Therapeutic and Preventive Effects of a New Type Healthy and Viable Food Supplement on Fatty Liver and Blood Lipids in Animal Model

Azam Bayat (azam.bayat@modares.ac.ir)
Tarbiat Modares University
Aref Khalkhali
NBS organic Company
Ali Reza Mahjoub
Tarbiat Modares University

Research

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Therapeutic and preventive effects of a new type healthy and viable food supplement on fatty liver and blood lipids in animal model

Azam Bayat [1, 2, *], Aref Khalkhali [2], Ali Reza Mahjoub [1]

[1]Department of Chemistry, Tarbiat Modares University, Tehran 14155-4383, Iran

[2]NBS organic Company, Istanbul, Turkey

Corresponding author

Dr. Azam Bayat

Department of Chemistry, Tarbiat Modares University, Tehran 14155-4383, Iran. E-mail: azam.bayat@modares.ac.ir

Abstract

Background: Fatty liver disease is one of the most common liver complications worldwide. Also, blood lipids are elevated cholesterol or triglyceride levels from the normal amount in the blood that causes fatty liver disease. The aim of this study was to investigate the therapeutic effects of a new healthy and viable food supplement from wheat on fatty liver disease and blood lipids in animal model.

Methods: The new NBS healthy and viable food supplement was prepared by a green route. The NBS healthy and live food powder had various vitamins, macro and micro molecules, and ingredients such as B1, B2, B3, B5, B6, B9, C, K, A, E, D, phosphorus, potassium, sulfur, magnesium, calcium, iron, manganese, zinc, copper, omega-3 and etc. For therapeutic and preventive effects of the new healthy and viable dietary supplement on fatty liver and blood lipids, 25 Wistar rats weighing 180-220 g were used. The rats were divided into 5 groups and were treated with 25, 50 and 100 mg/kg of healthy and live medication.

Results: Investigation of the interaction between the concentrations of the medication supplement showed that 100 mg/ kg has the most therapeutic effect against oasises in fatty liver disease. Also, it was found that the concentration of 1000 mg/ kg has the most reducing effect on the level of lipid profile.

Conclusions: The new food supplement reduces the level of hepatic macrovesicles, microvesicles, and the steatosis symptoms without specific hepatic complications. Also, the healthy food causes reduction of lipid parameters.

Keywords: therapeutic, preventive, healthy and viable food, fatty liver, animal model, blood lipids

Introduction

Fatty liver disease is a chronic inflammation of the human body that has led to a substantial increase in our population. The importance of this disease is due to the destruction of liver cells and in the absence of early diagnosis and proper treatment in which can lead to progressive and irreversible liver disease called cirrhosis. Hypertension, hyperlipidemia, obesity and diabetes that all of them are components of metabolic syndrome, have been observed with fatty liver disease. For this reason, some researchers may know fatty liver disease as insulin resistance, or metabolic syndrome. Insulin resistance has adverse effects on vital organs such as the heart and brain blood vessels, kidney, peripheral nerves and ultimately the liver. In the other word, fatty liver disease can be a sign of resistance to insulin and for this reason, early diagnosis and appropriate treatment will prevent of the liver damages and the cardiovascular complications that are the most important cause of death in patients with fatty liver [1]. Lipid-lowering drug gemfibrozil is that medicines improve symptoms in patients with fatty liver is laboratory [2]. Statins are other lipid-lowering drugs, particularly cholesterol, which also caused symptoms improvement in laboratory [3], however, the Ursodeoxycholic acid as a protective liver cells is not very useful in recent studies [4]. Based on the available information, the treatments of fatty liver are weight loss, elimination of drugs and toxins as well as diabetes and blood lipid controls [1]. However, lack of proper treatment and numerous side effects of existing chemical drugs including gastric bloating, stomach ache and heartburn, cutaneous rash and nausea or vomiting may continue to occur in the area of drug medications. Thus, the aim of this study was to evaluate the therapeutic activity of a new healthy and viable food supplement, without side effects, against fatty liver disease.

On the other hand, blood lipids are elevated cholesterol or triglyceride levels from the normal amount in the blood that causes fatty liver disease. The amount of these substances in the blood increases as a result of dietary intake is more than needed or when the metabolism of fat

develops. High levels of fat or cholesterol can lead to complications such as atherosclerosis (stiff and vasodilated), high blood pressure, cardiovascular disease, increased risk of stroke, fatty liver, and so on.

Inappropriate diet therapy, the use of fatty and high-calorie foods, inappropriate culture of over consumption of fast foods, inappropriate culture of the consumption of red meat in society and machinery life has caused many people have a problem with high fat and blood cholesterol or obesity. Cardiovascular diseases are now the leading cause of death in industrialized countries. Reports suggest that the disease has caused death in 95,000 people in the United States in 1998, and in 2000, this country incurred about $ 118 billion in costs for this disease. Increased blood lipids, especially cholesterol as an important factor in exacerbating this disease. Now, there are over 100 million Americans adult with high blood cholesterol and about 50 million of these need treatment [5-7]. Over the past few decades, most countries have increased the use of alternative therapies, especially herbal therapies and dietary supplements, to improve a variety of diseases, including high levels of blood lipids. One of the major problems of physicians and consumers of herbal medicines is insufficient information on drug health and its effect on disease. Fortunately, over the past 30 years, there has been a great deal of research on the effectiveness of medicinal herbs used in the traditional medicine that prove their efficient or inefficient. Recent research into the nutritional supplements and herbal drugs used in traditional medicine suggests that their compounds, including dietary fiber, vitamins, flavonoids, sterols, and other antioxidant compounds, can reduce LDL oxidation and free radical oxygen uptake and probably, with effect on the immune system and metabolic disorders improve this disease [8-12]. Considering the mentioned issues and the increasing in the number of diseases associated with lipid profile and the lack of definitive treatment of this disease, the present study was designed to investigate the reducing effect of live and healthy food powder on the level of lipid profile.

Methods and methods

Fatty liver disease

Biochemical evaluation of serum of rats in two groups of control and high fat diet

In order to create steatosis, rats were fed with high fat diet. The fat emulsion according to the method described by Zou et al. [13], were contains 400 grams of corn oil, 150 grams of sucrose, 80 grams of whole milk powder, 100 grams of cholesterol, 10 grams of sodium deoxycholate, 36.4 grams of polysorbate 80, 31.1 grams of propylene glycol, 2.5 grams multivitamin, 10 grams of salt, 1.4 gram of mixed minerals and 300 ml of distilled water. The mice were fed a high fat emulsion via gavage, in the amount of 10 ml/kg, daily for 4 weeks. At the same time the control group were given equal volumes of saline via gavage daily.

After 30 days of treatment, to evaluate the effect of healthy and viable dietary supplement on fatty liver induced by high fat diet in rats, blood sampling and evaluation of the parameters level was performed using capillary tube and biochemical kits, respectively. Also, for histopathological study of the studied animals, they killed using mechanical method (spinal cord dissection). From these samples, sections of 4-6 micron was prepared using standard methods of the tissue processing and histopathological sections, and stained with hematoxylin-eosin.

Blood biochemical parameters of rats receiving different concentrations of dietary supplement and healthy living

After 30 days of treatment, to evaluate the effect of different concentrations of healthy and live dietary supplement on fatty liver caused by high fat diet in rats, capillary blood sampling was performed. The levels of the parameters were measured using biochemical kits, the results of these experiments were recorded.

Liver pathology of mice treated with live and healthy dietary supplements

The rats in the treatment groups at concentrations of 25, 50 and 100 mg/ ml, healthy and viable dietary supplement, after 4 weeks of treatment, blood samples were collected using

mechanical method (spinal cord dissection). All mice were sacrificed and tissue samples were fixed in 10% formalin. The above specimens were prepared using high-pressure silica gel and from three phylogenetic bases, 4-6 mm slices were prepared and stained with hematoxylin-eosin. The results of these experiments were recorded.

Blood lipids profiles

In fatty diet groups, high fat emulsion was used for fatty diet induction, according to the method presented by Zou et al. [13], as mentioned in fatty liver section. At the end, blood samples were taken from the mice and the level of blood lipid parameters was measured. After a 30 day treatment, the effect of food powder (healthy and alive) on fatty profiles of high fat diet recipients was performed using a blood sampler tube from the rat's eye. The level of the parameters was measured using biochemical kits.

