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Protection from Steatohepatitis and Its Risk Factors by Plants Food Mixtures.

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ABSTRACT

The objective of the present study was to prepare and evaluate two mixtures of plants food in non-alcoholic fatty liver (NAFL) rat model. NAFL was induced in rats by feeding high fructose diet (HFD). Mixture I consists of pumpkin seed, oat, *Nigella sativa* seed and grape seed. Defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger and tomato powder represented mixture II. Proximate composition, dietary fibers and total phenolic contents of the mixtures were assessed. The results revealed that both mixtures I and II contain high percentage of protein. Mixture I showed higher content of fat and carbohydrate, while mixture II contain higher amount of dietary fiber, ash and total phenolic compounds. Feeding rats HFD for 35 days produced significant reduction in plasma high density lipoprotein cholesterol (HDL-Ch) and significant elevation in the activities of plasma alkaline phosphatase, transaminases, plasma total cholesterol (T-Ch), triglycerides (TG), low density lipoprotein cholesterol, the ratio of T-Ch/HDL-Ch, tumor necrosis factor-α and malondialdehyde. Liver total fat, T-Ch and TG were increased significantly in HFD-fed rats. Plasma levels of creatinine and urea as indicator of kidney function showed significant elevation in HFD-fed rats. Plasma glucose, insulin and insulin resistance were elevated significantly in HFD-fed rats. Feeding HFD mixed with either mixture I or II protected rats from the severe aforementioned biochemical changes and produced significant reduction in final body weight compared to HFD fed rats.

Keywords: Non-alcoholic fatty liver, plants food mixtures, oxidative stress, rats, fructose.

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INTRODUCTION

Metabolic syndrome is an emerging global epidemic, which comprises a cluster of metabolic disorders such as abdominal obesity and insulin resistance. One of the metabolic diseases often associated with metabolic syndrome is non-alcoholic fatty liver disease (NAFLD). NAFLD ranges from steatosis to non-alcoholic steatohepatitis (NASH), with or without fibrosis and cirrhosis[1]. NAFLD is the most prevalent chronic liver disease affecting 10–30% of people in developed countries[2, 3] and a cause of raised liver enzymes[4, 5]. The increasing prevalence of NAFLD, which is closely linked with the increasing prevalence of obesity and type 2 diabetes mellitus [2], has been associated with increased cardiovascular morbidity and mortality[4]. Dietary factors that influence NAFLD have become a focus of attention. Dietary fructose was accused in inducing NAFLD [6, 7] along with increasing visceral adiposity and lipids [8]. The mechanism underlying this assumption was ascribed to the induction of de novo lipogenesis by fructose. Fructose consumption might induce hepatic lipid accumulation by activating lipogenic gene expression and/or by the direct flow of fructose carbon into the glycolytic pathway, bypassing a key regulatory enzyme of glycolysis and phosphofructokinase [9]. For this reason, a higher proportion of the carbon from ingested fructose, as compared with glucose, is metabolized into triglycerides. Fructose consumption can also contribute to the inflammatory progression of NAFL into NASH by inducing bacterial overgrowth in the small intestine with a concomitant increase in endotoxin levels in the portal vein [10]. This might trigger the non-alcoholic steatohepatitis (NASH) pathology. Continuous accumulation of fat in liver could lead to elevated oxidative stress and inflammation resulting in NASH [11]. NASH is described as fatty liver with inflammation that could initiate the progression to liver cirrhosis and cancer. Plants food that are rich in antioxidant, anti-inflammatory and lipid lowering functional food ingredient could have a good impact in protection from fatty liver, steatohepatitis and their risk factors represented by cardiovascular disease, diabetes and liver cirrhosis. Previously, *Nigella sativa* seed oil and pumpkin seed oil showed great impact in protection from fatty liver in rats [12, 13]. Grape seed, tomatoes, turmeric and ginger were reported to possess antioxidant and anti-inflammatory activity[14-17]. Green coffee seeds were proved to reduce hepatic fat and guard against incidence of NASH [18]. Oat, which rich in dietary fibers has plasma lipid lowering effect and could be efficient in reducing heapatic fat. So it is hypothesized that combination of the aforementioned food sources could reduce fatty liver and its risk factors. The objective of the present study was to prepare two formulas from the above mentioned food sources and to evaluate their protective effect towards the induced NASH in rats. Bioactive constituents represented by dietary fibers and phenolic contents besides the proximate composition of the two formulas were analyzed.

