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Research Article
In vitro Anticancer Activity of Quinoa and Safflower Seeds and Their Preventive Effects on Non-alcoholic Fatty Liver

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Abstract

Background and Objective: Non-alcoholic fatty liver disease (NAFLD) is not only the most common cause of liver diseases in humans but also it may complicate and become a risk factor for liver cancer. The present work aimed to evaluate the anticancer activity (*in vitro*) of quinoa and safflower seeds powder and their beneficial effects against NAFLD (*in vivo*). **Materials and Methods:** Proximate analysis, fatty acids profile, total phenolic and phytic acid of quinoa and safflower seeds were assessed. Also their anticancer activities (*in vitro*) against liver cancer were evaluated. The preventive effect of both seeds on NAFLD was evaluated using twenty four male rats. NAFLD was induced in rats by high fructose diet (HFD) for 4 weeks. The effects of HFD and HFD supplemented with 20% quinoa or safflower powder on plasma and liver lipids, lipid peroxidation, total protein, albumin as well as liver and kidney functions were determined. **Results:** Quinoa seeds powder was promising in cytotoxicity against hepatocarcinoma cell line HEPG2 (IC_{50} was 14.6 µg). Feeding rats on HFD produced dyslipidemia and significant increase in liver functions and lipid peroxidation with significant elevation in liver triglycerides and total cholesterol. Quinoa and safflower seeds powder produced improvement in the biochemical parameters with different degrees. **Conclusion:** Quinoa and safflower seeds powder possessed cytotoxicity against hepatocarcinoma cell line HEPG2 and afford hepato-protection against NAFLD.

Key words: Quinoa, safflower, non-alcoholic fatty liver, hepato-protection, anticancer activity, hepatocarcinoma

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver diseases in humans. NAFLD is characterized by extravagant aggregation of triglycerides in the hepatocyte not due to the alcohol consumption[1]. A wide variety of complications may be accompanied to NAFLD, from simple steatosis to non-alcoholic steatohepatitis (NASH) and can eventually lead to cirrhosis, liver failure and/or liver cancer[2,3]. It was reported that NASH might develop to liver cirrhosis in about 10-29% of patients and liver cirrhosis might progress to hepatocellular carcinoma (HCC) in about 4-27% of patients[4]. NAFLD is associated with hyperlipidemia, overweight/or obesity and type 2 diabetes, so it is considered the hepatic component of the metabolic syndrome (MetS)[5]. The exact mechanism of starting and advancement of NAFLD till now unclear; inflammation and elevation of oxidative stress play an important role in the progression of NAFLD[6]. Fructose is a monosaccharide sugar found in fruits, honey, sucrose and high fructose corn syrup (HFCS). Fructose is able to elevate either plasma triglycerides or liver fat, which differentiate it from glucose. Fat accumulation in the liver due to fructose consumption (via the lipogenesis activation and the blocking in the fatty acid oxidation) is the key in elevation of triglycerides in liver[7]. Food can play an important role in treatment and prevention of different diseases. One of the newest concept in the prevention and treatment of NAFLD is the intake of dietary supplements that possess various health benefits besides to their nutritional value. Fruits, vegetables, spices, herbs are among these dietary supplements that contain high levels of biologically active compounds/or nutrients such as omega-3 fatty acids, phenolic compounds, antioxidant vitamins, fibers and minerals which play an important role in diseases prevention and treatment[8,9]. Biologically active compounds present in dietary supplements possess different biological activities such as anti-inflammatory, hypoglycemic, hypolipidemic, hypotensive, anti-atherosclerotic, anti-thrombotic, hepatoprotective and hypocholesterolemic effects[10,11]. Liver lipid-lowering activity of biologically active compounds may be attributed to their ability to regulate the expression of genes that implicated in *de novo* lipogenesis and fatty acid oxidation[12]. Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal, which used like rice and wheat[13]. Quinoa seeds are rich source of carbohydrates, high-quality protein and dietary fiber[14,15]. Recently, the biological activities of quinoa seeds have been studied through their effective role as antimicrobial[16] and anti-inflammatory[17]. Phenolic compounds and saponins were the most studied phytochemicals in quinoa seeds[18]. Safflower (*Carthamus tinctorius* L.) belongs to Asteraceae family. The Carthamus species have been cultivated in China, India, Iran and Egypt almost from prehistoric times. The seeds of safflower contain oil, protein and crude fiber by 30, 20 and 35%, respectively. Safflower seeds are rich source of minerals, tocopherols (α, β and δ), carotenoids and thiamine[19]. Safflower seeds have been used in the folk medicine for prevention and treatment of different diseases such as diabetes, cardiovascular diseases and bone diseases[20,21]. Safflower seeds powder possessed anti-androgenic effect in rats[22] and ameliorated benign prostate hyperplasia in rats[23]. Due to the plenty of biologically active compounds in quinoa and safflower seeds, it was hypothesized that these plants may protect liver from NAFLD and cancer. So, the present research aimed to evaluate the anticancer activity of these plants and their preventive effect on NAFLD in rats.

