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Nutrition Intervention by Functional Foods and Dietary Regimen for Management of Nonalcoholic Fatty Liver Disease

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ABSTRACT

The aim of the present study is evaluation of dietary advice with or without functional foods in form of bakery products in non-alcoholic fatty liver disease (NAFLD) patients. Twenty four hours dietary recall and anthropometric measurements were assessed for volunteers at the start and the end of the study. Different plasma biochemical parameters reflecting NAFLD and metabolic syndrome were determined in normal and NAFLD patients; in the start of the study and after two months of dietary intervention. Proximate composition, dietary fibers and total phenolic content of the two functional foods were assessed. Results revealed that functional food II contained higher protein and fat percentage, while functional food I contained higher levels of carbohydrate and dietary fibers. Patients understudy were obese and hyper-caloric in the start of the work. After two months of intervention by functional foods with dietary advice; body mass index was reduced significantly while dietary advice produced only insignificant improvement; also all patients reduced their caloric intake significantly. In the start of the study; plasma transaminases (AST, ALT) and alkaline phosphatase activities, malondialdehyde, oxidized-low density lipoprotein, high sensitive-C-reactive protein, plasma glucose, insulin and insulin resistance, creatinine and urea were significantly higher in NAFLD patients than normal. Dyslipidemia was noticed in NAFLD patients. All biochemical parameters of patients consumed functional food I or II with dietary advice were improved significantly except for AST and plasma glucose. Consumption of functional foods with dietary advice was superior in management of NAFLD and related biochemical changes than dietary advice alone.

Keywords: Nonalcoholic fatty liver, functional foods, dyslipidemia, insulin resistance, inflammatory biomarker.

INTRODUCTION

Metabolic syndrome and obesity are major health problem and their prevalence increased in young and older individuals. Fatty acids deposition in tissues other than adipose tissue could lead to obesity-related metabolic abnormalities. Adipose tissue controls the flux of lipids in systemic circulation during the postprandial period. If the flux capacity of adipose tissue is impaired, then other tissues, such as skeletal muscle and liver may store excess lipids [1]. In healthy subjects, the liver could be adapted to altered nutrient fluxes that occur. Accumulation of fat in liver contributes to the pathology of metabolic diseases. When fatty acids exceed the capacity of liver for removal they are stored as triglycerides. The retention of triglycerides may develop non-alcoholic fatty liver disease (NAFLD). NAFLD is an important health problem worldwide. The prevalence of NAFLD ranges from 10% to 35%

in the general population with a median prevalence of 20% worldwide [2]. NAFLD is associated with obesity, type 2 diabetes mellitus, and dyslipidemia. Non-alcoholic fatty liver is considered the hepatic manifestation of the metabolic syndrome [3]. It is presumed that the underlying common pathophysiology among these conditions is insulin resistance (IR). IR and metabolic syndrome are involved in the pathogenesis and disease progression of NAFLD, causing, among other symptoms, increased free fatty acid influx to the liver, oxidative stress, mitochondrial toxicity, deregulation of adipokines followed by inflammation and fibrosis [4]. Patients with NAFLD [NAFL and non-alcoholic steatohepatitis (NASH)] have an increased risk of liver-related mortality and increased risk of cancer, kidney disease and cardiovascular disease [5]. NAFLD therapy remains a challenge for the scientific community, and there are no definite therapies for NAFLD. Lifestyle modifications, through diet and physical exercise, are proven to be effective, but they are challenging to implement [6].The lipid-lowering properties, anti-inflammatory and antioxidants activities of plants phytochemicals motivated our research team to prepare and evaluate two powderd food mixtures in rat model of NAFLD. These powders showed significant improvement in experimental NAFLD and its complications [7]. Mixture I consists mainly of pumpkin seed, oat, *Nigella sativa* seeds and grape seeds. Ingredients of mixture II were defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger and tomato powder. So the results from the previous study encouraged the research team to extend the evaluation of the beneficial effects of these two powder mixtures in form of bakery products (Functional food I & II) in NAFLD patients which is the objective of the present study. The aim also includes assessment of proximate composition, dietary fibers and total phenolic contents of the two functional foods that could be responsible of functional foods bioactivity. The prepared functional foods were sensory evaluated.

