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Therapeutic and Preventive Effects of a New Type Healthy and Viable Drug Supplement on Fatty Liver and Blood Lipids

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Abstract

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 drug supplement from wheat on fatty liver disease and blood lipids in animal model. The new NBS healthy and viable drug supplement was prepared by a green route. The NBS healthy and live drug powder had various vitamins, macro and micro molecules, as vitamin B_1, B_2, B_3, B_5, B_6, B_9, C, K, A, E, D, Phosphorus, Potassium, Sulfur, Magnesium, Calcium, Iron, Manganese, Zinc, Copper, Omega-3 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 gm-220 gm were used. The rats were divided into 5 groups and were treated with 25 mg/kg, 50 mg/kg and 100 mg/kg of healthy and live medication. 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. The new drug supplement reduces the level of hepatic macrovesicles, microvesicles, and the steatosis symptoms without specific hepatic complications. Also, the healthy drug causes reduction of lipid parameters.

Keywords: Healthy and Viable Food; Fatty Liver; Animal Model; Blood Lipids

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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.

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 drugs, inappropriate culture of over consumption of fast drugs, 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 dollar 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 [5-6]. 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 [7-11].

The healthy and viable drug supplement in the current research may be comparable to chemical supplements. The majority of the multivitamins that are available on the market only meet the needs of the human body. In addition, special attention was paid to their regulation and balance. This highlights the importance of the balanced cellular, molecular, and metabolic function of the human body, which has often been overlooked in other chemical and herbal drugs. In general, emphasis on balance is associated with the improvement and treatment of various diseases.

Another example in this regard is Ganoderma fungi, which has recently been introduced as a therapeutic drug owing to its active compounds for the body, some of which require further investigation. These fungi contain some chemicals that are unknown to the body, including three types of toxins, which may be hazardous to liver health. In addition, the long-term consumption of this material at high doses could lead to adverse complications. With this background in mind, no comparable foreign and domestic products have been registered that are produced in a similar manner to the processing of cereal grains in the form of a powder supplement for the disease control and treatment. 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 drug supplement, without side effects, against fatty liver disease. 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 drug powder on the level of lipid profile.

Methodology

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. [12], 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 grams, 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 was 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 mg/ml, 50 mg/ml 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 drug powder (healthy and alive) on fatty profiles of high fat diet recipients was

performed using a blood sample 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 and Discussion

Analysis of new healthy and live drug supplement is **Table 1**. In the electronic supplementary material. Also, analysis of phenolic compounds in the Nutritional supplement and healthy living (extracted with using HPLC) are shown as **Figure 1**. The percentage of phenolic compounds of new healthy and live drug supplement are Arctigenin 2.34, Gallic Acid 2.41, Quercetin 9.42, Alpha Linoleic Acid 26.80, Linoleic Acid 19.46, Inulin 2.64, Oleic acid 13.24, Palmitic acid 14.98, Stearic acid 3.14 and unknown compound.

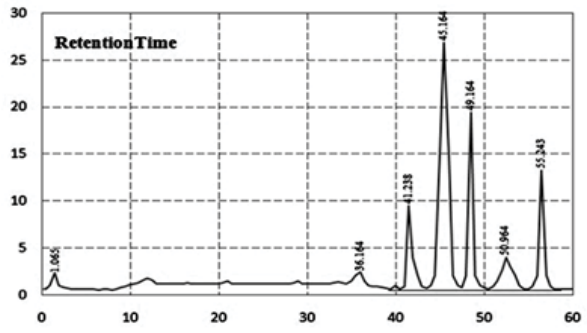

Figure 1 Analysis of phenolic compounds in the Nutritional supplement and healthy living (extracted with using HPLC).

Table 1: Analysis of the new NBS healthy and live food powder with various vitamins, macro and micro molecules, and ingredients.