MATERIALS AND METHODS

The present study was carried out in the National Research Centre, Egypt. It was started in March, 2018 and end in August, 2018.

Quinoa seeds were purchased from local markets, while safflower seeds (Giza 1) were purchased from Agriculture Research Centre, Cairo, Egypt.

Cancer cells: Liver human tumor derived cell lines (HEPG2) were supplied from National Cancer Institute, Cairo University, Egypt and used for cytotoxicity study.

Animals: Male Sprague Dawley rats of 129.8±12.17 g as Mean±SD 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*.

Preparation of plant materials: Quinoa and safflower seeds were dried separately in an air-circulated oven at 40°C till complete dryness and then they were reduced into powder form and stored in airtight containers and kept at 5-7°C until used.

Chemical analysis of seeds powder: Quinoa and safflower seeds powder samples were sieved through 100-mesh sieve. The samples were analyzed for moisture, protein, fat, crude fiber and ash contents using standard AOAC[24] procedures. Different chemical analysis were carried out in triplicate and averaged.

Assessment of fatty acids of quinoa and safflower seed oils: Oil of both seeds was extracted using Soxhlet apparatus; petroleum ether 40-60°C was used for extraction of oil. The solvent was evaporated under vacuum using rotary evaporator. Fatty acid methyl esters of the both oils were prepared according to AOAC[24] to be subjected to GLC analysis of fatty acids. Assessment of the methyl ester was carried out by injecting 2 µL into a Hewlett Packard HP-system 6890 gas chromatograph equipped with FID. HP-5 capillary column (30 m×0.32 mm i.d.; 0.25 µm film thickness) was used to separate the different methyl esters. The chromatographic analysis conditions were: initial temperature 70°C with a hold for 1 min, then rose to 120°C at a rate of 40°C min^{-1} with 2 min hold then the temperature was finally raised to 220°C at a rate of 4°C min^{-1} with another 20 min hold. The injector and detector temperatures were 250 and 280°C, respectively. Identification of the fatty acid methyl esters was carried out by direct comparison of retention times of each of the separated compounds with standards of the fatty acid methyl esters analyzed under the same conditions. Quantization was based on peak area integration.

Determination of total phenolics: Quinoa and safflower seeds powder were extracted twice with 80% ethanol according to the optimized extraction conditions described in a previous work[25]. Total phenolics were determined using Folin-Ciocalteu reagent[26]. Absorbance was measured at 765 nm using UVPC spectrophotometer. The results were expressed as milligrams of gallic acid equivalents (GAE) per100 g on dry weight basis. Three replicates were measured and the results were expressed as Mean±SE.

Determination of phytic acid (PA): Phytic acid content was determined in the quinoa and safflower seeds powder according to the method of Gao *et al.*[27]. A series of calibration standards containing 0, 1.12, 2.24, 3.36, 5.6, 7.84, or 11.2 mg L^{-1} PA were prepared from sodium phytate (Sigma, St. Louis, MO). The PA content of the sample was calculated from the standard curve and was represented as PA content/100 g sample. Three replicates were measured and the results were expressed as Mean±SE.

Anticancer activity: Anticancer activity of quinoa and safflower seeds was tested using the cell line technique according to Cordero *et al.*[28]. Cells of HEPG2 (liver carcinoma cell) were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment to be attached to the wall of plate. All plants powder was dissolved in dimethyl sulfoxide (DMSO) at 10 mM as a stock solution. Different concentrations of the plants powder (0, 5, 12.5, 25 and 50 µg mL^{-1}) were added to the cell monolayer, triplicate wells were prepared for each individual dose of each plant powder. Plates were incubated for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain, then excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction of cancer cell and plants powder dose was plotted. IC_{50} was obtained from the curves.