MATERIALS AND METHODS

Subjects, materials and methods

Materials

- Pumpkin seed, oat, *Nigella sativa* seed, red grape seed, green coffee seeds, tomato, turmeric root, ginger and whole wheat flour were purchased from local markets, while flaxseed and defatted soybean were purchased from Agriculture Research Centre, Cairo, Egypt.

Subjects

The subjects included in the clinical study were thirty patients (male and female) diagnosed with fatty liver. Their age ranged from 37 to 65 years old (average: 49.8±6.91 as mean±SD). Fifteen healthy subjects (11 females and 4 males) of matched age were enrolled in the study as control healthy subjects.

Methods

- **Preparation of plant materials.** Fresh tomato was washed by tap water and cut into small pieces. Seeds of red grape were separated from the fruits and washed while other parts were discarded. Pumpkin seeds were peeled. The aforementioned prepared plants´parts and all other purchased plants were dried separately in an air-circulated oven at 40 °C till complete dryness, and then they were reduced into powder form.
- **Preparation of Functional Food I in form of crackers**

Pumpkin seeds, oat, *Nigella sativa* seeds, grape seeds and whole wheat flour were made into crackers form representing functional food (I). Yeast (1.5%) was mixed with water (25 ^{o}C), salt (1%) and very little amount of sugar to form a suspension, to which all the ingredients of formula I were then added and kneaded to form smooth dough. The dough was later proofed for 2 hours in a proofer, followed by sheeting to 1.0 mm thickness using a dough sheeter. The dough was then cut into pieces rolled and baked at 170 ^{o}C for 15 min.

- **Preparation of functional food II in form of Syrian Bread**

Defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger, tomato powder and whole wheat flour were mixed and made into Syrian bread to give functional food (II).The straight dough method was used to produce the Syrian bread. This method involves the addition of all the ingredients of formula II with water and yeast at mixing stage and kneading to obtain the dough. The dough was spread into sheet and cut into rectangular pieces then placed in baking pans smeared with vegetable oil and was covered for the dough to ferment resulting in gas production and gluten development for about 1 hour. The dough was then baked in the oven at 350°C for 5 minutes. The baked Syrian bread were carefully removed from the pans and allowed to cool and packaged in polyethylene bags for analysis.

- **Chemical analysis of functional foods.** Moisture**,** protein, fat, ash, and crude fiber contents were determined in the functional foods according to A.O.A.C. [8]. Carbohydrates were calculated by difference. Total dietary fiber content of both functional foods was determined according to the method of dietary fiber kit (K-ACHDF 09/11, Megazyme, Ireland).Total phenolics were determined in the dried baked functional foods using Folin-Ciocalteu

reagent [9]. Absorbance was measured at 765 nm using UVPC spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in grams per 100 gram.