MATERIALS AND METHODS

Materials

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

Animals

Male Sprague Dawley rats of 140-160 g body weight were used in the present study. Animals were obtained from Animal house of National Research Centre, Cairo, Egypt. Animals were kept individually in stainless steel cages; water and food were given ad-libtium.

Methods

Preparation of plant materials

Fresh tomato was washed by tap water and cut into small pieces. Seeds of red grape were removed from fruits and washed. Pumpkin seeds were peeled. All 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 formulas of plants food

Pumpkin seed, oat, *Nigella sativa* seed and grape seed powders were homogeneously mixed to form mixture I. Defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger and tomato powders were mixed to give mixture II. All samples were stored in airtight containers and kept at 5-7°C until used.

Chemical analysis of powder mixtures

Powder mixtures samples were re-dried and sieved through 100-mesh sieve. The samples were analyzed for moisture, protein, fat, crude fiber and ash contents using standard AOAC[19] procedure. Total dietary fiber content of both mixtures was determined according to the method of AOAC[20]. Total phenolics were determined in the powder mixtures using Folin-Ciocalteu reagent[21]. 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 dry mixture. Different chemical analysis were carried out in triplicate and averaged.

Diets

Experimental diets were prepared as in table; 1. High fructose diet was prepared similar to Kawasaki *et al*.[22] with some modification to induce NASH (nonalcoholic fatty liver with inflammation). The main ingredient in the diet that causes NASH is fructose complemented by lard. Twenty grams from mixture I and II were mixed with the high fructose diet to give diet I and II, respectively. The contents of protein, fat and carbohydrate of the 20 g mixtures were reduced from casein protein, corn oil and starch, respectively without affecting fructose or lard levels.

Table 1: Composition of different experimental diets (g/100 g).

Ingredients	Diets			
	Balanced diet	High fructose diet	Diet I[a]	Diet II[b]
Casein	12*	12*	5.9	1.2
Corn oil	10	4.1	-	0.7
Lard	-	5.9	5.9	5.9
Fructose	-	63.7	63.7	63.7
Starch	68.5	9.8	-	4
Salt mix.	3.5	3.5	3.5	3.5
Vit. mix.	1	1	1	1
Fiber	5	-	-	-
Powder (mix. I)	-	-	20	-
Powder (mix. II)	-	-	-	20

* 12 casein has been estimated to contain 10 g protein using AOAC (2000).

[a] Diet I: High fructose diet supplemented by mixture I.

[b] Diet II: High fructose diet supplemented by mixture II.

EXPERIMENTAL PROCEDURES

Twenty-four rats were divided into four groups, each of six rats. The first group was considered as the normal healthy group where rats received a balanced diet. The second group was named control NASH where rats were fed on high fructose diet. Rats of group three and four were fed on high fructose diet containing 20% powder mixture I and II (diet I and II, respectively). During the experiment, body weight and food intake were recorded weekly. After thirty-five days (end of the study) total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Blood samples were collected from all rats after an overnight fast for the determination of plasma total cholesterol (T-Ch)[23], high density lipoprotein cholesterol (HDL-Ch)[24], low density lipoprotein cholesterol (LDL-Ch)[25] and triglycerides (TG)[26]. T-Ch / HDL-Ch ratio was calculated as indicator of cardiovascular risk. Plasma malondialdehyde (MDA) was estimated as an indicator of lipid peroxidation[27]. Plasma tumor necrosis factor-α (TNF-α)[28] was determined as an inflammatory biomarker. The activity of plasma aspartate transaminase (AST) and alanine transaminase (ALT)[29] and alkaline phosphates (ALP)[30] were estimated as indicator of liver function. Plasma level of creatinine[31] and urea[32] were determined to study any possible changes in kidney function. Fasting plasma glucose (FPG) and insulin (FPI) were determined according to Trinder[33] and Turkington *et*