Preventive effect of quinoa and safflower seeds on NAFLD in rats

Diets: Diets were formulated according to the following scheme given in Table 1. High fructose diet was prepared similar to Chang *et al.*[29] for inducing of fatty liver. Twenty grams from quinoa and safflower seeds powder were mixed with the high fructose diet to give quinoa diet and safflower diet, respectively. The contents of protein, fat and carbohydrate of the 20 g powder were reduced from casein protein, corn oil and starch, respectively without affecting fructose levels.

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

	Diets			
Ingredients	Balanced diet	High fructose diet	Quinoa diet	Safflower diet
Casein	12.0*	12.0*	9.10	9.04
Corn oil	9.0	-	-	-
Saturated fat	-	7.0	5.58	0.98
Fructose	-	60.0	60.00	60.00
Starch	69.5	16.5	0.82	5.48
Salt mixture	3.5	3.5	3.50	3.50
Vitamin mixture	1.0	1.0	1.00	1.00
Cellulose	5.0	-	-	-
Quinoa	-	-	20.00	-
Safflower	-	-	-	20.00

*12 casein has been estimated to contain 10 g protein using AOAC[24]

Animals' treatments: 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 NAFLD where rats were fed on high fructose diet. Rats of group three and four were fed on high fructose diet supplemented with 20% quinoa or safflower powder. During the experiment, body weight and food intake were recorded weekly. After four weeks (end of the study) total food intake, body weight gain and feed efficiency ratio (Body weight gain/total food intake) were calculated.

Analysis of blood and tissues: Blood samples were collected from all rats after an overnight fast for the determination of plasma total cholesterol (T-Ch)[30], high density lipoprotein cholesterol (HDL-Ch)[31], low density lipoprotein cholesterol (LDL-Ch)[32] and triglycerides (TG)[33]. The T-Ch/HDL-Ch ratio was calculated as indicator of cardiovascular disease risk. Plasma total protein[34] and albumin[35] were determined. Plasma malondialdehyde (MDA)[36] was estimated as an indicator of lipid peroxidation. Plasma uric acid[37] was determined. The activity of plasma transaminases[38] aspartate transaminase (AST) and alanine transaminase (ALT) were estimated as an indicator of liver functions. Plasma levels of creatinine[39] and urea[40] were determined to study any possible changes in kidney functions. 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.*[41]. In brief, liver tissues were homogenized with chloroform:methanol (2:1) for lipids extraction. After the extraction and evaporation, tissue lipids were re-dissolved in isopropanol and liver cholesterol[30] and triglyceride[33] levels were estimated enzymatically.

This study has been carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

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

Phytochemical analysis of quinoa and safflower seeds: Table 2 summarized the chemical composition of quinoa and safflower seeds powder respectively. Safflower seeds contained high amount of protein (17.3%) and fat (30.1%) than quinoa seeds. Quinoa seeds were found to contain the highest amount of carbohydrate (72.3%) than safflower seeds. Total phenolic compounds (Table 2) were present in safflower and quinoa seeds by 176 and 36.4 as mg GAE/100 g, respectively. Phytic acid was present in safflower seeds by 2.2% and in quinoa seeds by 1.03%.

Table 2: Chemical composition of quinoa and safflower seeds powder

Ingredients	Quinoa seeds	Safflower seeds
Protein (g/100 g)	14.40±0.125	17.3±0.234
Fat (g/100 g)	7.10±0.114	30.1±0.215
Ash (g/100 g)	2.20±0.082	5.0±0.146
Crude fibers (g/100 g)	4.00±0.371	10.1±0.203
*Carbohydrate (g/100 g)	72.30±0.139	37.5±0.357
Total phenolic (mg GAE/100 g)	36.40±0.332	176.0±1.532
Phytic acid (g/100 g)	1.03±0.064	2.2±0.174

*Calculated by differences

Table 3: Fatty acids contents of quinoa and safflower seeds oils (as percentage of total fatty acids)

Fatty acids	Quinoa seeds	Safflower seeds
Saturated fatty acids		
Palmitic acid: C16 (0)	8.10	6.8
Stearic acid: C18 (0)	0.70	2.1
Arachidic acid: C20 (0)	0.43	-
Behenic acid: C22 (0)	0.57	-
Total identified saturated fatty acids	9.80	8.9
Unsaturated fatty acids		
Oleic acid: C18 (1)	26.80	13.8
Linoleic acid: C18 (2)	46.70	66.8
Linolenic acid: C18 (3)	8.83	5.1
Total identified unsaturated fatty acids	82.33	77.0