- **Sensory evaluation of functional foods.** Just after baking, the two functional foods were cooled to room temperature and subjected to sensory evaluation. Each patient was asked to assign scores on a ten-point scale for color, odor, taste, crispiness, appearance and overall acceptability. A sensory score of 5 or above was deemed acceptable, and a sensory score below 5 was considered unacceptable.
- **Design of the clinical study (intervention study):** The study was carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt. Patients with non-alcoholic fatty liver were divided into three groups. Group 1 and 2 each of 10 NAFLD patients (3 males and 7 females) were given daily quantity of 75g from functional food I and II, respectively on the expense of consumed carbohydrates together with following dietary regimen. Group three of ten NAFLD patients (3 males and 7 females) followed only dietary regimen. The study continued for 2 months. All fatty liver patients were assessed nutritionally through anthropometric measurements represented by WC and BMI [10] and questionnaire for dietary intake (one-day dietary recall in addition to frequency of food items consumed using the program of Nutrisurvey for Windows 2007. Patients were advised to reduce calories and carbohydrates specially sucrose and fructose rich foods and juices from their diets. They were also persuaded to substitute saturated fats, purified flour and its products by unsaturated fats, whole cereals and cereal products, respectively. Advice was also extended to replace full milk and full milk products with skimmed milk and low fat milk products, respectively. Patients were also advised to take daily specific amount of fresh vegetables and not to eat fatty meat. At the end of the study another questionnaire for one-day dietary recall was taken from all patients. The nutrient content of the functional foods were among the calculated nutrients' intake. Different nutrient intake of patients at the end of the study was compared with that at the start. Questionnaire concerning daily physical activity of patients was recorded. Biochemical analysis of blood was carried out at the start and end of the study. Fifteen normal healthy subjects were included in this study as control. Blood samples were obtained from fasted subjects. The blood samples were received on heparin for separation of plasma for determination of activity of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as indicator of liver function. Plasma total cholesterol (T-Ch), high density lipoprotein-cholesterol (HDL-Ch), low density lipoprotein-cholesterol (LDL-Ch) and triglycerides (TG) were determined as lipid profile. T-Ch/HDL-Ch ratio was calculated as risk factor for cardiovascular disease. Plasma malondialdehyde (MDA) and oxidized-LDL (ox-LDL) were assessed as indicator of lipid peroxidation. Plasma high sensitive C-reactive protein (hs-CRP) was determined as inflammatory biomarker. Plasma level of creatinine and urea were estimated for evaluation of kidney function. Plasma glucose and insulin were assessed. Insulin resistance (IR) based on homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting plasma glucose (FPG) and insulin (FPI), divided by a constant [FPG (mg/dl) × FPI (μU/ml)/2430] according to Cacho *et al*. [11]. All biochemical parameters were assessed using commercial kits (BioSystem, Spain). ELISA technique was used for determination of hs-CRP, ox-LDL and insulin (MyBiosource, Cat. No. MBS040244, CellBioLabs, Cat. No. STA-369 and abcam, ab100578, respectively). The biochemical parameters of patients at the start of clinical study were compared with those of the healthy control. Also, biochemical parameters of patients were compared before and after intervention with either dietary regimen or functional foods with dietary regimen.
- **Statistical analysis.** The results were expressed as the Mean ± SE and they were analyzed statistically using student's t-test. In all cases $p<0.05$ was used as the criterion of statistical significance.

RESULTS

Chemical analysis of functional foods:

Moisture was present as 2.35 ± 0.25 and 8.16 ± 0.46 in fresh functional food I and II, respectively. Chemical composition of dry functional food I and II revealed that functional food II contained high amount of protein (29.27 ± 1.15%) and fat (11.32± 0.63%) than functional food I. Functional food I contained higher amount of carbohydrate than functional food II (78.27± 2.38 % and 50.68 ± 1.66 %, respectively). Ash was present as 1.82 ± 0.12% in functional food I and 4.62 ± 0.68% in functional food II on dry basis. Crude fibers were 2.24 ± 0.11 % and 4.11 ± 0.34 % in dry functional food I and II, respectively. Dietary fibers were present as 25 ± 2.33 % in functional food I and 27 ± 1.42 % in functional food II. Total phenolic contents were 52.53 ± 2.28 and 73.96 ± 3.73 g GAE/100g functional food I and II, respectively.

Sensory evaluation of the two functional foods (Table 1) revealed that both of them are accepted by patients but preference belonged to functional food I.

Nutritional status of NAFLD patients.

Nutritional status of patients was assessed through determination of anthropometric parameters and food intake as seen in table 2 and 3, respectively. The average of BMI (kg/m^2) of all NAFLD patients collectively in the start of the study was 33.6±1.123 which revealed that patients were obese. The mean of waist circumference (cm) of all NAFLD patients was 108.7±1.006 in the start of the study. Table 3 showed that there was significant reduction in body mass index after receiving functional food I and II with dietary regimen, while non-significant reduction was observed after dietary regimen (dietary advice) only. Waist circumference was only reduced insignificantly at the end of the study in all patients´ groups. All NAFLD patients in the beginning of the study were hyper-caloric as could be seen from mean dietary intake. Daily physical activity of patients was low according to the questionnaire. After two months of intervention study all NAFLD patients reduced their caloric intake significantly. Total carbohydrates, saturated fat, cholesterol and protein intake of NAFLD patients of all groups were reduced while dietary fibers were significantly increased after dietary intervention.