Statistical Analysis

In order to analysis the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. Also, in this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. $P<0.05$ indicates statistical significance. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups.

Results

Fatty liver disease

Biochemical evaluation of serum of rats in two groups of control and high fat diet

According to the results and comparing the results of two groups of mice (control and a high fat diet), can be stated that the levels of the serum alanine amino transferase (ALT), aspartate aminotransferase (AST), serum total bilirubin (TB) and alkaline phosphatase (ALP) compared with healthy control group, have a significant increase and total protein (TP) and albumin

(Alb) have significant decrease. According to the biochemical tests results of the level of liver parameters and comparison of the results of healthy group with the patient group (high fat diet) (Table 1(a-b)) (Fig. 1(a-f)) and also according to histopathologic changes in the liver tissue of control rats (Fig. 2(a-d)), it can be concluded that the diet used in this study caused fatty liver disease. Fig. 2(a-b) show the microscopic examination of the liver tissue of a rat in the control group in which hepatocytes and liver tissue structure are normal. In contrast, the microscopic examination of liver tissue with high-fat diet fed that prove the formation of the large fat macrovesicles (rounded bodies, white and hollow), are shown in Fig. 2(c-d).

Blood biochemical parameters of rats receiving different concentrations of dietary supplement and healthy living

According to the results of tests of biochemical studied mice, it can be concluded that the dietary supplement alive can return biochemical parameters to normal levels. Investigation of the interaction between the concentrations of healthy and live medication supplement showed that 100 mg / kg showed the most effective therapeutic effect and 25 mg / kg the least effect against steatosis (5% probability level).

Results of liver pathology of mice treated with live and healthy dietary supplements

Also, according to the observations of the blood biochemical tests of mice receiving healthy and live medication supplement (Table 2(a-c)), and also the results of liver pathology images (Fig. 3(a-f), It can be concluded that a healthy and viable dietary supplement return the level of hepatic parameters to normal state and reduces the level of hepatic macrovesicles, microvesicles, and the steatosis symptoms without specific hepatic complications. In microscopic studies, no abnormal state in the mice liver of the control group were observed. While, in mice fed with only high fat diet for 30 days, severe liver steatosis as macrovesicles, and sometimes microvesicles fat, accompanied by hepatocytes swelling, had been created (Fig. 2). In the high fat diet group treated with healthy dietary supplements, the incidence of fatty change in hepatocytes significantly was prevented. Pathology of liver tissue of mice

treated with a concentration of 25 show no changes on the large fat macrovesicles (rounded bodies, white and hollow) in patient groups (Fig. 3(a-b)). In contrast, pathology of liver tissue of mice treated with a concentration of 50 and 100 show significant changes on the large fat macrovesicles (rounded bodies, white and hollow) in which fats are sporadic and mild. (Fig. 3(c-f)).

Investigation of the interaction between the concentrations of healthy and live medication supplement showed that 100 mg/ kg showed the most effective therapeutic effect and 25 mg/ kg the least effect against steatosis (5% probability level).

Meanwhile, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. The results of this test showed that there was a significant difference between the groups with 5% probability level. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups. The results can be seen in Table 3.

Blood lipids profiles

In this research, comparing the results of two groups of control and high fat diet, it can be concluded that in rats fed with high fat diet, serum levels of glycoside, LDL increased significantly and with attention the results of biochemical tests on blood lipids can be found that the diet used in this study induced lipid-related disease in rats (Tables 4-8). According to the results of blood biochemical tests in the studied mice, it can be concluded that dietary powder (healthy and alive nutrition) causes reduction of blood lipid parameters in the studied mice. In the study of the interaction of the concentrations of food powder (healthy and alive nutrition), it was found that the concentration of 1000 mg/ kg had the most and the concentration of 250 mg/ kg had the least therapeutic effect on increasing the blood lipid profiles.

In order to analysis the data in this study, we first make sure that the distribution of data is normal, using Kolmogorov-Smirnov test. In this regard, by using the information test

obtained by observing Sig, it can be stated that these data have a normal distribution. Therefore, parametric tests should be used to analysis the differences between groups. In this study, in order to evaluate the significance of the data, it is recommended to use ANOVA test. ANOVA test was used to investigate the differences between and within groups. The results of this test showed that there was a significant difference between the groups with 5% probability level. In order to clarify this issue, by Scheffe post hoc test, this significance was tested one by one between groups. The results can be seen in Table 9.

Discussion

The liver is one of the important members of the body that detoxify from drugs, disposal of waste products resulting from demolition and renovation as bile red blood cells, the production of blood clotting factors, glucose stored as glycogen and the regulation of sugar and fat metabolism are the most important roles of liver in the body. However, the role of liver should not be ignored in fat absorption and defense against microbes and toxins absorbed by fatty food. Fatty liver in medicine is a reversible condition of the accumulation of fat vacuoles in the liver cells, which is characterized by liver inflammation. This condition may occur in people who drink alcohol, but in Iran the disease has other causes and is called non-alcoholic fatty liver. The non-alcoholic form of the disease occurs in a number of clinical disorders such as diabetes, obesity and malnutrition. Fat existence in the liver is normal, but if this amount exceeds 2 to 5 percent of the total liver weight, the person develops fatty liver disease. It has no specific symptoms, but not observing and progression this disease, it was causes indigestion and eventually leads to death [14]. Background disorders should be treated to improve fatty liver. Treatment is currently focused on controlling the medical problems and conditions that underlie fatty liver. Various new drugs, such as metformin, have been introduced for the treatment of fatty liver, but their definitive effect has not been established. Daily exercise and regular consumption of fruits and vegetables have beneficial effects on the disease. Gradual weight loss is necessary and beneficial in obese people, but sudden weight

loss exacerbates the disorder. Vitamin E at a dose of 4 mg/ day reduces the damage to liver cells and improves liver enzymes, but because it is associated with an increased risk of heart disease, long-term use is not recommended.

Increase the activity of liver function biomarkers, includes AST, ALP and ALT in serum, is indicative of liver damage [15], since the change in the above markers during the liver steatosis previously have been reported [16-17]. So in this study, serum levels of these enzymes were studied. In this research, the levels increase of the serum ALT, AST and ALP in the serum of the high fat diet mice was observed that indicate damage to the liver cells and is consistent with the findings Chidambarama et al. in 2010 [13].

Treatment with medicinal food supplement relatively prevent from serum levels increase of the mentioned enzymes due to the high fat diet fed. In this study, biochemical results obtained with histopathological findings were also confirmed. In any case, histopathological evaluation showed anti-hepatic steatosis effect of the healthy and viable dietary supplement in rats fed with high fat diet [18-31].

Today, the importance of medicinal plants and herbs and their vital role in advancing national, regional and global goals for achieving medicinal self-sufficiency, employment creation, economic development, food security, and preserving genetic reserves with active participation in global markets is unknown. Therefore, distribution of indigenous populations and subspecies of medicinal plants and economic importance of these species in the pharmaceutical and spice industry, gathering and evaluating indigenous populations under identical cultivation conditions and selecting the most suitable chemotypes for domestication, modification and use in the food and pharmaceutical industries can be effective.

The new NBS healthy and live food powder has various vitamins, macro and micro molecules, and ingredients such as B1, B2, B3, B5, B6, B9, C, K, A, E, D, phosphorus, potassium, sulfur, magnesium, calcium, boron, iron, manganese, zinc, coper, omega-3, omega-6, omega-9, and etc. Omega 3 fatty acids have a protective role in the fatty liver. The best sources of omega-3

fatty acids have many health benefits, including reducing inflammation and lowering triglyceride levels. Polyphenols are a group of compounds that act as antioxidants and anti-inflammatory and improve liver metabolism. Deficiencies of some minerals such as copper, selenium and iron have been observed in patients with fatty liver and supplement of these minerals can be effective in these patients. Copper and selenium act as antioxidants in the body, and iron plays a role in oxygen transfer and genetic material synthesis. Vitamin D reduces the risk of diabetes, hypertension and heart disease and vitamin E reduces inflammation and improves fatty liver. The grains contain of fiber, protein, potassium, minerals and vitamin B can improve blood flow and lower cholesterol.

