

DEVELOPMENT OF ANIMAL MODEL FOR NON ALCOHOLIC FATTY LIVER DISEASE BASED ON MODIFIED DIETS

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is considered to be among the most common liver diseases worldwide. The objective of the study was to develop an animal model for NAFLD using a high fat diet and spices used in the regular diet of humans. Wistar rats were divided into 7 groups of 10 rats each. Control group was fed with normal rat pellets diet and remaining groups were fed with the six different modified fat diets for 28 weeks. Assessment of body weight, AST, ALT, ALP, triglycerides, cholesterol, and glucose levels was done on four weekly bases. Histopathological changes in liver were also observed. Among the six diets used, diet I, Diet II, Diet V and Diet VI showed a significant and consistent increase in the biochemical parameters assessed. Histopathological studies revealed a significant change in the hepatological architecture in the groups I, II, V and VI where conditions like changes in the blood vessels, swelling and vacuolation of hepatocytes and kupffer cell proliferation was observed; which are similar to the conditions in NAFLD. These abnormalities appear within 12 weeks of study and remain persistent till 32 weeks. The changes in biochemical parameters and histology of liver mimic the conditions of NAFLD. Thus the developed model may be used to study NAFLD and to screen new drugs for the treatment of same.

Keywords: High fat diet; Fatty liver disease; Rat model.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a liver disorder observed in patients without a history of significant alcohol consumption that histologically resembles alcohol induced liver damage [1, 2]. Dietary effects on whole-body metabolism and its regulation via effects on hormones, transcription factors, and lipid metabolic pathways are considered to play an important role in NAFLD [3, 4].

As NAFLD is now considered as life style disease, its major causes are abnormal and imbalanced nutrition, decreased physical activity with disproportionate high fat food intake, obesity, type 2 diabetes mellitus and metabolic syndrome [5, 6]. The prevalence of NAFLD has shown substantial increase during past decades and it is going to further increase in future [7]. The mechanism responsible for NAFLD is not well established. The lack of knowledge for its etiology and mechanism are hampering the development of an effective therapeutic approach. Hence it is necessary to develop animal models which can be used to study the pathophysiology and treatment of NAFLD [8, 9]. Studying mechanisms of NAFLD development and for testing potential drugs for NAFLD treatment, most essential requirement is appropriate animal models. These models should show as much similarity as in human NAFLD. However models used in current studies matches partially to human NAFLD. These models include nutritional, drug induced [10], toxic and genetic models [11] of NAFLD in rats. Currently used nutritional models include a high fat diet, methionin cholin deficient diet [12], high saccharose, fructose [13] and cholesterol model [14]. Nutritional high fat animal models seem to simulate human pathogenesis of primary NAFLD most closely [12]. As it is a lifestyle disease, we focused on daily diet fat ingredients and spices to prepare high fat diet used in the current study.

The aim of the present study was to develop a high fat diet rat model to study progression of NAFLD. During the study, six different types of modified fat diets were chosen to monitor the effects of the dietary elements in the development of NAFLD.

Methods

Chemicals

All the chemicals used in the study were procured from Sigma Aldrich, USA. The diagnostic kits required to determine the various biochemical

parameters were purchased from Erba Diagnostics, India. Materials required for the preparation of the high fat diets were purchased from the local market.

Experimental animals

Wistar albino rats (80 to 120g) of either sex were used for the study. Animals were procured from the Haffkine Institute, Mumbai, India. The protocol for the animal study was approved by the Institutional Animal Ethics Committee (IAEC). Wistar rats were housed at $22 \pm 3^{\circ}\text{C}$ in the central animal house facility with standard rat chow and water supply during the period of acclimatization.

Diet preparation

All diets were prepared in the laboratory as per concentration provided in table 1.

Treatment

After acclimatization to the laboratory conditions for one week, the animals were divided into 7 groups containing 10 animals in each. Animals in control group were fed with normal rat pellet diet and other six groups were fed with the six different modified fat diets (Table 2). Each group was fed with approx 100 gm of the diet daily for 28 weeks. After 28 weeks all animals were fed with normal rat food till 32 weeks. Parameters assessed

Body weight

Weight of the rats from each group was monitored every week in order to determine the variation in weight.

Biochemical parameters

Blood samples collection was done using the retro orbital blood extraction method. Plasma was separated by centrifugation (15 Min at 3000 rpm) and stored at -20°C until chemical analysis.