al.[34], respectively. Insulin resistance (IR) was calculated based on homeostasis model assessment of insulin resistance (HOMA-IR), according to Cacho *et al*.[35]. The equation was [FPG (mmol/l) × FPI (µU/mL)]/22.5. Liver was immediately removed, weighed and stored at -20 °C till analyzed. Total hepatic lipids were extracted and weighed according to the procedure of Folch *et al*.[36] and Cequier-Sànchez *et al*.[37] and the concentration of triglycerides and cholesterol was assesses utilizing the methods of Megraw *et al*.[26] and Watson[23], respectively. This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt.

Statistical analysis

The results of animal experiments were expressed as the mean±SE and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

RESULTS

Chemical composition of the powder mixtures shown in Table 2 clarified that both mixtures contain high percentage of protein (25.83 and 44.93) and fat (20.22% and 17.05 %) in mixture I and II, respectively. Percentage carbohydrate was 41.99% and 24.29%, crude fibers were 1% and 1.5% and the ash contents were 2.22 and 5.9% in mixture I and II, respectively. Dietary fibers were present as 22% in mixture I and 23% in mixture II. Total phenolic contents were 4.6 and 8.15g GAE/100g mixture I and II, respectively.

Table 2: Chemical composition of powder mixtures. (Mean±SD)

Ingredients/100g dry sample	Mixture I	Mixture II
Moisture (g)	8.73 ± 0.525	6.29 ± 0.581
Protein (g)	25.83 ± 1.027	44.93 ± 0.899
Fat (g)	20.22 ± 0.569	17.05 ± 0.756
Ash (g)	2.22 ± 0.311	5.93 ± 0.899
Crude fibers (g)	1 ± 0.327	1.5 ± 0.408
Carbohydrate*	41.99 ± 1.424	24.29 ± 2.812
Dietary fiber (g)	22 ± 0.816	23 ± 0.816
Total phenolic compounds (g GAE)	4.6 ± 0.432	8.15 ± 0.645

* Calculated by differences

Table (3) showed the nutritional parameters of different studied groups. Nutritional parameters and liver weight % to body weight of different experimental groups are shown in table 3. It could be noticed that there was no-significant changes when all the nutritional parameters of HFD fed rats were compared to rats fed on balanced diet. Final body weight, body weight gain and food efficiency ratio of rats fed on HFD containing 20% of mixture I, or II were reduced significantly when compared with HFD group. Liver weight % to body weight of rats fed on HFD was significantly higher than that of normal rats. Liver weight % to body weight of rats fed on diet I was reduced significantly when compared with HFD fed rats or rats fed on diet II, but still significantly higher than normal healthy rats.

Table 3: Nutritional parameters of different experimental groups.

Parameters	Normal control	High fructose control	Diet I	Diet II
Initial BW(g)	149.7±3.954^{a}	149.7±3.611^{a}	149.7±3.756^{a}	150±8.199^{a}
Final BW (g)	195.3±7.077^{a}	195.7±6.327^{a}	177.2±3.952^{b}	178.7±9.706^{b}
Body weight gain (g)	45.7±4.424^{a}	46±4.781^{a}	27.3±2.333^{b}	28.2±1.249^{b}
Total Food intake (g)	401.2±19.765^{a}	407±18.694^{a}	493±10.728^{b}	499.3±16.745^{b}
Food efficiency ratio	0.114±0.009^{a}	0.113±0.010^{a}	0.056±0.004^{b}	0.056±0.001^{b}
Liver weight/body weight %	2.7±0.070^{a}	3.5±0.117^{b}	3.1±0.085^{c}	3.5±0.123^{b}

In each row same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability.