Table (1): Parameters of sensory evaluation of the functional foods

Parameters	Functional food I	Functional food II
Odor	7.83±0.82	7.33±0.85
Color	8.0±1.095	7.5±1.048
Crispiness	8.17±0.79	8.0±1.26
Taste	7.67±2.065	6.83±2.56
Appearance	7.83±0.98	6.78±1.47
Overall acceptability	7.65±1.33	7.50±1.64

Table (2): Mean body mass index (BMI) and waist circumference (WC) of NAFLD patients before and after intervention study

Parameters	All Fatty liver patients collectively at the beginning of the study	Dietary advice		Functional Food I+ dietary advice		Functional Food II+ dietary advice	
		Before	After	Before	After	Before	After
BMI	33.6±1.123	32.7±0.985	30.2±1.005	35.1±0.746	31.8*±0.901	33.9±0.666	31.9*±0.644
WC (cm)	108.7±1.006	106.6±3.255	97.4±3.403	108.2±3.548	99.3±3.359	104.6±2.847	96.8±2.539

Values significantly differ from before values:: p<0.05.*

Table (3): Mean dietary intake of different nutrients/day in the beginning of the study and after intervention study

Parameters	All Fatty liver patients collectively at the beginning of the study	Dietary advice		Functional Food I + dietary advice		Functional Food II+ dietary advice	
		Before	After	Before	After	Before	After
Energy (Kcal)	2622.6±41.27	2423.9±17.27	1910.8*±26.06	2569.1±15.86	2121.3*±16.25	2874.8±63.66	2243.6*±10.69
Carbohydrate (g)	397.3±10.39	351±10.84	251.9±10.21	399.6±12.53	299.5±10.14	441.2±17.52	306.8±10.96
Protein (g)	96.8±3.409	84.9±4.69	76.1±5.15	93.9±2.83	76.1±2.19	111.6±6.34	84.7±3.79
Fat (g)	74.75±3.31	78.2±5.25	66.1±4.24	68.6±5.49	70.9±4.74	77.5±6.32	72.7±4.079
Saturated Fat (g)	28.2±1.50	32±2.57	29±2.54	25.8±2.41	22.73±3.52	26.9±2.58	25.74±2.07
Cholesterol (mg)	326.2±6.05	307.5±9.94	288±7.86	333.5±10.42	303.7±8.49	337.6±9.13	298.7±5.46
Dietary fibers (g)	29.8±0.52	28.4±0.83	37.3*±0.70	30.2±1.02	36.7*±1.12	30.9±0.68	33.1*±0.84

Values significantly differ from before values:: p<0.001.*

Biochemical parameters of fatty liver patients

Table 4 illustrates the different biochemical parameters of all NAFLD patients collectively at the beginning of the study in comparison to healthy subjects. High significant plasma activities of ALP, AST and ALT were noticed in NAFLD patients compared to normal subjects. Plasma lipid profile (T-Ch, TG, LDL-Ch, and T-Ch/HDL-Ch) of NAFLD patients showed significant elevation along with significant reduction of HDL-Ch compared to normal healthy subjects. Plasma levels of MDA and ox-LDL as markers of lipid peroxidation were significantly higher in NAFLD patients than in normal subjects. Also plasma level of hs-CRP as inflammatory biomarker was significantly high in NAFLD patients compared to normal subjects. Plasma glucose level, plasma insulin and insulin resistance were significantly higher in NAFLD patients than in normal subjects. Plasma creatinine and urea showed significant higher levels in NAFLD patients than normal subjects reflecting an initiation of kidney dysfunction.

Table (4): Different biochemical parameters of all NAFLD patients collectively before dietary intervention in comparison to healthy control subjects

Plasma parameters	Healthy control subjects	NAFLD patients
	Mean ± SE	Mean ± SE
Glucose (mg/dl)	81.7±1.344	100.4*±1.912
Insulin (µU/ml)	3.41±0.393	11.22*±0.632
IR	0.693±0.085	2.856*±0.215
hs-CRP (µg/ml)	2.11±0.156	3.84*±0.129
AST (U/l)	15.9±0.491	35.6*±1.285
ALT (U/l)	13.2±0.449	20.7*±0.616
ALP(IU/l)	50.4±2.346	66.5*±1.268
T-Ch (mg/dl)	176.1±6.507	257.6*±3.779
TG (mg/dl)	87±3.402	120.4*± 2.385
HDL-Ch (mg/dl)	55.5± 2.990	38.8*±0.469
LDL-Ch (mg/dl)	47.7±2.758	50.4*±0.759
T-Ch/HDL-Ch ratio	3.563±0.179	6.67*± 0.098
Ox-LDL (ng/ml)	64.1±2.236	118.9*±2.468
MDA (nmol/l)	4.9±0.248	16.5*±0.361
Creatinine (mg/dl)	0.739±0.027	1.02*±0.024
Urea (mg/dl)	28.9±1.154	29.4*±0.831