Liver enzymes like aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were estimated after four weeks interval. Biochemical parameters like Triglycerides (TG), Cholesterol and Glucose were also determined after four week interval. Liver enzymes and biochemical parameter estimation was done using standard Erba Diagnostic kits and auto analyzer Erba, Germany [15].

Histopathology

At every four weeks, one rat was sacrificed from

each group and its liver was extracted. Histology of the extracted liver was done under the expert advice of a veterinary pathologist. Liver tissues extracted were fixed in 10% formalin for 24 h, and were embedded in paraffin; 5–6 μm sections were stained with haematoxylin and eosin (H&E) and assessed in a light microscope [16].

Data analysis

Statistical data analysis was performed by using *Sigma stat* software. The data was analyzed by using one way ANOVA (at $\alpha = 0.05$). Data was expressed as mean \pm standard error of mean (SEM).

Results

Body weight

There was significant increase in body weight of experimental groups treated fed with diet group I (237 \pm 2.6gms), diet group II (212 \pm 7.1gms), diet group V (210 \pm 0.5gms) and diet group VI (233 \pm 1.1gms) as compared to control group (180 \pm 4.1gms) throughout the study. Animals from other diet groups did not showed significant change in body weight (fig.1).

Biochemical parameters

Significant changes were observed in the biochemical parameters; triglycerides, cholesterol, and glucose. Also there was significant increase in liver enzymes like alanine transaminase, aspartate aminotransferase and alkaline phosphatase.

Triglycerides

There was significant increase in the triglycerides levels on 12th week of high fat diet feeding in groups. After 24th weeks triglycerides levels significantly increased for high fat diet group I (199.1 \pm 2.073 mg/dL), II (176.9 \pm 1.79 mg/dL), IV (256.2 \pm 9.59 mg/dL) and VI (261.1 \pm 13.47 mg/dL) as compared to the control group fed with normal diet as depicted in figure 2.

Cholesterol

During the study cholesterol levels of all high fat diet fed group showed significant increase as compare to the control diet group after 12 weeks. High fat diet fed group I (187.1 \pm 4.53 mg/dL), group II (154.6 \pm 2.24 mg/dL), group V (178.7 \pm 2.105 mg/dL) and group VI (172.8 \pm 1.622 mg/dL) when compared to the control diet group (67.11 \pm 1.342 mg/dL) after 20th weeks as shown in figure 3.

Alanine transaminase (ALT)

Alanine transaminase (ALT) was significantly increased in high fat diet fed groups I (56.73 \pm 1.806 IU/L) and diet group V (51.98 \pm 2.373 IU/L) when compared to the control diet group (33.41 \pm 1.232 IU/L) after 32 weeks of the study as shown in figure 4.

Aspartate aminotransferase (AST)

Serum aspartate aminotransferase (AST) levels was started showing significant increase after feeding high fat diet for 12 weeks in diet group I, II, V and VI. After 32 weeks of study AST levels significantly high in diet group I (50.75 \pm 1.318 IU/L), group II (78.08 \pm 1.834 IU/L), group V (52.17 \pm 1.821 IU/L) and group VI (56.53 \pm 1.738 IU/L) where as control diet group was showed normal level of AST (37.5 \pm 0.6396 IU/L) as depicted in figure 5.

Alkaline phosphatase (ALP)

Similar pattern of increased levels of alkaline phosphatase (ALP) was observed in high fat diet fed groups I, II V and VI as depicted in figure 6. When compared to the control diet group (75.16 \pm 1.895 IU/L) there was significant increase of ALP levels in group I (124.03 \pm 2.141 IU/L), group II (93.01 \pm 1.14 IU/L), group V (150.4 \pm 1.992 IU/L) and group VI (126.9 \pm 0.8055 IU/L) where as other diet groups did not showed any significant change in the ALP levels.

Glucose

High fat diet fed group I, II, V and VI started showing significant increase in serum glucose from 12 weeks of the diet feeding. After 24 weeks study serum glucose levels were remain significantly high in high fat diet group I (156 \pm 2.69), II (187.5 \pm 7.693), V(181.9 \pm 1.319) and VI (165.8 \pm 6.029) where as control diet group (115.8 \pm 5.273) showed normal levels of serum glucose levels as represented in figure 7.