Table (4) illustrated the biochemical changes in different experimental groups. Significant increase in the activities of plasma ALP, AST and ALT were noticed in control HFD-fed rats compared to normal healthy rats. Treatment by mixture I and II resulted in significant decrease in ALP, AST and ALT activity compared to the HFD-fed rats. Control HFD-fed rats exhibited a significant reduction in plasma HDL-Ch and a significant increase in total cholesterol, triglycerides, LDL-Ch and the ratio of T-Ch/HDL-Ch compared with control normal healthy rats. In addition, significant increase was observed in total fat, T-Ch and TG in the liver tissue of HFD-fed rats compared to control normal. Rats fed on diet I and II showed significant improvement in plasma lipid profile and reduction in the contents of liver total fat, T-Ch and TG with different degrees. Plasma levels of MDA in control HFD fed rats was significantly higher than that of normal control rats. Rats fed diet I and II showed significant reduction in plasma MDA levels compared to HFD-fed rats but still higher than normal rats. Plasma level of TNF-α was significantly higher in HFD group than in normal healthy rats. This elevation was reduced significantly in rats fed on diet I and II. Control rats fed on high fructose diet showed significant elevation in plasma levels of creatinine and urea as indicator of kidney function. Feeding rats on diet I and II showed significant reduction in plasma levels of both creatinine and urea indicating improvement in kidney function. Plasma glucose was elevated significantly in control HFD-fed rats compared with normal control group. Rats fed on diet I and II showed significant reduction in plasma glucose levels compared to HFD-fed rats. Plasma insulin and IR were elevated significantly in HFD-fed rats compared with normal control group. Rats fed on diet I and II showed significant reduction in plasma insulin and IR levels compared to HFD-fed rats.

Table 4: Biochemical parameters of different experimental groups.

Biochemical Parameters	Normal control	High fructose control	Diet I	Diet II
Plasma Parameters				
Total cholesterol (mg/dl)	87.2±1.519^{a}	166.0±2.955^{b}	138.7±2.472^{c}	143.5±5.376^{c}
HDL-Ch (mg/dl)	43.5±0.619^{a}	25.7±0.558^{b}	35.5±0.764^{c}	34.8±0.477^{c}
LDL-Ch (mg/dl)	21.5±0.885^{a}	99.0±1.505^{b}	73.2±2.574^{c}	75.0±1.788^{c}
TCh/HDL-Ch ratio	2.01±0.062^{a}	6.48±0.168^{b}	3.9±0.119^{c}	4.1±0.115^{c}
Triglycerides (mg/dl)	91.7±1.148^{a}	117.0±1.769^{b}	102.3±1.686^{c}	105.5±1.821^{c}
Glucose (mg/dl)	71.3±1.519^{a}	82.8±2.315^{b}	70.8±1.922^{a}	72.0±1.238^{a}
Insulin (mU/l)	4.03±0.092^{a}	6.18±0.145^{b}	5.25±0.173^{c}	5.4±0.19^{c}
Insulin resistance	0.711±0.026^{a}	1.26±0.048^{b}	0.919±0.046^{c}	0.964±0.424^{c}
Malondialdehyde (nmol/ml)	5.9±0.187^{a}	8.7±0.356^{b}	6.0±0.302^{c}	6.6±0.207^{c}
Tumor necrosis factor-α (pg/ml)	20.2±0.593^{a}	34.2±0.792^{b}	25.1±0.546^{c}	27.0±0.856^{c}
AST (IU/l)	42.8±1.137^{a}	84.7±2.108^{b}	61.7±0.989^{c}	64.3±1.282^{c}
ALT (IU/l)	56.5±1.359^{a}	89.0±1.712^{b}	69.3±0.803^{c}	73.5±0.991^{c}
Alkaline phosphatase (IU/l)	169.8±3.159^{a}	187.2±2.441^{b}	165.7±1.429^{c}	167.5±1.231^{c}
Creatinine (mg/dl)	0.621±0.09^{a}	0.769±0.013^{b}	0.628±0.016^{a}	0.626±0.013^{a}
Urea (mg/dl)	30.8±0.946^{a}	38.8±1.195^{b}	31.2±0.703^{a}	31.8±1.137^{a}
Liver Tissue:				
Total fat (mg/g tissue)	23.8±0.872^{a}	48.0±1.807^{b}	32.5±1.057^{c}	33.3±0.882^{c}
Total Cholesterol (mg/g tissue)	2.03±0.147^{a}	7.2±0.144^{b}	3.7±0.143^{c}	4.1±0.367^{c}
Triglycerides (mg/g tissue)	5.07±0.183^{a}	14.2±0.797^{b}	5.3±0.336^{a}	5.5±0.136^{a}