Conclusions and implications

In this research, based on the results of the biochemical tests of blood of the studied mice, it can be concluded that the new NBS healthy and viable food supplement will restore the level of liver parameters to normal. Interaction effects of the concentrations of healthy and live dietary supplements showed that 100 mg/ kg showed the most effective therapeutic effect and 25 mg / kg showed the least effect against acaiosis. Based on the observations from liver pathology images, it can be concluded that a healthy and viable dietary supplement reduces the level of hepatic macrovesicles, microvesicles, and the steatosis symptoms without specific hepatic complications. Also, the results of this study showed that the use of the new NBS healthy and viable dietary supplement would normalize the fat-related factors in blood serum, so that can use this compound as a lipid lowering agent. Given the similar results, clinical studies are needed to extend the results to the community.

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Authors' contributions

Ak performed the statistical analysis and wrote the first draft of the manuscript. AB helped analyze and interpret the data and contributed in preparing the draft. Project development performed by AK and AM. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures involving animals were approved by the Ethics Committee of the Tarbiat Modares University Medical School and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]NBS organic Company, Istanbul, Turkey.

[2]Department of Chemistry, Tarbiat Modares University, Tehran 14155-4383, Iran.

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Tables

Table 1. Test results of liver enzymes and protein levels in control and patient rats (a and b).

Analysis **a**	Result					Range
	Mice (1)	Mice (2)	Mice (3)	Mice (4)	Mice (5)	
AST (S.G.O.T)	26 U/L	30 U/L	32 U/L	29 U/L	31 U/L	0.000 - 37.0
ALT (S.G.P.T)	24 U/L	23 U/L	30 U/L	25 U/L	28 U/L	0.000 – 41.0
Albumin	4 g/dl	5 g/dl	4.1 g/dl	4 g/dl	4 g/dl	2.5 – 5.8
Total Bilirubin	0.8 mg/dl	0.8 mg/dl	0.7 mg/dl	0.8 mg/dl	0.8 mg/dl	0.1 _1.2
Total Protein	6.2 g/dl	7.2 g/dl	7.1 g/dl	7.1 g/dl	6.3 g/dl	6.3 - 8
ALP	191 IU/L	186 IU/L	180 IU/L	168 IU/L	179 IU/L	80.00-306.0

Analysis **b**	Result					Range
	Mice (1)	Mice (2)	Mice (3)	Mice (4)	Mice (5)	
AST (S.G.O.T)	84 U/L	93.65 U/L	90 U/L	89 U/L	91 U/L	0.000 - 37.0
ALT (S.G.P.T)	77 U/L	82.46 U/L	80 U/L	78 U/L	76 U/L	0.000 – 41.0
Albumin	2.6 g/dl	2.34 g/dl	1.2 g/dl	3.4 g/dl	2.1 g/dl	2.5 – 5.8
Total Bilirubin	4.2 mg/dl	4.41 mg/dl	4.4 mg/dl	4.1 mg/dl	4.6 mg/dl	0.1 _1.2
Total Protein	5.4 g/dl	5.92 g/dl	6.1 g/dl	5.4 g/dl	5.3 g/dl	6.3 - 8
ALP	326 IU/L	312.37 IU/L	308 IU/L	298 IU/L	323 IU/L	80.00-306.0

Table 2. Test results of liver enzymes and protein levels of mice treated with the concentrations of (a) 25, (b) 50, and (c) 100 healthy and alive nutrients.

Analysis **a**	Result					Range
	Mice (1)	Mice (2)	Mice (3)	Mice (4)	Mice (5)	
AST (S.G.O.T)	54 U/L	56 U/L	58 U/L	54 U/L	49 U/L	0.000 - 37.0
ALT (S.G.P.T)	52 U/L	47 U/L	48 U/L	46 U/L	44 U/L	0.000 – 41.0
Albumin	2.6 g/dl	3.2 g/dl	3.2 g/dl	2.7 g/dl	2.6 g/dl	2.5 – 5.8
Total Bilirubin	1.8 mg/dl	1.9 mg/dl	1.6 mg/dl	1.8 mg/dl	1.7 mg/dl	0.1 _1.2
Total Protein	5.6 g/dl	5.9 g/dl	5.4 g/dl	5.3 g/dl	6.1 g/dl	6.3 - 8
ALP	289 IU/L	248 IU/L	284 IU/L	264 IU/L	231 IU/L	80.00-306.0

Analysis **b**	Result					Range
	Mice (1)	Mice (2)	Mice (3)	Mice (4)	Mice (5)	
AST (S.G.O.T)	44 U/L	42 U/L	44 U/L	39 U/L	39 U/L	0.000 - 37.0
ALT (S.G.P.T)	45 U/L	40 U/L	43 U/L	42 U/L	41 U/L	0.000 – 41.0
Albumin	3.2 g/dl	3.3 g/dl	3.6 g/dl	4.2 g/dl	4.6 g/dl	2.5 – 5.8
Total Bilirubin	1.5 mg/dl	1.9 mg/dl	1.9 mg/dl	2.4 mg/dl	2.3 mg/dl	0.1 _1.2
Total Protein	6.3 g/dl	5.8 g/dl	5.1 g/dl	5.2 g/dl	5.2 g/dl	6.3 - 8
ALP	224 IU/L	209 IU/L	237 IU/L	263 IU/L	272 IU/L	80.00-306.0

Analysis **c**	Result					Range
	Mice (1)	Mice (2)	Mice (3)	Mice (4)	Mice (5)	
AST (S.G.O.T)	36 U/L	34 U/L	39 U/L	38 U/L	38 U/L	0.000 - 37.0
ALT (S.G.P.T)	42 U/L	36 U/L	41 U/L	41 U/L	40 U/L	0.000 – 41.0
Albumin	3.8 g/dl	4.2 g/dl	4.4 g/dl	4.8 g/dl	4.7 g/dl	2.5 – 5.8
Total Bilirubin	0.7 mg/dl	0.6 mg/dl	0.9 mg/dl	1.8 mg/dl	0.8 mg/dl	0.1 _1.2
Total Protein	6.8 g/dl	6.2 g/dl	6.2 g/dl	7.8 g/dl	7.2 g/dl	6.3 - 8
ALP	217 IU/L	241 IU/L	214 IU/L	234 IU/L	245 IU/L	80.00-306.0

Table 4. Results of ANOVA and Scheffe post hoc test for the (a) S.G.O.T, (b) S.G.P.T, (c) Albumin, (d) Total Bilirubin, (e) Total Protein and (f) ALP results of studied mice.

Multiple Comparisons

SGOT
Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	-59.80000*	1.74585	.000	-65.7113	-53.8887
	Concentration-25	-24.60000*	1.74585	.000	-30.5113	-18.6887
	Concentration-50	-12.00000*	1.74585	.000	-17.9113	-6.0887
	Concentration-100	-7.40000*	1.74585	.009	-13.3113	-1.4887
Patient group	Healthy group	59.80000*	1.74585	.000	53.8887	65.7113
	Concentration-25	35.20000*	1.74585	.000	29.2887	41.1113
	Concentration-50	47.80000*	1.74585	.000	41.8887	53.7113
	Concentration-100	52.40000*	1.74585	.000	46.4887	58.3113
Concentration-25	Healthy group	24.60000*	1.74585	.000	18.6887	30.5113
	Patient group	-35.20000*	1.74585	.000	-41.1113	-29.2887
	Concentration-50	12.60000*	1.74585	.000	6.6887	18.5113
	Concentration-100	17.20000*	1.74585	.000	11.2887	23.1113
Concentration-50	Healthy group	12.00000*	1.74585	.000	6.0887	17.9113
	Patient group	-47.80000*	1.74585	.000	-53.7113	-41.8887
	Concentration-25	-12.60000*	1.74585	.000	-18.5113	-6.6887
	Concentration-100	4.60000	1.74585	.182	-1.3113	10.5113
Concentration-100	Healthy group	7.40000*	1.74585	.009	1.4887	13.3113
	Patient group	-52.40000*	1.74585	.000	-58.3113	-46.4887
	Concentration-25	-17.20000*	1.74585	.000	-23.1113	-11.2887
	Concentration-50	-4.60000	1.74585	.182	-10.5113	1.3113