Histopathology

Hepatic tissue analysis from rats fed with high fat diet revealed a progressive increase in hepatic cell necrosis and inflammatory damage in comparison with control diet fed animals. As observed in fig. 8, control group has shown normal hepatic architecture with no sign of liver disease. Histology of liver from groups fed with diet I (60 % fat), diet V (30% fat + chilli powder + black pepper powder) and diet VI (30% fat + chilli powder + black pepper powder+ mustard powder) showed similar

histological pattern as observed in the liver disease, characterized by ballooning of the hepatic cells, necrosis of the hepatic cells, proliferation of the kupffer cells and change in the blood vessels. Some mild changes of the hepatic cells and blood vessels were observed in the group fed with diet II (30% fat). There were no significant changes observed in the group fed with diet III (60% fat + chilli powder + black pepper powder+ mustard powder) and diet IV (30% fat + chilli powder).

Discussion

Epidemiological studies suggest that development of obesity and insulin resistance occurs more prominently in individuals having a diet rich in fat [17]. Furthermore, excessive amounts of consumption of these energy dense macronutrients increase the odds to develop obesity. Accordingly, diets rich in fat, e.g., 30% –75% of total calories have been proposed to be a useful tool to induce metabolic alterations and NAFLD. Duration of the feeding time and the combination of the fatty acids results in altered metabolism in the rodents; impaired glucose tolerance, insulin resistance, and dyslipidemia which can be studied while developing the rodent model for the NAFLD[18, 19].

Development of an animal model is essential to study the disease and to observe effect of various drugs for its treatment. To study NAFLD, it is important to have an animal model which reproduces the pathological expressions of the human disease and also focus on the period of the development and progress of the disease [20]. The aim of this work was to develop a simple high fat diet animal model to study NAFLD. The study was carried out for 32 weeks by feeding the animals with different composition high fat diets.

Serum aminotransferase levels were elevated in the animals fed with diet I, II, V, and VI as compared to the control group. Recent data from the US National Health and Nutrition Examination Survey (NHANES) reported elevated ALT (>30 U/L) as a marker of potential NAFLD [21, 22]. Similarly, in the study, elevated levels of ALT were observed in the animals fed with diet I, II, V, and VI after the 12th week of feeding and remained elevated till the completion of the study.

Also, elevated levels of AST and ALP were observed in the animals fed with diet I, II, V, and VI which can be correlated with the progression of NAFLD as it has been studied that elevated levels of the AST and ALP are related to the NASH relates progression

of the liver fibrosis [23, 24].

Hyperlipidemia is associated with NAFLD, hence increased levels of the serum triglycerides and cholesterols can be correlated with the advancement of NAFLD [19, 20]. In the study, it was found that the animals fed with diet I, II, V and VI showed hyperlipidemic conditions till 24 weeks which can be due to development of NAFLD. With continuous feeding of same diets after 24th week, decrease in the triglycerides and the cholesterol levels was noted which is possible due to advancement in the liver cirrhosis which results in the lower levels of serum triglycerides and cholesterols [15,25].

The biochemical parameter levels found in the animals fed with diet I, II, V and VI were similar to the condition present in NAFLD [26].

Histopathological studies revealed that there was significant change in the hepatological architecture in the groups I, II, V and VI when compared to the control group. The conditions such as changes in the blood vessels, swelling and vacuolation of the hepatocytes and kupffer cell proliferation were observed in the groups I, II, V and VI which are similar to the conditions found in NAFLD [27, 28]. There was no significant change observed in the hepatocellular architecture of the groups II, III and IV as compared to the control group.

The data of biochemical parameters, supported by histological findings indicates that the rats from group I, II, V and VI developed the conditions which mimic NAFLD, in week 12 of feeding the high fat diet. The results were consistent till the 32 week. Hence, it can be concluded that a rat model can be developed using diets I, V and VI, to study NAFLD disease. Thus, although HFD models require longer feeding periods, they more closely resemble the pathophysiology observed in human NAFLD [29].

The study demonstrated that an NAFLD-like condition can be developed in the rats within 12 weeks after fed with diets I, II, V, and VI with modified fat content and these developed conditions remains till 32 weeks. Hence this model can be used for the study of NAFLD. Use of the diet ingredients commonly used in the regular diet make this model inexpensive and easy to develop.