In each row same letters means non-significant difference; different letter means the significance among the tested groups at 0.05 probability.

DISCUSSION

Fatty liver is considered as the hepatic component of metabolic syndrome and its prevalence is continually increasing due to increased outcomes of obesity. Fatty liver itself does not represent any hazards on health, however changing fatty liver to steatohepatitis is considered as a major health problem that needs combating. This is because steatohepatitis is one of the major causes of cardiovascular disease and liver carcinoma. Steatohepatitis is associated by IR that may lead to type 2 diabetes.

Experimental model of metabolic syndrome with fatty liver in rats were induced previously by feeding high fructose diet[22]. This effect of fructose was supported by Angulo [38], Nomura & Yamanouchi[10] and Al-Okbi *et al*.[13]. Fructose stimulates fat accumulation in liver by increasing fat synthesis and blocking fat

oxidation[39]. Fructose not only increases fat accumulation but also induced elevation of oxidative stress and inflammation[11].

So, in the present research this model was utilized and was complemented by using lard and reducing fibers to zero to enhance lipid accumulation in the liver. The model in the current study proved induction of steatohepatitis and metabolic syndrome represented by the accumulated fat, dyslipidemia, elevated MDA as indicator of oxidative stress and the increased tumor necrosis factor-α as an inflammatory biomarker, increased plasma glucose and IR. An extra negative health effect is the induced kidney dysfunction which may speculate an initiation of hepatorenal syndrome in this model. This result agreed with the results of Fan *et al*.[40] who reported that high-fructose diet-induced renal damage involving renal inflammation, insulin resistance and lipid accumulation in rats. High dietary intake of fructose is an important factor in the development of the cardiorenal metabolic syndrome (CRS). The CRS is a constellation of cardiac, kidney and metabolic disorders including insulin resistance, obesity, metabolic dyslipidemia, high blood pressure, and evidence of early cardiac and kidney disease. The consequences of fructose metabolism may result in intracellular ATP depletion, increased uric acid production, oxidative stress, inflammation, and increased lipogenesis, which are associated with endothelial dysfunction. Endothelial dysfunction is an early manifestation of vascular disease and a driver for the development of CRS[41]. High fructose intake causes metabolic syndrome, being an increased risk of chronic kidney disease development and affects the pancreatic islet function in humans and animals[42].

The model in the present study was used to evaluate the impact of combining different food sources that are rich in bioactive constituents to prevent induction of fatty liver and associated disorders. The selected food sources of mixture I were pumpkin seed, oat, *Nigella sativa* seed and grape seed while that of mixture II were defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger root and tomato powders. Combination of different bioactive food sources in one formula act synergistically and induced significant health benefits towards fatty liver as could be seen from the results.

Pumpkin seed and *Nigella sativa* seed were selected because their oil contents showed great impact in reducing fat content of liver, improved plasma lipid profile and reducing oxidative stress and inflammation in rat model of steatoheptitis in previous works[12, 13]. The effect of *Nigella* might be due to presence of thymoqinone, linoleic acid and phytosterol. Pumpkin effect might be attributed to the presence of phytosterol, unsaturated fatty acids (oleic and linoleic), antioxidant vitamins such as carotenoids, tocopherol and tocotrienols as reported by Stevenson *et al*.[43] and Al-Okbi *et al*.[44]. Barakat & Mahmoud[45] and Al-Okbi *et al*.[44] showed pumpkin seed oil to possess hypocholesterolemic effect which agreed with the present work. As an approval of these works and the current study pumpkin seed oil was reported to possess anti-inflammatory, hypolipidemic and antioxidant effect [46-48]. Thus this component of the studied functional food might also reduce the progression of fatty liver to NASH. Strong anti-obesity effect of pumpkin seed was reported in animal study which was ascribed to inhibition of lipid synthesis and enhanced lipid degradation in the body[49].