Values statistically significant when patients compared with healthy subjects:
**: $p < 0.001$*

Table 5 shows the biochemical parameters of patients before and after nutritional advice with or without functional food intervention. Plasma lipid profile of NAFLD patients consumed functional food I or II with dietary advice for two months improved significantly with different degrees. NAFLD patient given only dietary advice showed significant improvement in lipid parameters except for TG level. Plasma activity of ALT and ALP as indicator of liver function of NAFLD patients were reduced significantly in both groups consumed functional food I or II with dietary advice, while only the activity of ALT was reduced significantly in the group followed dietary advice only. NAFLD patients consumed functional food I or II with nutritional advice showed significant reduction of plasma MDA and ox-LDL, while plasma MDA reduced significantly in group given only dietary advice. High sensitive-CRP as inflammatory biomarker reduced significantly in all NAFLD groups under different dietary interventions. Plasma glucose level showed non-significant reduction in all NAFLD groups followed different dietary interventions. NAFLD groups consumed functional food I or II showed significant reduction in plasma insulin and insulin resistance, while were reduced non-significantly in group given only dietary advice. Plasma levels of creatinine and urea were reduced significantly in NAFLD groups consumed functional food I or II, while reduced non-significantly in group given only dietary advice.

Table (5): Different biochemical parameters of NAFLD patients before and after 2-month of dietary advice with or without functional foods.

Plasma parameters	Dietary advice		Functional Food I + dietary advice		Functional food II+ dietary advice	
	Before	After	Before	After	Before	After
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Glucose (mg/dl)	108.9±3.822	103.3 ± 3.569	94.9±2.373	90±0.943	97.3±1.64	95±1.350
Insulin (µU/ml)	14.6±0.945	13.8±0.757	9.3±0.592	7.8*±0.215	9.6±0.581	7.69*±0.369
IR	3.98±0.362	3.04±0.259	2.19± 0.197	1.73*±0.054	2.32± 0.176	1.81*±0.100
hr-CRP (µg/ml)	3.93±0.192	3.26*±0.129	4.2±0.201	3.6*±0.113	3.4±0.217	2.69*±0.197
AST (U/l)	41.3±1.350	37.2±1.289	35.2±1.919	30±1.764	30.5±1.990	25.7±1.961
ALT (U/l)	17.7±0.716	13.7*±0.804	23±0.989	18.5**±0.500	21.3±0.746	17.5**±0.719
ALP(IU/l)	67.9±2.735	62.3±2.409	65.1± 2.168	59.4*±2.177	66.6± 1.714	60.8*±1.504
T-Ch (mg/dl)	255.3±7.764	217.5**±8.002	250.7±6.803	209**±2.582	266.7±4.072	238**±5.363
TG (mg/dl)	113.7±4.058	106.5±3.368	122±3.751	100*±1.761	125.4±4.059	111.5*±2.892
HDL-Ch (mg/dl)	38.8±1.052	46**±1.096	39.7±0.539	49.1**±0.836	37.8±0.727	46.7**±1.086
LDL-Ch (mg/dl)	52±1.256	46**±1.202	46.9± 0.836	40.1**±0.722	52.3±1.116	43.6**±1.166
T-Ch/HDL-Ch ratio	6.59±0.179	4.73**±0.125	6.3±0.180	4.27**±0.093	7.08±0.168	5.12**±0.162
Ox-LDL (ng/ml)	114.6±5.082	104±4.801	116.2±3.559	106.2*±3.373	125.8±3.505	113.9*±4.065
MDA (nmol/l)	14.9±0.348	11.9*±0.314	16.6±0.562	13.5*±0.719	18.1±0.504	15.6**±0.521
Creatinine (mg/dl)	0.981±0.026	0.91±0.028	1.05± 0.051	0.843*±0.047	1.02±0.044	0.825**±0.034
Urea (mg/dl)	31.1±1.139	29±0.516	27.3±1.687	22.5*±0.764	29.9±1.304	25.1*±1.100

Values statistically significant when data after intervention were compared with that before:
**: $p < 0.05$, ***: $p < 0.001$*

DISCUSSION

Non-alcoholic fatty liver disease is a spectrum of liver disease manifested by the presence of ectopic fat in the liver. NAFLD is mainly associated with obesity, insulin resistance (IR), type 2 diabetes mellitus and the metabolic syndrome. Obesity, particularly central obesity, is a predictor of hepatic steatosis (deposition of fat in liver) and disease progression. In obese subjects, the prevalence of steatosis is at least two times more frequent than in lean subjects, being directly related to the increase of BMI. Metabolic syndrome is a cluster of cardiovascular risk factors that accompanies IR, which are central obesity, hypertension, dyslipidemia and glucose intolerance. At least one third of subjects with NAFLD have the metabolic syndrome and 80% at least one of its components. The prevalence of NAFLD increases with the number of components of the metabolic syndrome [12].