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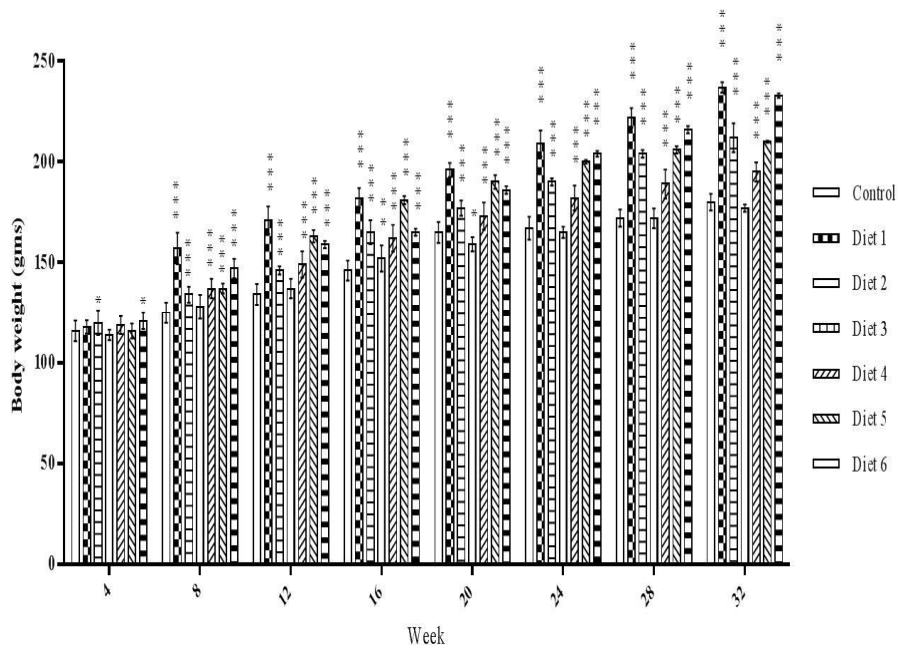


Figure 1 Effect of the various diets on body weight of rats.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

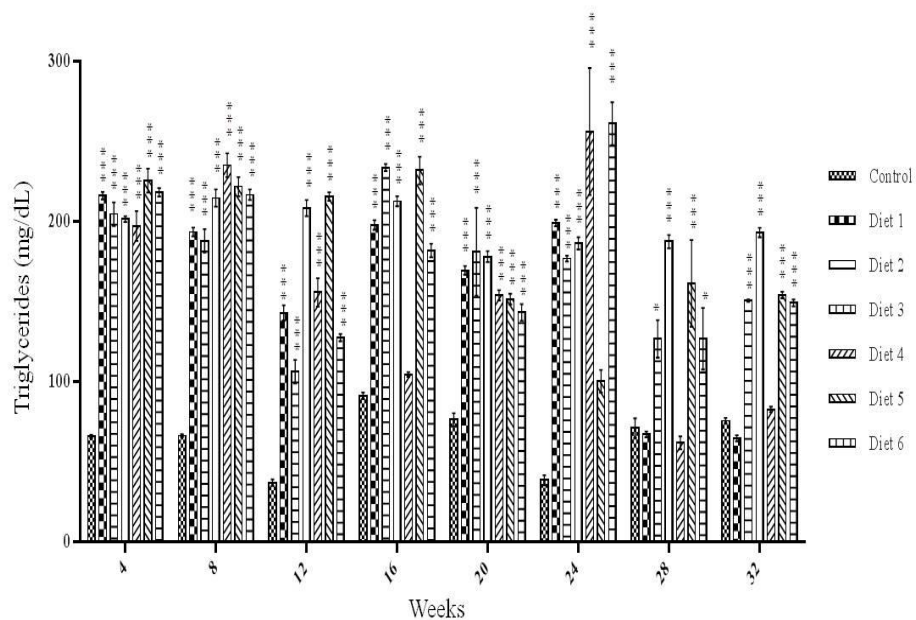


Figure 2 Effect of the various diets on the triglycerides values in rats.*
 $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