Oat was incorporated in mixture I because of its previously reported hypolipidemic effect due to presence of β-glucan. So, it has a metabolic-regulating and liver-protecting effect. Consumption of oat reduced obesity, abdominal fat, and improved lipid profiles and liver functions. Taken as a daily supplement, oat could act as an adjuvant therapy for metabolic disorders[50].

Grape seed which is one component of mixture I is a source of many bioactive ingredients. Grape seed is a source of polyphenols-flavonoids, essential fatty acid-linoleic acid, vitamin E, and oligomeric proanthocyanidin, gallic acid. Grape seed procyanidins regulate the main gene signal involved in inflammation (NF-kappaB), thereby reducing inflammation and preventing the release of the inflammatory form of nitric oxide (iNOS)[14]. Grape seed extract was reported to have beneficial effect on low-grade inflammatory diseases, through inhibition of the proinflammatory molecules CRP, IL-6 and TNF-α and the enhanced production of the anti-inflammatory cytokine adiponectin. Procyanidins reduced obesity-related adipokine dysregulation to manage cardiovascular and metabolic risk factors[51]. Procyanidins also lowered plasma triglycerides, free fatty acids, apolopoprotein B (apoB), LDL-cholesterol and slightly increased HDL-cholesterol. Procyanidins improve the atherosclerotic risk index in the postprandial state, and thereby induce overexpression of cholesterol 7α-hydroxylase increase cholesterol elimination via bile acids in the liver[52]. Grape seed extract showed previous health benefit in rat model of fatty liver, where it reduced mRNA of

receptors of IL-6, TNF-α and leptin that are elevated in fatty liver. It also improved the level of adiponectine which is reduced in fatty liver i.e. restore the level of adipocytokine receptors in fatty liver disease[53].

Defatted soybean as component in mixture II is rich in isoflavones which was previously suggested as a useful alternative medicine in preventing NAFLD and pathological adiposity and this action may be partially related to ChREBP and Wnt signaling[54]. Soy isoflavone can reduce the hepatic lipid deposition and increase antioxidant capacity; the mechanism may be related to inhibition of SREBP-1c and activation of PPARα expression in liver[55]. Soy protein may improve the liver function in patients with non-alcoholic steatohepatitis by lowering lipid levels in the blood and liver and by increasing the anti-oxidative capacity and improving insulin resistance[56].

Flaxseed, a component of powder mixture II, contains high levels of dietary fibers and phytochemicals such as lignans (phenolic compound) with potential weak estrogenic activity[57-60]. Lignans may block androgen or progesterone receptors, thereby may alter cardiovascular disease risk by changing HDL-cholesterol metabolism[57]. Lignans, which are converted by gut bacteria into the bioactive mammalian lignans enterolactone and enterodiol with a potent antioxidant activity[61] that may result in reduction of MDA in the present study. Flaxseed was reported to reduce LDL oxidation in obese insulin resistance subjects[62].

Flaxseed is a rich source of omega-3 fatty acid, which is α-linolenic acid[63]. Polyunsaturated fatty acids are efficient in the prevention and therapy of cardiovascular diseases, dyslipidemia and metabolic syndrome [64-66]. Polyunsaturated fatty acids possess anti-inflammatory, antithrombotic, antiarrhythmic, and vasodilatory properties[67, 68]. They decrease insulin resistance and cytokine synthesis[67, 68]. α-Linolenic acid can act as the precursor of longer chain omega-3 polyunsaturated fatty acids (EPA and DHA) or compete with linoleic acid to reduce arachidonic acid content or may directly interact with ion channels and nuclear receptors, and thus may exert numerous beneficial effects in the human body, including antiarrhythmic and anti-inflammatory effect[69]. Recently, α-linolenic acid was reported to reduce liver size and hepatic lipids contents and thus attenuate NAFLD[70]. The presence of phenolic content in flaxseed may reduce oxidative stress and inflammation[71].