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

SGPT

Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	-50.80000*	1.58745	.000	-56.1750	-45.4250
	Concentration-25	-20.60000*	1.58745	.000	-25.9750	-15.2250
	Concentration-50	-14.40000*	1.58745	.000	-19.7750	-9.0250
	Concentration-100	-12.20000*	1.58745	.000	-17.5750	-6.8250
Patient group	Healthy group	50.80000*	1.58745	.000	45.4250	56.1750
	Concentration-25	30.20000*	1.58745	.000	24.8250	35.5750
	Concentration-50	36.40000*	1.58745	.000	31.0250	41.7750
	Concentration-100	38.60000*	1.58745	.000	33.2250	43.9750
Concentration-25	Healthy group	20.60000*	1.58745	.000	15.2250	25.9750
	Patient group	-30.20000*	1.58745	.000	-35.5750	-24.8250
	Concentration-50	6.20000*	1.58745	.018	.8250	11.5750
	Concentration-100	8.40000*	1.58745	.001	3.0250	13.7750
Concentration-50	Healthy group	14.40000*	1.58745	.000	9.0250	19.7750
	Patient group	-36.40000*	1.58745	.000	-41.7750	-31.0250
	Concentration-25	-6.20000*	1.58745	.018	-11.5750	-.8250
	Concentration-100	2.20000	1.58745	.750	-3.1750	7.5750
Concentration-100	Healthy group	12.20000*	1.58745	.000	6.8250	17.5750
	Patient group	-38.60000*	1.58745	.000	-43.9750	-33.2250
	Concentration-25	-8.40000*	1.58745	.001	-13.7750	-3.0250
	Concentration-50	-2.20000	1.58745	.750	-7.5750	3.1750

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Albumin
Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	1.82000*	.31736	.000	.7454	2.8946
	Concentration-25	1.36000*	.31736	.009	.2854	2.4346
	Concentration-50	.44000	.31736	.750	-.6346	1.5146
	Concentration-100	-.16000	.31736	.992	-1.2346	.9146
Patient group	Healthy group	-1.82000*	.31736	.000	-2.8946	-.7454
	Concentration-25	-.46000	.31736	.718	-1.5346	.6146
	Concentration-50	-1.38000*	.31736	.008	-2.4546	-.3054
	Concentration-100	-1.98000*	.31736	.000	-3.0546	-.9054
Concentration-25	Healthy group	-1.36000*	.31736	.009	-2.4346	-.2854
	Patient group	.46000	.31736	.718	-.6146	1.5346
	Concentration-50	-.92000	.31736	.119	-1.9946	.1546
	Concentration-100	-1.52000*	.31736	.003	-2.5946	-.4454
Concentration-50	Healthy group	-.44000	.31736	.750	-1.5146	.6346
	Patient group	1.38000*	.31736	.008	.3054	2.4546
	Concentration-25	.92000	.31736	.119	-.1546	1.9946
	Concentration-100	-.60000	.31736	.486	-1.6746	.4746
Concentration-100	Healthy group	.16000	.31736	.992	-.9146	1.2346
	Patient group	1.98000*	.31736	.000	.9054	3.0546
	Concentration-25	1.52000*	.31736	.003	.4454	2.5946
	Concentration-50	.60000	.31736	.486	-.4746	1.6746

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

TotalBilirubin
Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	-3.56000*	.18243	.000	-4.1777	-2.9423
	Concentration-25	-.98000*	.18243	.001	-1.5977	-.3623
	Concentration-50	-1.22000*	.18243	.000	-1.8377	-.6023
	Concentration-100	-.18000	.18243	.910	-.7977	.4377
Patient group	Healthy group	3.56000*	.18243	.000	2.9423	4.1777
	Concentration-25	2.58000*	.18243	.000	1.9623	3.1977
	Concentration-50	2.34000*	.18243	.000	1.7223	2.9577
	Concentration-100	3.38000*	.18243	.000	2.7623	3.9977
Concentration-25	Healthy group	.98000*	.18243	.001	.3623	1.5977
	Patient group	-2.58000*	.18243	.000	-3.1977	-1.9623
	Concentration-50	-.24000	.18243	.783	-.8577	.3777
	Concentration-100	.80000*	.18243	.007	.1823	1.4177
Concentration-50	Healthy group	1.22000*	.18243	.000	.6023	1.8377
	Patient group	-2.34000*	.18243	.000	-2.9577	-1.7223
	Concentration-25	.24000	.18243	.783	-.3777	.8577
	Concentration-100	1.04000*	.18243	.000	.4223	1.6577
Concentration-100	Healthy group	.18000	.18243	.910	-.4377	.7977
	Patient group	-3.38000*	.18243	.000	-3.9977	-2.7623
	Concentration-25	-.80000*	.18243	.007	-1.4177	-.1823
	Concentration-50	-1.04000*	.18243	.000	-1.6577	-.4223

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

TotalProtein
Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	1.16000*	.31138	.026	.1057	2.2143
	Concentration-25	1.12000*	.31138	.034	.0657	2.1743
	Concentration-50	1.26000*	.31138	.014	.2057	2.3143
	Concentration-100	-.06000	.31138	1.000	-1.1143	.9943
Patient group	Healthy group	-1.16000*	.31138	.026	-2.2143	-.1057
	Concentration-25	-.04000	.31138	1.000	-1.0943	1.0143
	Concentration-50	.10000	.31138	.999	-.9543	1.1543
	Concentration-100	-1.22000*	.31138	.018	-2.2743	-.1657
Concentration-25	Healthy group	-1.12000*	.31138	.034	-2.1743	-.0657
	Patient group	.04000	.31138	1.000	-1.0143	1.0943
	Concentration-50	.14000	.31138	.995	-.9143	1.1943
	Concentration-100	-1.18000*	.31138	.023	-2.2343	-.1257
Concentration-50	Healthy group	-1.26000*	.31138	.014	-2.3143	-.2057
	Patient group	-.10000	.31138	.999	-1.1543	.9543
	Concentration-25	-.14000	.31138	.995	-1.1943	.9143
	Concentration-100	-1.32000*	.31138	.009	-2.3743	-.2657
Concentration-100	Healthy group	.06000	.31138	1.000	-.9943	1.1143
	Patient group	1.22000*	.31138	.018	.1657	2.2743
	Concentration-25	1.18000*	.31138	.023	.1257	2.2343
	Concentration-50	1.32000*	.31138	.009	.2657	2.3743

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Alp

Scheffe

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Healthy group	Patient group	-132.60000*	11.61516	.000	-171.9278	-93.2722
	Concentration-25	-82.40000*	11.61516	.000	-121.7278	-43.0722
	Concentration-50	-60.20000*	11.61516	.001	-99.5278	-20.8722
	Concentration-100	-49.40000*	11.61516	.009	-88.7278	-10.0722
Patient group	Healthy group	132.60000*	11.61516	.000	93.2722	171.9278
	Concentration-25	50.20000*	11.61516	.008	10.8722	89.5278
	Concentration-50	72.40000*	11.61516	.000	33.0722	111.7278
	Concentration-100	83.20000*	11.61516	.000	43.8722	122.5278
Concentration-25	Healthy group	82.40000*	11.61516	.000	43.0722	121.7278
	Patient group	-50.20000*	11.61516	.008	-89.5278	-10.8722
	Concentration-50	22.20000	11.61516	.475	-17.1278	61.5278
	Concentration-100	33.00000	11.61516	.131	-6.3278	72.3278
Concentration-50	Healthy group	60.20000*	11.61516	.001	20.8722	99.5278
	Patient group	-72.40000*	11.61516	.000	-111.7278	-33.0722
	Concentration-25	-22.20000	11.61516	.475	-61.5278	17.1278
	Concentration-100	10.80000	11.61516	.926	-28.5278	50.1278
Concentration-100	Healthy group	49.40000*	11.61516	.009	10.0722	88.7278
	Patient group	-83.20000*	11.61516	.000	-122.5278	-43.8722
	Concentration-25	-33.00000	11.61516	.131	-72.3278	6.3278
	Concentration-50	-10.80000	11.61516	.926	-50.1278	28.5278

*. The mean difference is significant at the 0.05 level.