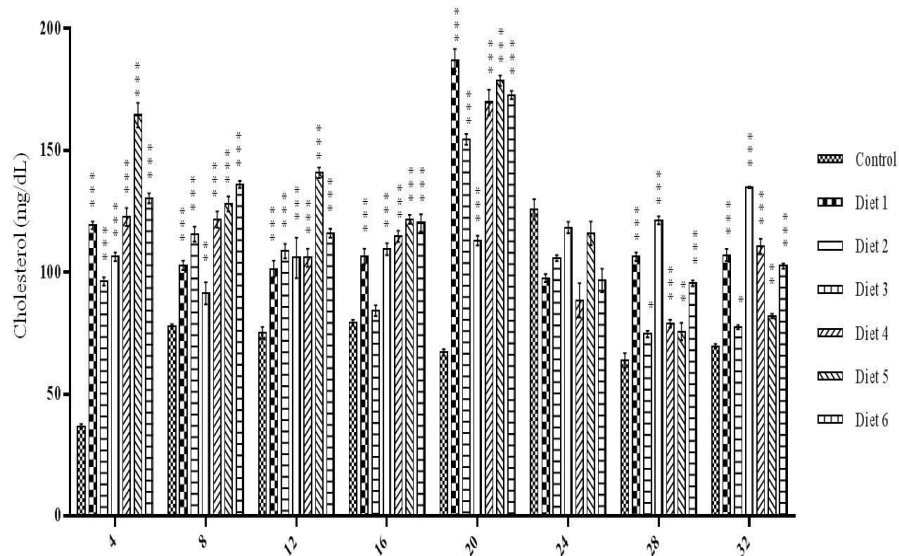


Figure 3 Effect of the various diets on cholesterol values in rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

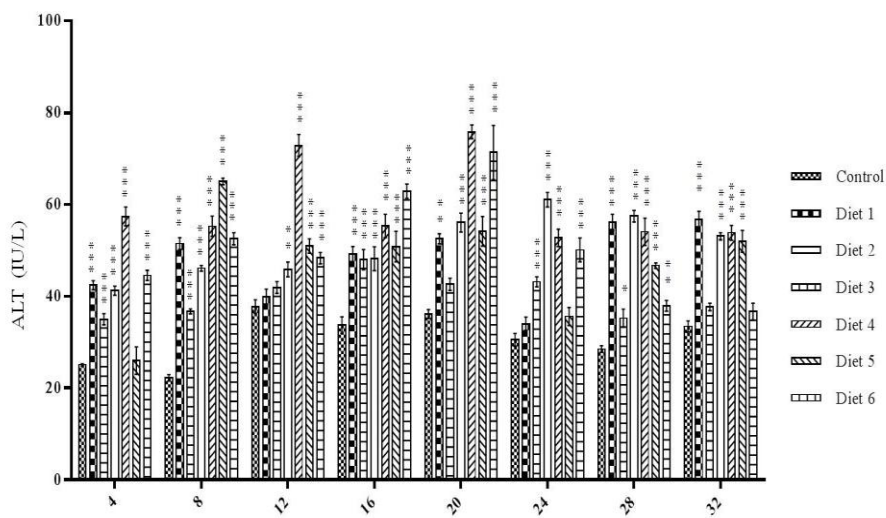


Figure 4 Effect of the various diets on ALT values in rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

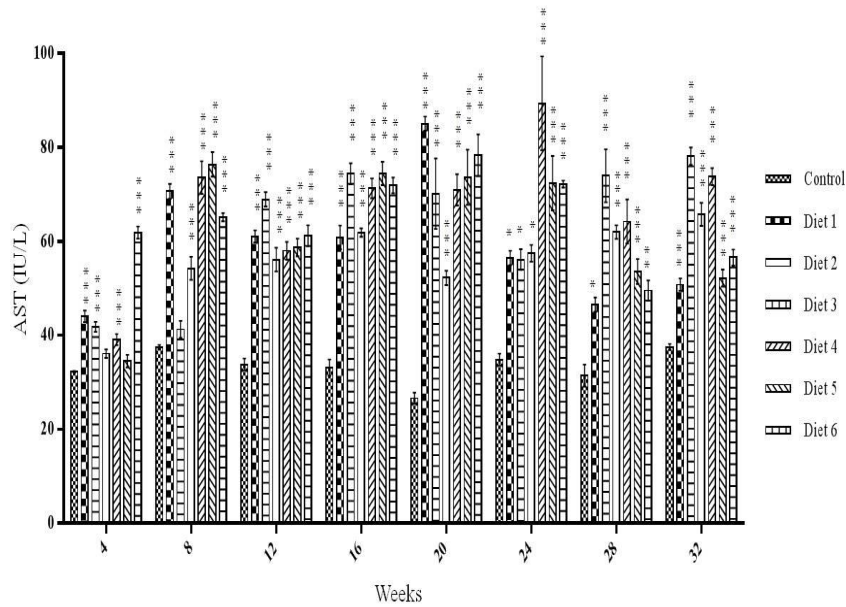


Figure 5 Effect of the various diets on AST values in rats.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

