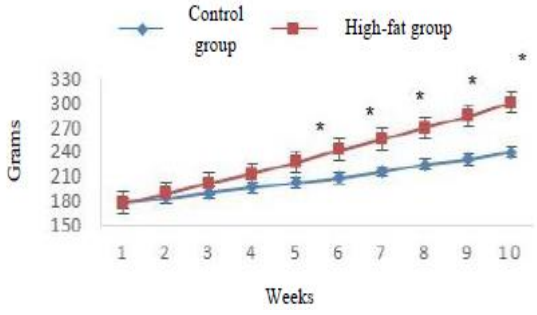

Figure 1. Weight changes during the experiment in SD and HFD treat animals. *p<0.05 and **p<0.01 compared with control group. Weight gain in high-fat group started to become significant from the sixth week compared with control group.

The effect of high-fat diet on liver enzymes: The activity of AST, ALT and alkaline phosphatase (ALP) in serum of HFD increased significantly compared with SD group. The AST activity was (89.85±12.7 IU/L and 147.84±17.8 IU/L) in SD and HFD groups respectively ($p<0.05$), and ALT activity was

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(46.28±7.2 IU/L and 86.85±9.2 IU/L) in SD and HFD ($p<0.05$) (Fig 3).

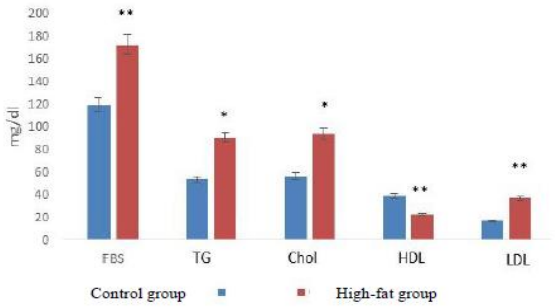

Figure 2. Comparation of serum glucose and lipid profiles in SD and HFD animals. *p<0.05 and **p<0.01 compared with control group. Changes in all indices of high-fat group were significant compared with control group.

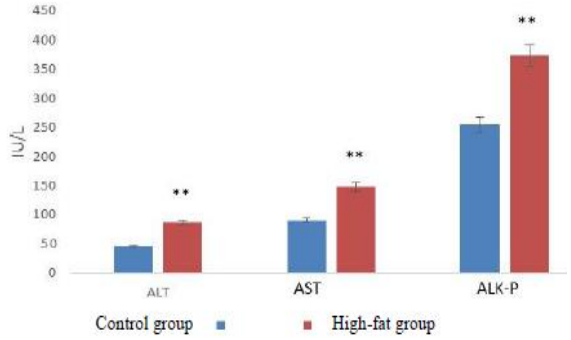

Figure 3. Serum enzyme activities in SD and HFD groups. *p<0.05 and **p<0.01 compared with control group. Changes in liver enzymes of high-fat group were significant compared with control group

Histologic study liver tissue: Obvious changes were observed in HFD hepatocytes compared to SD in appearance and according to macroscopic view (Fig 4). In microscopic studies, no abnormal sign was observed in liver of SD group (Fig5). However, histopathological changes including microvesicular and macrovesicular fat accumulation accompanied by hepatocyte swelling were observed in HFD group (Fig 6).

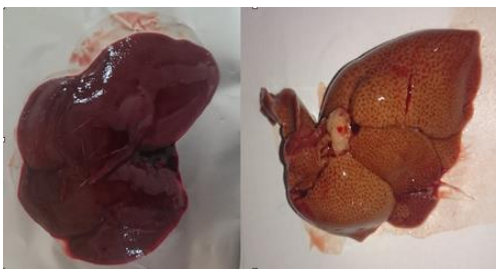

Figure 1. The macroscopic view of fatty liver induced by high-fat diet compared with normal liver. Liver of a rat in high-fat group on the right and liver of a rat in control group on the left