In the present study fatty liver patients showed significant elevation in plasma T-Ch, TG, LDL-Ch, T-Ch/HDL-Ch in addition to significant reduction in HDL-Ch compared with normal subjects. These changes in plasma lipid profile reflected that NAFLD is associated with dyslipidemia and increased risk for cardiovascular diseases (CVD). The present results are in agreement with a previous study [13], which reported that NAFLD is associated with hyperlipidemia. The high level of Ox-LDL-Ch in NAFLD patients in the present study pointed to the risk of deposition of fat and inflammation in the arteries leading to atherosclerosis, hypertension and CVD. Oxidation of LDL-Ch might be due to elevated oxidative stress represented by the high level of MDA.

NAFLD patients showed significant elevation of plasma glucose, insulin and HOMA-insulin resistance compared with normal subjects, in the current study. It is reported in the literature that NAFLD patient have an increase in hepatic and peripheral insulin resistance [14]. Insulin resistance is an essential risk factor in the pathogenesis of NAFLD and is accompanied by dyslipideamia, atherosclerosis, and endothelial dysfunction. NAFLD is associated with atherogenic dyslipidaemia (an increase in LDL-Ch, TG, apolipoprotein B, and decrease in HDL-Ch) which is a risk of CVD [14]. Both metabolic syndrome and NAFLD are associated with high incidence of CVD and most characteristics of metabolic syndrome are present in subjects with NAFLD and diabetes.

In NAFLD patients of the present study, plasma activities of transaminases (AST & ALT) and ALP as liver function biomarkers showed significant high levels compared with normal subjects. Several cross-sectional studies have reported a relationship between elevated serum activity of ALT and metabolic syndrome, diabetes, and NAFLD. Elevated plasma activity of liver enzymes independently predicted the future development of metabolic syndrome and diabetes mellitus as well as CV events and mortality in prospective studies. These associations can be partly ascribed to NAFLD and insulin resistance, in addition of other underlying mechanisms that contribute to the increased CV risk represented by inflammation and oxidative stress. The increased ALT may be related to increase in the risk of CVD. The interrelation between ALT and coronary heart disease events is significant, which proposed that NAFLD is associated with coronary heart disease independently of other features of the metabolic syndrome. Interestingly, serum ALT is also shown to be linked with an increased risk of carotid atherosclerosis in NAFLD. It was reported that AST and ALT levels can be modestly elevated or not changed [15].

NAFLD patients in the present study showed significant elevation in plasma levels of MDA as lipid peroxidation markers (oxidative stress) and hs-CRP as inflammatory marker. Inflammation is crucial in pathogenesis of NAFLD. Liver fat accumulation produced elevated oxidative stress and the pro-inflammatory cytokines TNF-α, IL-6, and IL-8 along with the activation of other inflammatory pathways such as CRP. Inflammation that accompanies insulin resistance possibly resulted in increased lipolysis in adipose tissue, increased NEFA uptake by hepatocytes and increased triglycerides synthesis in the liver. Mitochondrial fat oxidation and export of very low density lipoprotein particles are not in a parallel speed with triglycerides synthesis which leads to net fat deposition in the hepatocytes. As a result of the abnormal fat accumulation in the hepatocytes, there would be a marked derangement in the insulin signaling pathways in the liver. Inflammation is considered as the link between obesity and insulin resistance and could lead to pathogenesis of hepatic and systemic insulin resistance and CVD.

Plasma levels of creatinine and urea (kidney function marker) of fatty liver patients in the present study showed significant elevation compared with normal subjects. It was reported that subjects with NAFLD have an increased prevalence and incidence of both CVD and chronic kidney disease (CKD). It could be speculated that NAFLD, CVD and CKD have common risk factors (such as visceral obesity, insulin resistance, dysglycaemia, dyslipidaemia and hypertension) therefore NAFLD might be considered a marker rather than a causal risk factor of CVD and

CKD. In patients with steatohepatitis, several molecules released from the steatotic and inflamed liver might have a role in the development of atherosclerosis and kidney damage.