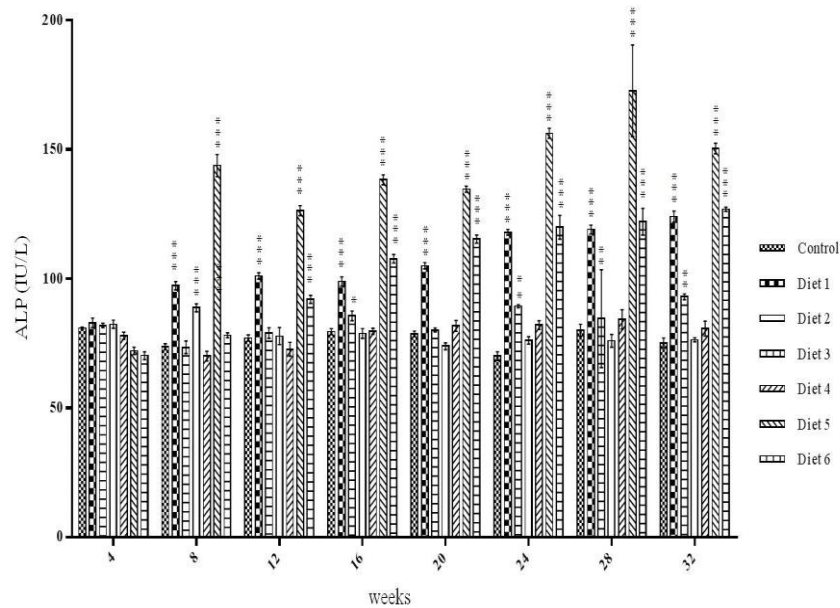


Figure 6 Effect of the various diets on ALP values in rats.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

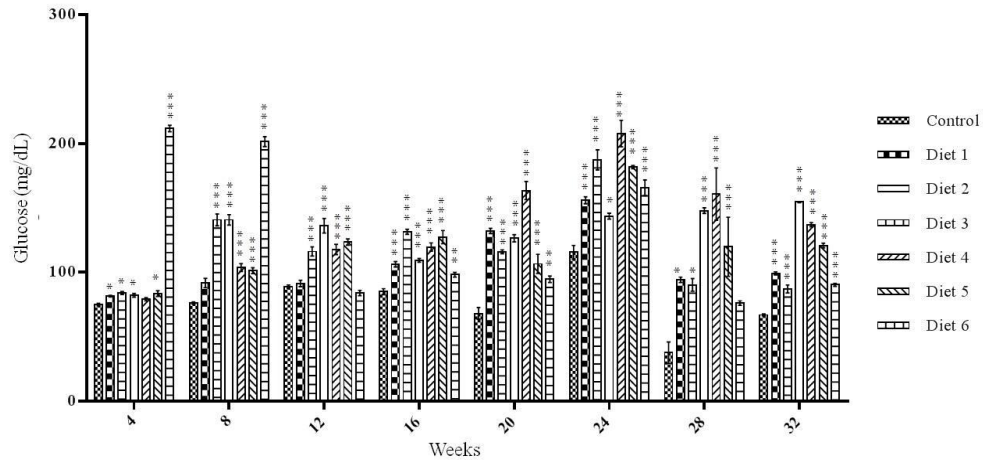


Figure 7 Effect of the various diets on plasma glucose values in rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant compared to Control.