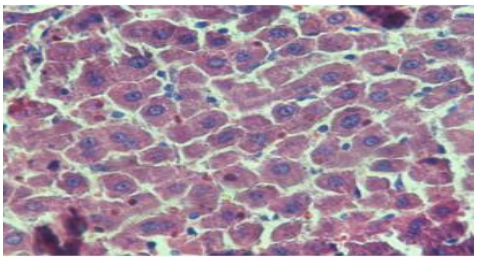

Figure 2. The microscopic view of liver tissue of a rat in control group, revealing normal hepatocytes and normal liver tissue structure

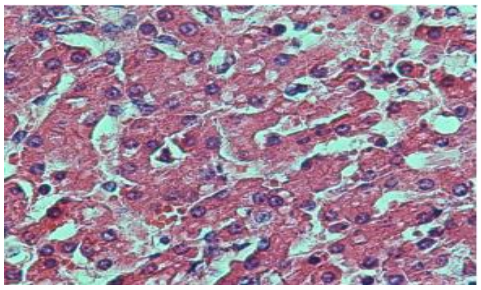

Figure 3. The microscopic view of liver tissue of a rat fed with high-fat diet, revealing microvesicular and macrovesicular fat accumulation.

Discussion

In the present study, nonalcoholic fatty liver disease was induced in rats (without genetic intervention) using a formulated high-fat diet. Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease that can ultimately convert to hepatic cirrhosis and hepatocellular carcinoma. This disease is associated with increased levels of liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood. The increase in cholesterol and triglyceride concentration was more than the concentrations observed in metabolic syndrome (15). Due to increased prevalence of this disease worldwide and particularly in Iran, the significance of studies in this field becomes more conspicuous. Since nonalcoholic fatty liver disease is directly related to people's lifestyle and particularly their diet, creating such a model that is highly similar to human model (diet) can be used by researchers as one of the most appropriate models for therapeutic and pathologic studies by medical researchers. An ideal animal model for fatty liver induction must reflect all aspects of human pathology and different stages of disease (16).

The animal model also needs to be reversible, reliable, simple, cost effective, practically possible and with the least disadvantages (16). Using a methionine and choline deficient (MCD) diet is one such model

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(11). Due to the absence of methionine and choline in this diet, lab animals fed with this diet will lose the ability to synthesize phosphatidylcoline and remove triglycerides from the liver, which ultimately leads to hepatic steatosis. This diet increases the level of reactive oxygen species and thus oxidative stress significantly (17). Therefore, using this diet imposes major drawbacks on the studied parameters. In addition, elimination of these two substances may cause side effects on the mechanisms of other cells, indirectly affecting biochemical and molecular parameters studied in nonalcoholic fatty liver. Moreover, the absence of these two substances is entirely at odds with the main causes of this disease in humans, namely excessive consumption and sedentary lifestyle.

Their absence also causes limitations in the preparation of the model. However, the model used in this study is much more appropriate in terms of similarity to human models and regarding absence of oxidative stress. Another high-fat diet is the one proposed by Zou et al. (12). This diet is more appropriate than methionine and choline deficient (MCD) diet for investigating the hepatic parameters induced by nonalcoholic fatty liver. However, we must note that contrary to the previous diet, this one is essentially possible through daily gavage and this is one of the limitations of this diet, considering the stress imposed on animals because of daily gavage. In addition to possible risk factors of physical damage to rats during this task, gavage imposes a great deal of stress on the animal, which probably affects the studied parameters, particularly indicators of oxidative stress and inflammation. Considering the aim of the study for finding a diet that is most similar to the diet used by the majority of people, a diet was prepared based on animal fat along with a certain percentage of cholesterol and colic acid to increase the level of fat absorption in the intestine. Fats of animal origin with higher levels of glucose, lipid profile and liver enzymes are associated with increased possibility of pathogenicity and risk of consumption. As the results of biochemical indices in this study show, the ingredients added to this diet were able to fulfill the goals of the study. The results regarding liver enzymes, which were in line with lipid profile, indicated hepatic steatosis. The result of histopathologic study indicated fat accumulation and hepatocyte swelling, which confirms the induction of fatty liver. This type of fat, known as animal fat, has so many fans among Iranian people. This type of fat is also associated with the risk of disrupting the lipid balance as well as nonalcoholic fatty liver. The results of this study indicate that the proposed model is an appropriate model for researchers in terms of efficiency, cost and accessibility.

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