	Analysis	Result	Range
a	T.G	98/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	64/5 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	42/36 mg/dl	≥ 35

	Analysis	Result	Range
b	T.G	92/3 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	76/8 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	44/51 mg/dl	≥ 35

	Analysis	Result	Range
c	T.G	86/5 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	62/3 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	37/69 mg/dl	≥ 35

	Analysis	Result	Range
d	T.G	91/6 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	69/8 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	41/32 mg/dl	≥ 35

	Analysis	Result	Range
e	T.G	84/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	72/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	41/84 mg/dl	≥ 35

Table 4. Blood fat level parameters results in rats No. 1-5 (Healthy group).

	Analysis	Result	Range
a	T.G	216/6 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	154/2 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	51/23 mg/dl	≥ 35

	Analysis	Result	Range
b	T.G	221/8 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	158/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	54/29 mg/dl	≥ 35

	Analysis	Result	Range
c	T.G	218/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	162/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	46/52 mg/dl	≥ 35

	Analysis	Result	Range
d	T.G	232/6 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	157/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	44/68 mg/dl	≥ 35

	Analysis	Result	Range
e	T.G	216/3 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	182/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	51/36 mg/dl	≥ 35

Table 5. Blood fat level parameters results in rats No. 1-5 (Patient Group).

Analysis	Result	Range
a T.G	164/5 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	137/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	48/38 mg/dl	≥ 35

Analysis	Result	Range
b T.G	186/2 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	134/8 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	44/52 mg/dl	≥ 35

Analysis	Result	Range
c T.G	216/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	125/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	41/36 mg/dl	≥ 35

Analysis	Result	Range
d T.G	154/6 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	132/7 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	41/29 mg/dl	≥ 35

Analysis	Result	Range
e T.G	154/6 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	128/3 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	44/62 mg/dl	≥ 35

Table 6. Blood fat level parameters results in rats No. 1-5 (The treatment group with 250 NBS healthy and viable drug supplement packs).

Analysis	Result	Range
a T.G	134/5 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	116/7 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	44/52 mg/dl	≥ 35

Analysis	Result	Range
b T.G	142/3 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	121/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	47/62 mg/dl	≥ 35

Analysis	Result	Range
c T.G	126/7 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	105/7 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	38/49 mg/dl	≥ 35

Analysis	Result	Range
d T.G	133/8 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	112/3 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	42/31 mg/dl	≥ 35

Analysis	Result	Range
e T.G	116/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
LDL	119/2 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
HDL	36/52 mg/dl	≥ 35

Table 7. Blood fat level parameters results in rats No. 1-5 (The treatment group with 500 NBS healthy and viable drug supplement packs).

	Analysis	Result	Range
a	T.G	126/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	104/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	42/65 mg/dl	≥ 35

	Analysis	Result	Range
b	T.G	116/7 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	92/7 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	44/7 mg/dl	≥ 35

	Analysis	Result	Range
c	T.G	106/4 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	98/4 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	38/61 mg/dl	≥ 35

	Analysis	Result	Range
d	T.G	112/3 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	102/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	40/54 mg/dl	≥ 35

	Analysis	Result	Range
e	T.G	121/7 mg/dl	Desirable ≤ 200 Moderate risk 200- 400 High risk ≥ 400
	LDL	97/6 mg/dl	Desirable ≤ 130 Moderate risk 130- 160 High risk ≥ 160
	HDL	42/86 mg/dl	≥ 35

Table 8. Blood fat level parameters results in rats (The treatment group with 1000 NBS healthy and viable drug supplement packs).

Table 9. (a) The Kolmogorov-Smirnov test to determine the data distribution normality, (b) results of ANOVA test between groups and data, and (c) the Scheffe post hoc test results to determine the differences between groups.

		T.G	LDL	HDL
N		25	25	25
Normal Parameters[a] **a**	Mean	1.4674E2	1.1564E2	43.7916
	Std. Deviation	4.88519E1	3.27046E1	4.34932
Most Extreme Differences	Absolute	.163	.083	.177
	Positive	.159	.083	.177
	Negative	-.163	-.081	-.083
Kolmogorov-Smirnov Z		.814	.413	.886
Asymp. Sig. (2-tailed)		.522	.996	.612
a. Test distribution is Normal.				

b		Sum of Squares	df	Mean Square	F	Sig.
T.G	Between Groups	53618.834	4	13404.708	73.303	.000
	Within Groups	3657.364	20	182.868		
	Total	57276.198	24			
LDL	Between Groups	24691.364	4	6172.841	126.131	.000
	Within Groups	978.796	20	48.940		
	Total	25670.160	24			
HDL	Between Groups	231.637	4	57.909	5.209	.005
	Within Groups	222.360	20	11.118		
	Total	453.998	24			

Dependent Variable	(I) VAR00001	Groaps	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
T.G	Healthy group	Patient group	-131.14000*	8.55262	.000	-160.0983	-102.1817
		Nbs 250	-85.34000*	8.55262	.000	-114.2983	-56.3817
		Nbs 500	-40.82000*	8.55262	.003	-69.7783	-11.8617
		Nbs 1000	-26.78000	8.55262	.079	-55.7383	2.1783
	Patient group	Healthy group	131.14000*	8.55262	.000	102.1817	160.0983
		Nbs 250	45.80000*	8.55262	.001	16.8417	74.7583
		Nbs 500	90.32000*	8.55262	.000	61.3617	119.2783
c		Nbs 1000	104.36000*	8.55262	.000	75.4017	133.3183
	Nbs 250	Healthy group	85.34000*	8.55262	.000	56.3817	114.2983
		Patient group	-45.80000*	8.55262	.001	-74.7583	-16.8417
		Nbs 500	44.52000*	8.55262	.001	15.5617	73.4783
		Nbs 1000	58.56000*	8.55262	.000	29.6017	87.5183
	Nbs 500	Healthy group	40.82000*	8.55262	.003	11.8617	69.7783
		Patient group	-90.32000*	8.55262	.000	-119.2783	-61.3617
		Nbs 250	-44.52000*	8.55262	.001	-73.4783	-15.5617
		Nbs 1000	14.04000	8.55262	.618	-14.9183	42.9983
	Nbs 1000	Healthy group	26.78000	8.55262	.079	-2.1783	55.7383
		Patient group	-104.36000*	8.55262	.000	-133.3183	-75.4017
		Nbs 250	-58.56000*	8.55262	.000	-87.5183	-29.6017
		Nbs 500	-14.04000	8.55262	.618	-42.9983	14.9183
LDL	Healthy group	Patient group	-93.88000*	4.42447	.000	-108.8608	-78.8992
		Nbs 250	-62.60000*	4.42447	.000	-77.5808	-47.6192
		Nbs 500	-45.90000*	4.42447	.000	-60.8808	-30.9192
		Nbs 1000	-30.02000*	4.42447	.000	-45.0008	-15.0392
	Patient group	Healthy group	93.88000*	4.42447	.000	78.8992	108.8608
		Nbs 250	31.28000*	4.42447	.000	16.2992	46.2608
		Nbs 500	47.98000*	4.42447	.000	32.9992	62.9608
		Nbs 1000	63.86000*	4.42447	.000	48.8792	78.8408
	Nbs 250	Healthy group	62.60000*	4.42447	.000	47.6192	77.5808
		Patient group	-31.28000*	4.42447	.000	-46.2608	-16.2992
		Nbs 500	16.70000*	4.42447	.024	1.7192	31.6808
		Nbs 1000	32.58000*	4.42447	.000	17.5992	47.5608
	Nbs 500	Healthy group	45.90000*	4.42447	.000	30.9192	60.8808
		Patient group	-47.98000*	4.42447	.000	-62.9608	-32.9992
		Nbs 250	-16.70000*	4.42447	.024	-31.6808	-1.7192
		Nbs 1000	15.88000*	4.42447	.034	.8992	30.8608
	Nbs 1000	Healthy group	30.02000*	4.42447	.000	15.0392	45.0008
		Patient group	-63.86000*	4.42447	.000	-78.8408	-48.8792
		Nbs 250	-32.58000*	4.42447	.000	-47.5608	-17.5992
		Nbs 500	-15.88000*	4.42447	.034	-30.8608	-.8992
HDL	Healthy group	Patient group	-8.07200*	2.10884	.021	-15.2123	-.9317
		Nbs 250	-2.49000	2.10884	.842	-9.6303	4.6503