Although the majority of biochemical parameters (AST, ALT, ALP and urea) showed significant changes compared to healthy control however they are still within the normal range. However these significant changes pointed to the initiation of possible severe changes.

Food intake of fatty liver patients revealed that all patients are hyper-caloric and take large amount of saturated fat. Recommended dietary allowance for total calories is 2200 Kcal for women and 2500 Kcal for men as reported by FAO/WHO in 1989. Consumption of high-saturated fat diet is one of the most leading causes of NAFLD because this diet results in obesity, abnormalities of lipid metabolism and insulin resistance, all of which have been speculated to be linked to NAFLD.

Anthropometric measurements in the present study revealed that NAFLD patients were obese (BMI was over 30 and waist circumference more than 100 cm). It was reported that increase in BMI is accompanied by high prevalence of NAFLD. The prevalence of NAFLD increased from 16.4% among people with normal BMI to 75.8% among obese subjects. The incidence of NAFLD is even higher with morbid obesity, and among morbidly obese undergoing bariatric surgery the incidence may be as high as 96%. A marked increase in BMI and waist/hip ratio was reported in NAFLD subjects. Insulin resistance is independent on BMI but is related to central obesity (reflected in waist circumference), which is a feature of NAFLD. In individuals with or without diabetes NAFLD is associated with increased risk of CVD [16].

In the present study two functional foods were evaluated in NAFLD patients. Pumpkin seed, oat, *Nigella sativa* seed, grape seed and whole wheat flour are components of functional food I. Defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger, tomato powder and whole wheat flour were the ingredients of functional food II.The prepared functional foods contain food sources expected to be rich in phenolic compounds, plant protein, dietary fibers, carotenoids and unsaturated fatty acids (omega 3 and 6).

After two months of consuming functional food I or II along with dietary advice by NAFLD patients, plasma lipid profile showed significant improvement which was associated by significant reduction of HOMA-insulin resistance, oxidative stress markers and inflammatory markers. Liver function of these patients showed improvement with different degrees. Plasma levels of creatinine and urea showed significant reduction reflecting improvement in kidney function. NAFLD patients after two months of dietary advices only showed improvement in all the studied parameters with different degrees.

After two months of study all fatty liver patients reduced their calories, saturated fatty acid and cholesterol intake with increase of dietary fibers. These changes in dietary intake were associated with reduction in body weight, waist circumference and BMI. Weight reduction appears to be the first-line treatment in NAFLD. Dietary restrictions are as essential as physical activity and changes in lifestyle.

The prepared functional foods contain phenolic compounds and dietary fibers as shown from the present study. Functional food II contains higher amount of total phenolic and dietary fibers than functional food I. Dietary phenolic contents could be used for treatment of metabolic syndrome due to their reported protective effect towards fatty liver, CVD and diabetes [17]. Also dietary fibers are known to possess weight reducing ability and have a great impact in improving lipid and carbohydrate metabolism thereby they could ameliorate fatty liver.

The presence of pumpkin seed in formula I could help in improving fatty liver and associated biochemical changes and in reducing body weight and waist circumference. Previously, pumpkin seed was proved to reduce body weight in animal experiment due to an inhibiting effect on fat compartment of the body according to Hyounjeong *et al*. [18]. Also, pumpkin seed oil was reported to have antioxidant, anti-inflammatory, and lipid lowering effect [13]. It could be speculated that pumpkin seed oil inhibiting effect on NASH in animal model may be related to its content of phytosterols, β-carotene, tocopherols, tocotrienols and unsaturated fatty acids [19]. The unsaturated fatty acids present in pumpkin seeds are oleic and linoleic acids which could play an important role as hypocholesterolemic.

Oat was reported as complementary therapy for metabolic syndrome which could be due to presence of beta-glucan that reported to have liver-protecting effect and ability to reduce obesity, abdominal fat, and to improve dyslipidemia [20]. It is worth mentioning that formula I in the present study contain oat.