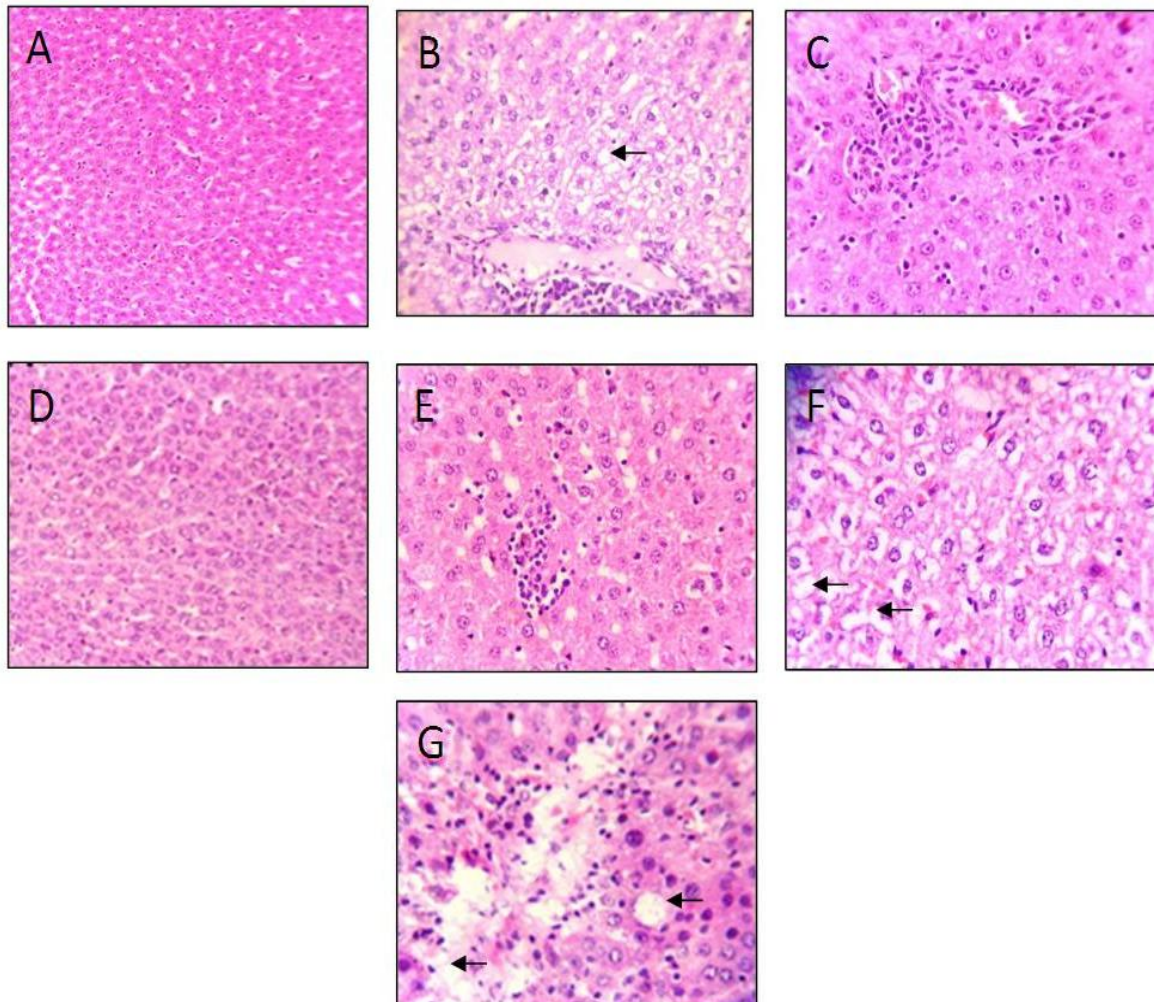


Figure 8 Histopathology of liver from animals fed with different diets (A: Control group B: Diet I; C: Diet II; D: Diet III; E: Diet IV; F: Diet V; G: Diet VI).

Table 1: Preparation of the diet with different concentration of the ingredients

Ingredients Diets	Wheat Flour (%)	Gram Flour (%)	Fats (%)			Chilli Powder (%)	Black pepper powder (%)	Mustard powder (%)	NaCl (%)
			Vanaspati ghee	Coconut oil	Ground nut oil				
I	25	14	20	20	20	--	--	--	1
II	55	14	10	10	10	--	--	--	1
III	19	14	20	20	20	2	2	2	1
IV	53	14	10	10	10	2	--	--	1
V	51	14	10	10	10	2	2	--	1
VI	49	14	10	10	10	2	2	2	1

Table 2: Various diets used in the study

Diets	Ingredients
Diet I	60 % fat
Diet II	30% fat
Diet III	60% fat + chilli powder+black pepper powder+ mustard powder
Diet IV	30% fat + chilli powder
Diet V	30% fat + chilli powder + black pepper powder
Diet VI	30% fat + chilli powder + black pepper powder+ mustard powder