Acceptable range	Test results	Unit	Factors	Row
-	8.6	%	Moisture	1
-	1.8	%	Total Ash	2
-	10.58	%	Fiber	3
5-71.7	60.7	%	Digestible Nutrients	4
-	42.53	gr in 100 gr	Carbohydrate	5
-	420	Calories in 100 gr	Calculate Raw Energy	6
-	12.8	gr	Fat	7
5.5-40.3	17.8	%	Raw Protein	8
-	3.8	%	Sugar Loaf	9
-	6	%	Cellulose	10

Table 2: Analysis of the new NBS healthy and live food powder with various vitamins, macro and micro molecules, and ingredients.

Test result	Unit	Vitamin	Row
0.62	mg	B_1	1
0.23	mg	B_2	2
2.6	mg	B_3	3
0.896	mg	B_5	4
0.84	mg	B_6	5
48	µg	B_9	6
52.4	mg	C	7
63.6	µg	K	8
530	IU	A	9
0.92	mg	E	10
432	IU	D	11

Table 3: Analysis of the new NBS healthy and live food powder with various vitamins, macro and micro molecules, and ingredients.

Acceptable range	Test results	Unit	Factors	Row
0.07-0.74	0.42	%	Phosphorus	1
0.42-9.36	2.31	%	Potassium	2
0.04-0.34	0.28	%	Sulfur	3
0.07-0.75	0.29	%	Magnesium	4
0.01-2.61	1.16	%	Calcium	5
0.07-0.75	0.62	%	Boron	6
-	266	mg/kg	Iron	7
6-265	49.3	mg/kg	Manganese	8
8-300	26.6	mg/kg	Zinc	9
-	13.1	mg/kg	Copper	10
-	43.51	mg/g	Omega-3	11
-	58.44	mg/g	Omega-6	12
-	19.24	mg/g	Omega-9	13

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 (STB) and Alkaline Phosphatase (ALP) compared with healthy control group, have a significant increase and Total Protein (TP) and Albumin 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 **Figure 2.** Also according to histopathologic changes in the liver tissue of control rats **Figure 3.** It can be concluded that the diet used in this study caused fatty liver disease. **Figure 3a, Figure 3b.** Shows 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 **Figure 3c, Figure 3d.**