		Nbs 500	-.34800	2.10884	1.000	-7.4883	6.7923
		Nbs 1000	-.32800	2.10884	1.000	-7.4683	6.8123
	Patient group	Healthy group	8.07200*	2.10884	.021	.9317	15.2123
		Nbs 250	5.58200	2.10884	.178	-1.5583	12.7223
		Nbs 500	7.72400*	2.10884	.030	.5837	14.8643
		Nbs 1000	7.74400*	2.10884	.029	.6037	14.8843
	Nbs 250	Healthy group	2.49000	2.10884	.842	-4.6503	9.6303
		Patient group	-5.58200	2.10884	.178	-12.7223	1.5583
		Nbs 500	2.14200	2.10884	.901	-4.9983	9.2823
		Nbs 1000	2.16200	2.10884	.898	-4.9783	9.3023
	Nbs 500	Healthy group	.34800	2.10884	1.000	-6.7923	7.4883
		Patient group	-7.72400*	2.10884	.030	-14.8643	-.5837
		Nbs 250	-2.14200	2.10884	.901	-9.2823	4.9983
		Nbs 1000	.02000	2.10884	1.000	-7.1203	7.1603
	Nbs 1000	Healthy group	.32800	2.10884	1.000	-6.8123	7.4683
		Patient group	-7.74400*	2.10884	.029	-14.8843	-.6037
		Nbs 250	-2.16200	2.10884	.898	-9.3023	4.9783
		Nbs 500	-.02000	2.10884	1.000	-7.1603	7.1203
*. The mean difference is significant at the 0.05 level.							

Figures

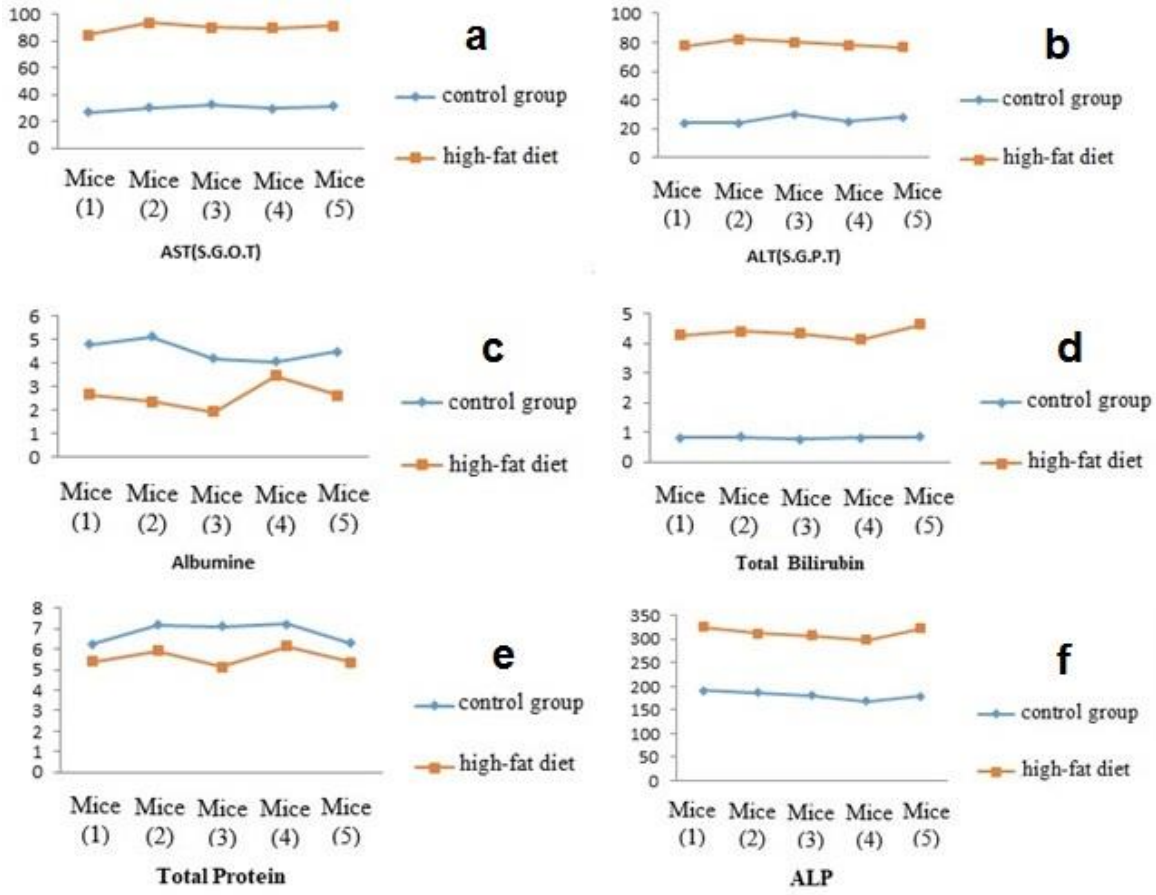

Fig. 1. Comparison of the (a) S.G.O.T, (b) S.G.P.T, (c) Albumin, (d) Total Bilirubin, (e) Total Protein and (f) ALP blood levels of healthy group and high fat diet.

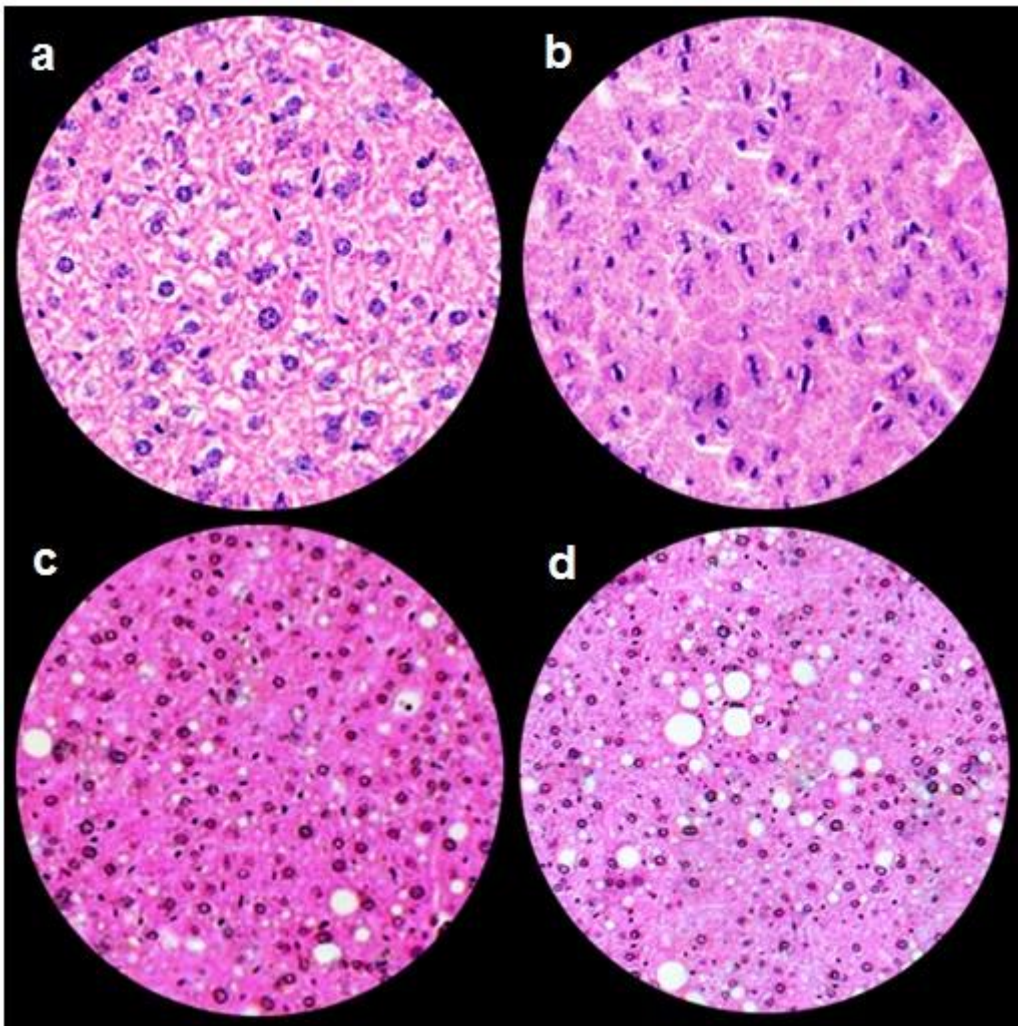

Fig. 2. Pathology of liver tissue of (a-b) the control and (c-d) patient rats.