Coffee is a complex mixture of more than 1,000 compounds with the major constituent being caffeine. The other two main components are diterpenes, such as cafestol and kahweol, and chlorogenic acids. Several studies have linked coffee consumption to an improvement in NAFLD[18, 72]. A recent study suggested that the serum aminotransferase levels in individuals suspected of having NAFLD are higher in those who consume lesser amounts of coffee[73]. A potential mechanism for this observation is that caffeine alters TGFβ signaling pathways by increasing the level of SMAD, which reduces the transcription of CTGF, a major stimulator of fibrosis[73-75]. Also Gutiérrez-Grobe *et al*.[76] reported that high intake of coffee has a protective effect against nonalcoholic fatty liver disease that could be due to antioxidant and anti-inflammatory activity of chlorogenic acids.

Lycopene and tomato extract were investigated and proved efficient for their relevant activity in controlling non-alcoholic steatohepatitis and cardiovascular risks[77-81]. So, tomato powder was incorporated in one of the studied mixtures.

Ginger was shown to possess antioxidant, anti-inflammatory and weight reducing effect[82, 83]. Turmeric was reported to have anti-inflammatory[15, 16] antioxidant[84], and cardiovascular protective effect[85]. So the presence of ginger and turmeric in mixture II could afford protective effect and prevent the progression of fatty liver to steatohepatitis with simultaneous protection from cardiovascular risk.

High phenolic contents especially in mixture II could render the mixtures some of their protective effects towards fatty liver and its risk factors. Since phenolic compounds were reported to reduce NAFLD, cardiovascular disease and diabetes[86] and could be of health benefit in metabolic syndrome. These therapeutic effects of phenolic compounds might be attributed to their antioxidant and anti-inflammatory activity reported previously[87, 88].

The two studied mixtures contain high quantity of dietary fiber to which improvement of plasma lipid profile, plasma glucose and IR may be ascribed. There is many documented emphasis that dietary fibers

possess lipid lowering effect[89] which was ascribed to its effect as inhibitor of intestinal fat absorption due to an effect on bile salt and prestaltic movement of the intestine[90] thereby improving plasma lipid profile. Soluble dietary fibers also have an impact in reducing blood sugar and improving carbohydrate metabolism [91]. All these activities could have an effect in reducing liver fats [92]. Dietary fibers are considered as prebiotic that could improve microflora profile and inducing anti-inflammatory activity that may share in their therapeutic effect [93]. Beside phenolic content and dietary fibers, both mixtures also contain polyunsaturated fatty acids ω3 and ω6, phytosterols and antioxidant vitamins that could render the two mixtures their therapeutic effect reflected in the improvement in biochemical parameters, and reduction in body weight gain. Although there was significant increase in total food intake on feeding diet I and II, a significant reduction in body weight was noticed compared with HFD fed rats and normal rats fed balanced diet. This might be due to high levels of dietary fibers or the presence of functional ingredients that elevate energy expenditure thereby reducing body fat.

Diet II reduce different liver fat content significantly without affecting % liver weight/body weight compared to HFD-fed rats which may indicate an increase in other liver compartments than fat.

The efficiency of both mixtures was comparable in improving biochemical parameters. Although a significant improvements in all biochemical parameters were noticed; however these parameters still not matching the normal levels. The only biochemical parameters that were normalized were plasma glucose, creatinine and urea and liver triglyceride.

CONCLUSION

Mixture I containing pumpkin seed, oat, *Nigella sativa* seed and grape seed and mixture II containing defatted soybean, flaxseed, green coffee seeds, turmeric root, ginger and tomato powder showed therapeutic efficiency in rat model of fatty liver. This effect could be attributed to the presence of phenolic compounds, dietary fibers, phytosterols and polyunsaturated fatty acids.

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