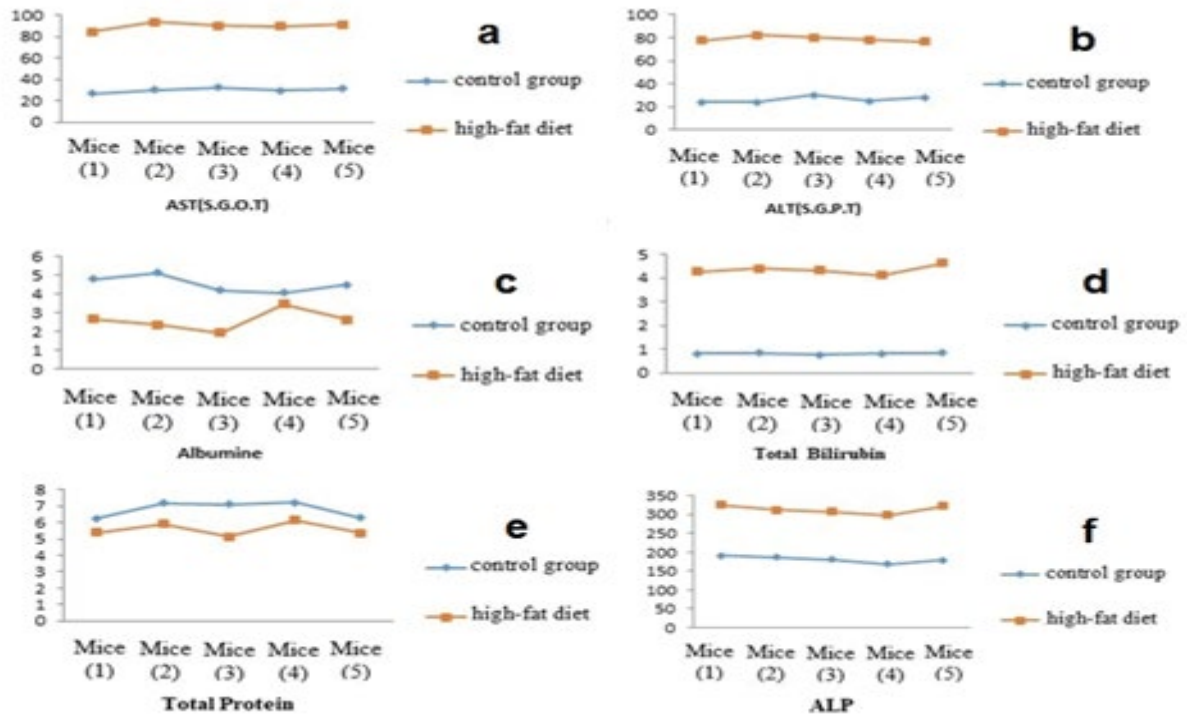

Figure 2 Comparison of the (a): S.G.O.T; (b): S.G.P.T; (c): Albumin; (d): Total Bilirubin; (e): Total Protein; (f): ALP blood levels of healthy group and high fat diet.

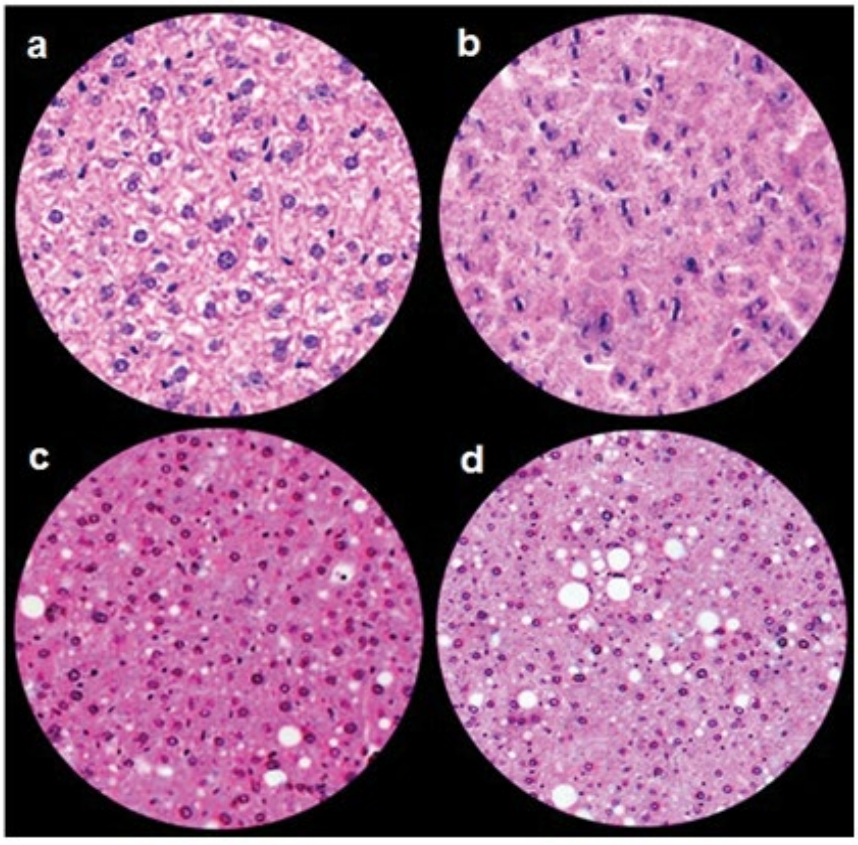

Figure 3 Pathology of liver tissue of (a-b): The control; (c-d): Patient rats.