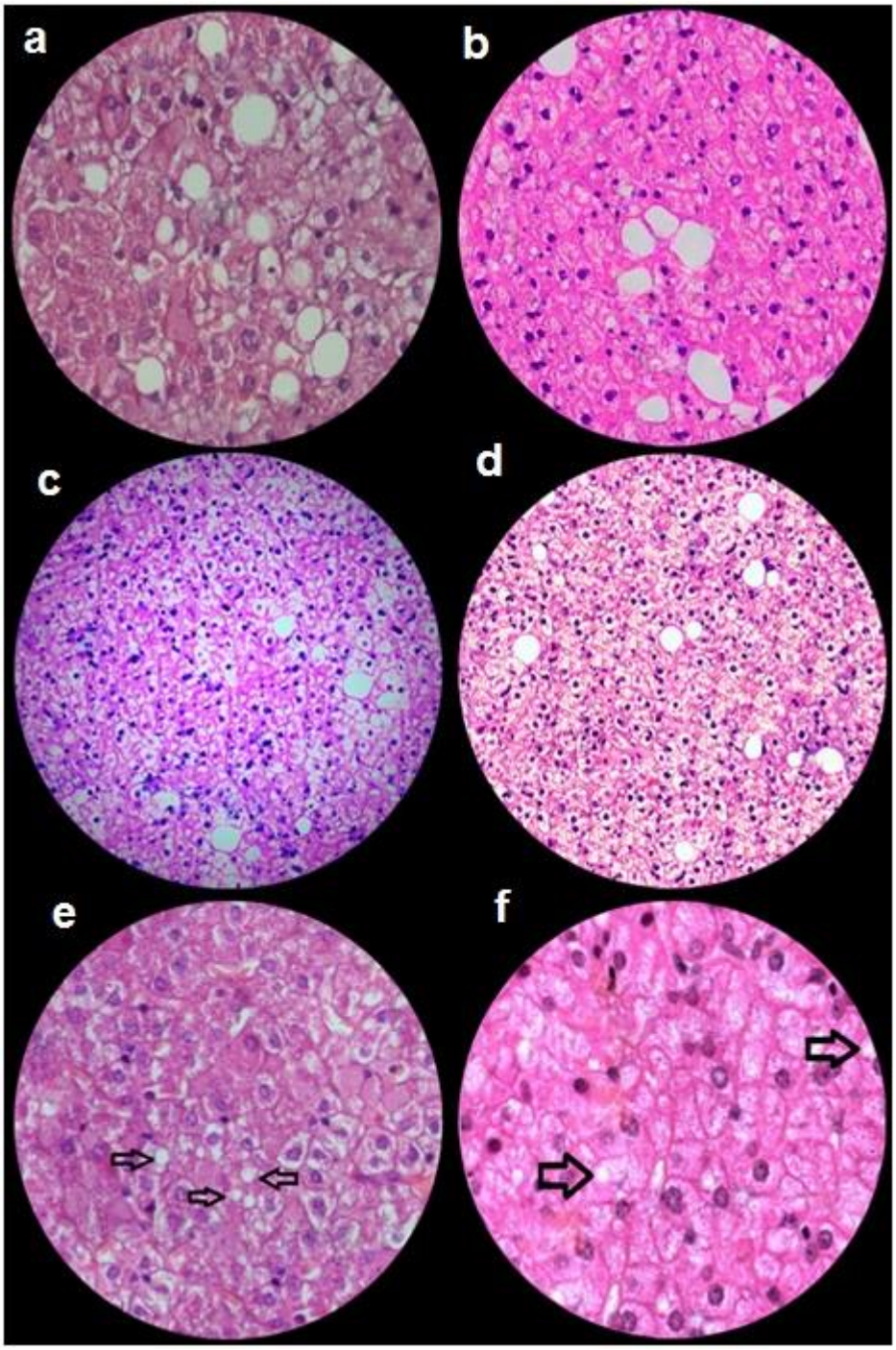

Fig. 3. Pathology of liver tissue of mice treated with a concentration of (a-b) 25, (c-d) 50, and (e-f) 100 healthy and healthy dietary supplement.

Figures

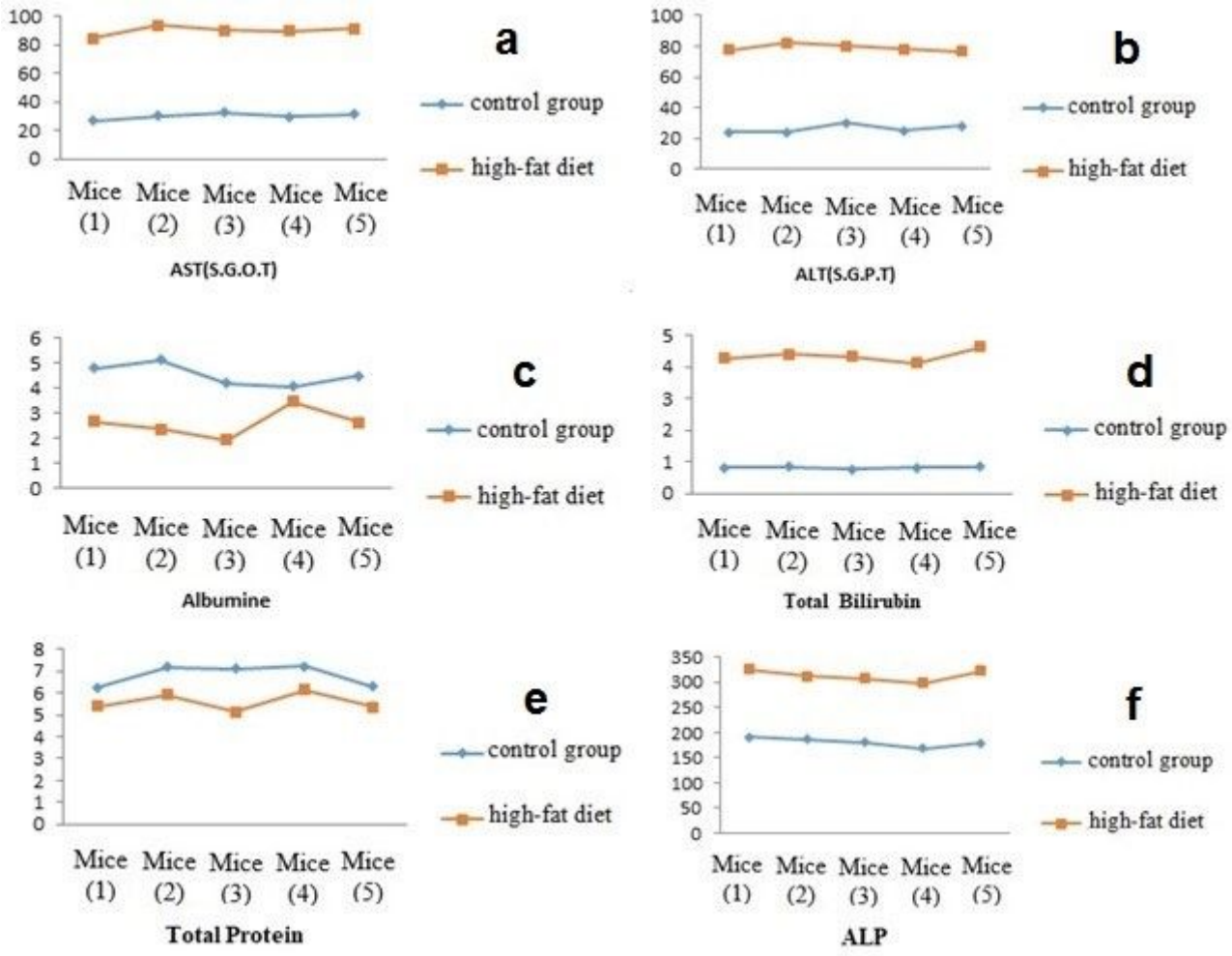

Figure 1

Comparison of the (a) S.G.O.T, (b) S.G.P.T, (c) Albumin, (d) Total Bilirubin, (e) Total Protein and (f) ALP blood levels of healthy group and high fat diet.