It could be noticed from previous researches that both the defatted part and the oil compartment of *Nigella sativa* possess therapeutic effect towards liver diseases through improving liver function, reducing liver fat, and improving dyslipidemia in addition to their antioxidant, and anti-inflammatory activity [21, 22]. So the presence of *Nigella sativa* seed in the present functional food I might have an important role in ameliorating fatty liver and associated disorders.

Grape seed which present in functional food I is rich in procyanidins that have antioxidant effect and attenuate steatosis and liver injury and reduce blood cholesterol and abdominal fats in experimental animals [23].

Isoflavones present in soy, a component of functional food II, inhibit lipogenic enzyme. So, isoflavones could help in reducing accumulation of liver fat thereby preventing NAFLD. Soy isoflavone also increases antioxidant capacity in liver and improve insulin resistance as reported by Leng *et al*. [24].

Flaxseed, an ingredient of functional food II, is rich in dietary fibers and the phenolic compound lignans. Lignans reduces CVD risk through elevating HDL-cholesterol and blocking androgen receptors. Bioactive metabolites of high antioxidant activity were produced from Lignans, by gut microbiota. These metabolites could be responsible of inhibiting MDA and ox-LDL levels in the current study. Reduction in LDL oxidation on consumption of flaxseed in the present study coincided with previous clinical study in obese adults with insulin resistance [25]. Alpha-linolenic acid (LNA) present up to 60% in flaxseed oil is considered as preventive therapy for metabolic syndrome including CV risk and dyslipidemia. The prevention of metabolic syndrome could be related to its anti-inflammatory, antithrombotic, antiarrhythmic, and vasodilatory properties and reduction of insulin resistance and cytokine synthesis. It may also elicit its therapeutic effect through acting as precursor of longer chain omega-3 fatty acids like eicosapentaenoic or decosahexaenoic acid or by competition with linoleic acid to reduce arachidonic acid synthesis. LNA was demonstrated to reduce hepatic lipids levels thereby inhibits the progression of NAFLD. However, since LNA in flaxseed oil could lead to elevated lipid peroxidation due to the unsaturation, this may have an adverse effect on hepato-protection. However the presence of phenolic content in FO or the consumption of the whole flaxseed as in the present study may prevent this adverse effect and may contribute in prevention of fatty livers by reducing hepatic lipid accumulation and oxidative stress.

Functional food II contains green coffee beans. Bio active constituents present in coffee are caffeine, chlorogenic acids and diterpenes represented by cafestol and kahweol. NAFLD and serum aminotransferase activities were reported to be improved by coffee consumption. Also, Caffeine reduced liver fibrosis on molecular level [26] which reflects the inhibition of fatty liver progression to more advanced state.

The progression of Non-alcoholic steatohepatitis were reported to be reduced by phytonutrients and phytochemicals namely lycopene and polyphenolic compounds. Lycopene is present in high amount in dry tomato powder [27] while poly phenols are present in turmeric and ginger. Curcumin, the main bioactive constituents in turmeric, attenuated hepatic fat accumulation in rats and prevented inflammation, oxidative stress and insulin resistance in high fat fed rats [28]. Also, turmeric extracts were shown previously to possess anti-inflammatory and antioxidant activity in rat oxonate model [29]. Ginger has anti-obesity effect in mice and gingerol, the bioactive constituent from ginger possess blood cholesterol lowering effect in animal [30]. Both ginger and turmeric has anti-fatty liver effect. So it is speculated that both could have great therapeutic effect in fatty liver and metabolic syndrome patients as in the present study.

In the present study the use of whole wheat flour in both functional foods could be of beneficial effect to fatty liver patients due to its high content of dietary fibers that could have a hand in reducing body weight and blood and liver lipids.

CONCLUSION

NAFLD is mainly related to obesity, so change in lifestyle aiming at weight loss and increased physical activity is essential for all patients with NAFLD. The prepared functional foods together with dietary advice in the present

study were superior in management of NAFLD than dietary advice alone. The improvement in the NAFLD patients' status was reflected in reduction of dyslipidemia, insulin resistance, oxidative stress and inflammation. The prepared functional foods are beneficial towards NAFLD and associated disorders such as metabolic syndrome, cardiovascular disease and kidney dysfunction. The beneficial effects of functional food could be related to their contents of dietary fibers and total phenolics assessed in the present study in addition to previously determined constituents such as plant protein, carotenoids, tocoperols, phytosterols and unsaturated fatty acids (omega 3 and 6)**.**

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