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 and also the results of liver pathology images **Figure 4.** 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, micro vesicles, 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 **Figure 3**. 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 shows no changes on the large fat macrovesicles (rounded bodies, white and hollow) in patient groups **Figure 4a**, **Figure 4b**. 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 **Figure 4c**, **Figure 4d**, **Figure 4f**.

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).

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 **Figure 5**, **Figure 6** and **Figure 7**. 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 drug 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.

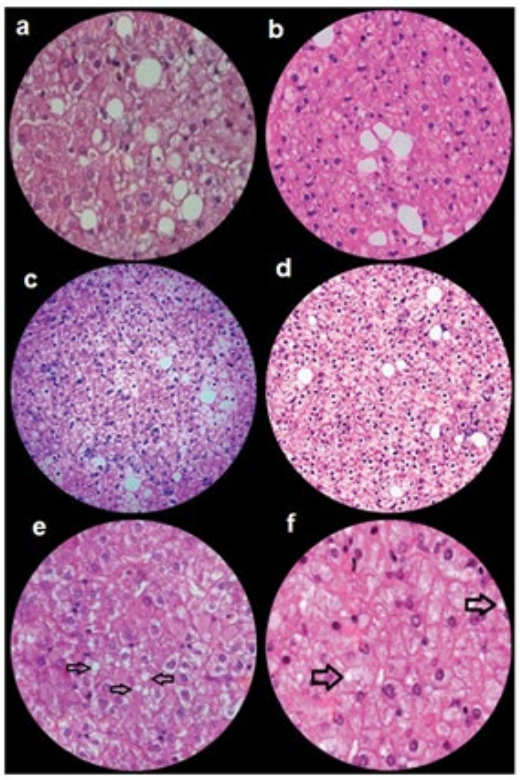

Figure 4 Pathology of liver tissue of mice treated with a concentration of (a-b): 25; (c-d): 50; (e-f): 100 healthy and healthy dietary supplements.

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

Figure 5 Test results of liver enzymes and protein levels in control and patient rats.

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

Figure 6 Test results of liver enzymes and protein levels of mice treated with the concentrations of (a): 25; (b): 50; (c): 100 healthy and alive nutrients.

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 drug. 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% 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 [13]. 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.

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

Figure 7 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

Figure 8 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

Figure 9 Blood fat level parameters results in rats No. 1-5 (The treatment group with 250 NBS healthy and viable drug supplement packs).

a

Analysis	Result	Range
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

b

Analysis	Result	Range
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

c

Analysis	Result	Range
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

d

Analysis	Result	Range
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

e

Analysis	Result	Range
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

Figure 10 Blood fat level parameters results in rats No. 1-5 (The treatment group with 500 NBS healthy and viable drug supplement packs).

a

Analysis	Result	Range
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

b

Analysis	Result	Range
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

c

Analysis	Result	Range
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

d

Analysis	Result	Range
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

e

Analysis	Result	Range
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

Figure 11 Blood fat level parameters results in rats (The treatment group with 1000 NBS healthy and viable drug supplement packs).

Increase the activity of liver function biomarkers, includes AST, ALP and ALT in serum, is indicative of liver damage [14], since the change in the above markers during the liver steatosis previously have been reported [15-16]. 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 drug 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 (**Figures 8-11**) [17-25].

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, drug 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 drug and pharmaceutical industries can be effective.

The new NBS healthy and live drug powder has various vitamins, macro and micro molecules, and ingredients such as Vitamin B_1, Vitamin B_2, Vitamin B_3, Vitamin B_5, Vitamin B_6, Vitamin B_9, Vitamin C, Vitamin K, Vitamin A, Vitamin E, Vitamin D, Phosphorus, Potassium, Sulfur, Magnesium, Calcium, Boron, Iron, Manganese, Zinc, Coper, Omega-3, Omega-6, Omega-9 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.

Conclusion

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 drug 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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