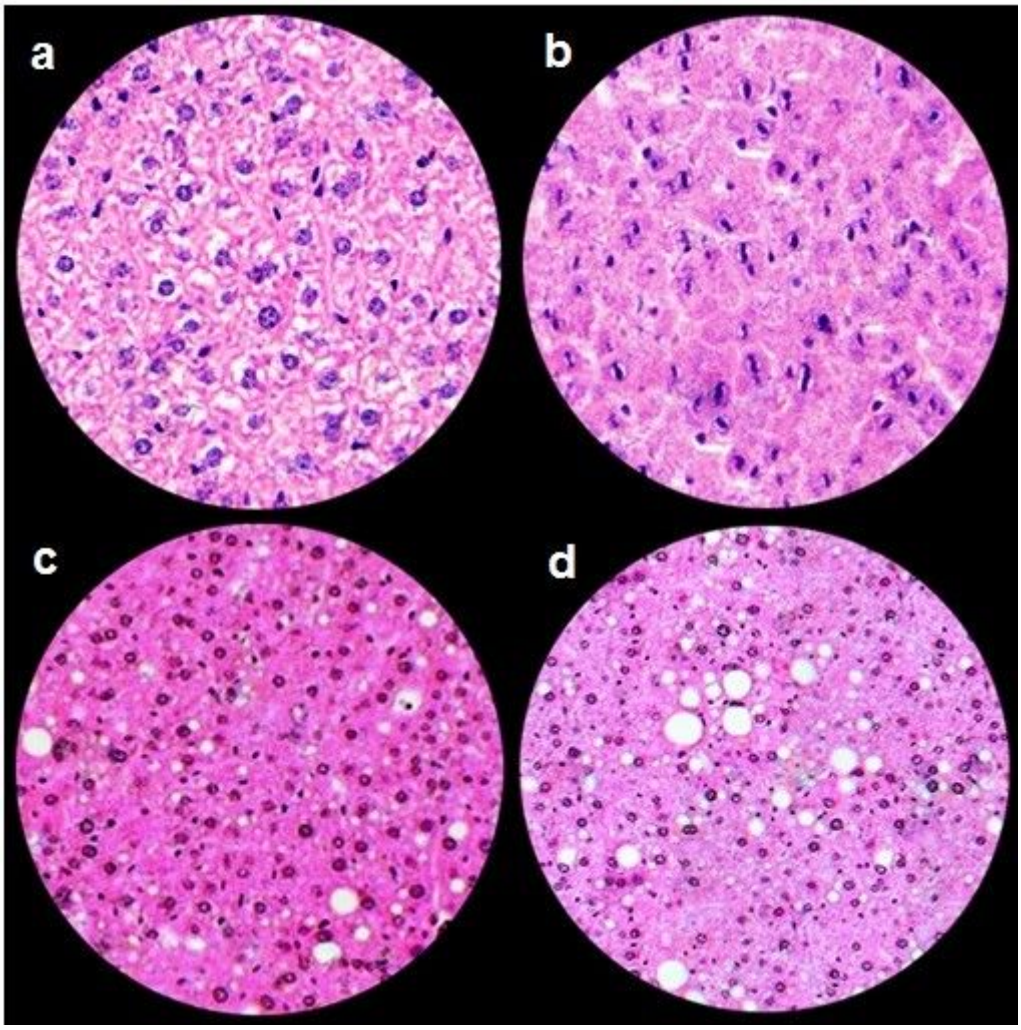

Figure 2

Pathology of liver tissue of (a-b) the control and (c-d) patient rats.

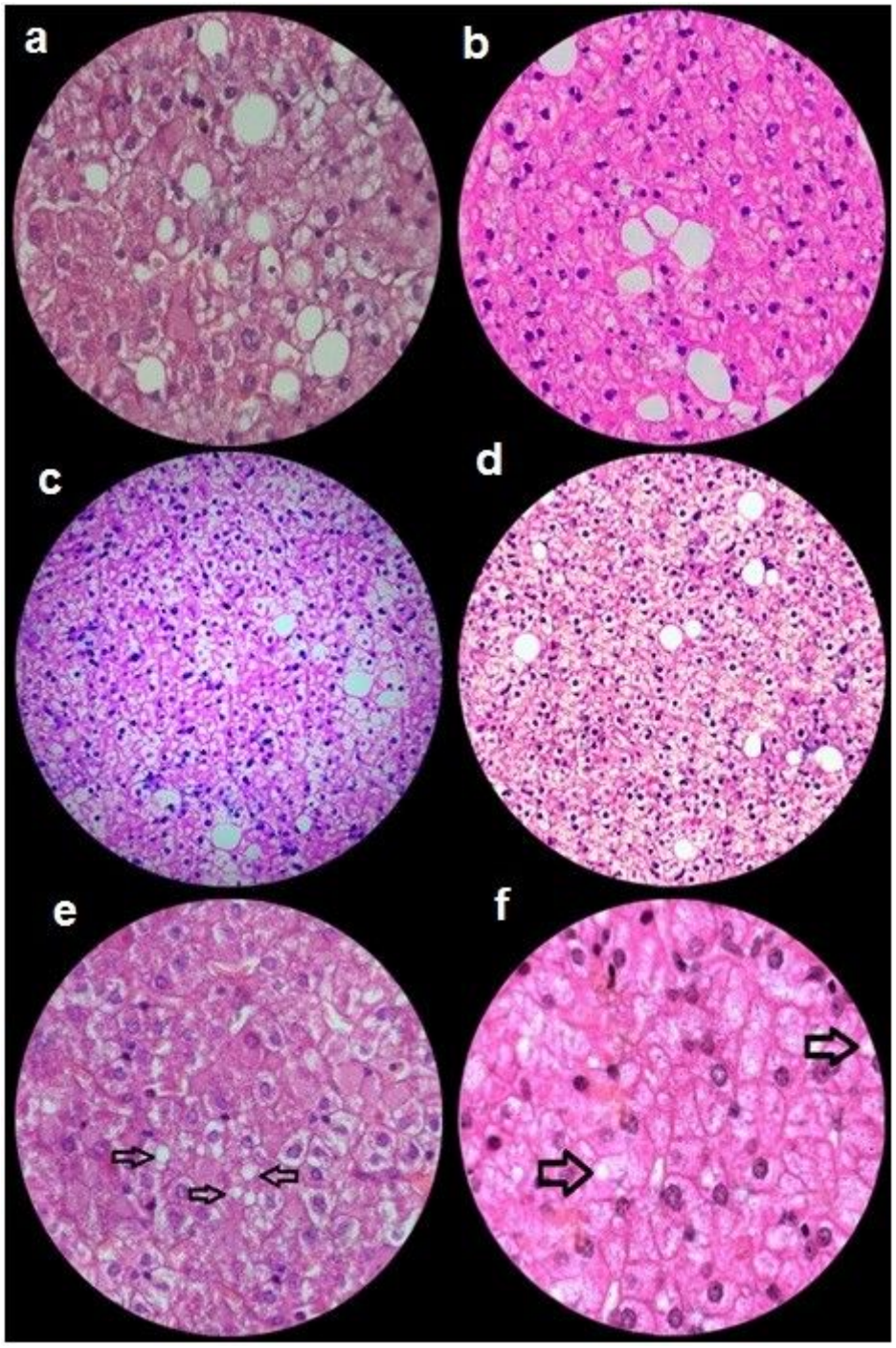

Figure 3

Pathology of liver tissue of mice treated with a concentration of (a-b) 25, (c-d) 50, and (e-f) 100 healthy and healthy dietary supplement.