Table 4: IC_{50} dose of the different plants powders under study

Ingredients	IC_{50} of liver carcinoma (HEPG2) cell line (µg)
Doxorubicin	3.6
Quinoa seeds	14.6
Safflower seeds	19.4

Fatty acid profile of quinoa and safflower seeds oil: The identified FAMEs percentages of quinoa and safflower seeds oil were presented in Table 3. Quinoa and safflower contained four major fatty acids, including palmitic, oleic, linoleic and α-linolenic acids. These results showed that approximately 82.33 and 77% of total fatty acids are represented by unsaturated fatty acids. Linoleic acid was the highest fatty acid in both seeds it was present by 46.7% and 66.8% in quinoa and safflower seeds oil, respectively. Palmitic acid was the highest saturated fatty acid present in both seeds oil. α-Linolenic acid was present in quinoa and safflower seeds oil by 8.83 and 5.1%, respectively which may provide favorable nutritional implications and beneficial physiological effects.

Anticancer activity of quinoa and safflower seeds: The cytotoxicity effect of quinoa and safflower seeds powder was shown in Table 4 and Fig. 1. Data in Table 4 showed the IC_{50} dose (concentration which reduces survival of the exposed

Table 5: Plasma lipid profile and liver fat, total cholesterol and triglycerides of normal and different fatty liver groups

Lipid profile	Normal	NAFLD control	Quinoa seeds	Safflower seeds
Plasma				
T-Ch (mg dL^{-1})	68.19±3.00^{a}	159.98±7.51^{c}	116.75±5.54^{b}	126.35±4.13^{b}
TG (mg dL^{-1})	54.00±3.98^{a}	93.83±2.50^{c}	64.60±4.21^{ab}	67.53±3.68^{b}
HDL-Ch (mg dL^{-1})	42.52±0.70^{b}	27.69±0.79^{a}	42.42±1.30^{b}	41.43±1.70^{b}
LDL-Ch (mg dL^{-1})	19.83±0.48^{a}	72.17±2.09^{c}	58.67±1.85^{b}	59.33±1.58^{b}
T-Ch/HDL-Ch ratio	1.61±0.08^{a}	5.81±0.35^{c}	2.77±0.16^{b}	3.07±0.12^{b}
Liver tissue				
Total fat (mg g^{-1} tissue)	20.50±0.43^{a}	47.00±1.24^{d}	30.00±1.06^{b}	39.17±0.83^{c}
T-Ch (mg g^{-1} tissue)	1.98±0.11^{a}	6.85±0.16^{d}	3.32±0.18^{b}	5.58±0.18^{c}
TG (mg g^{-1} tissue)	5.00±0.15^{a}	14.05±0.74^{d}	7.47±0.42^{b}	10.92±0.64^{c}

In each row same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as mean values±standard error

Table 6: Plasma malondialdehyde, total protein and albumin of the different experimental groups

Groups	MDA (nmol mL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})
Normal	4.98±0.22^{a}	7.76±0.17^{c}	3.98±0.21^{b}
NAFLD control	7.55±0.48^{b}	6.36±0.18^{a}	3.10±0.10^{a}
Quinoa seeds	5.61±0.19^{a}	7.45±0.15^{c}	3.31±0.12^{a}
Safflower seeds	5.89±0.31^{a}	6.95±0.09^{b}	3.22±0.13^{a}

In each row same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as mean values±standard error

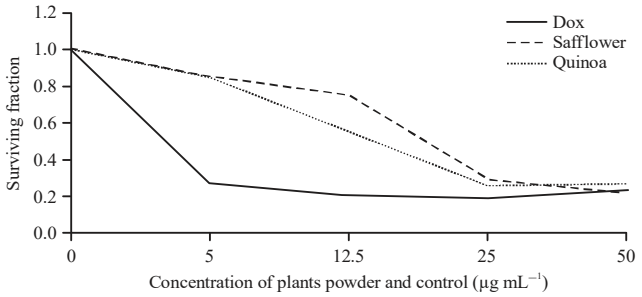

Fig. 1: Survival curve of liver carcinoma cell line (HEPG2)

cancer cells to 50%) of plants powder and Doxorubicin, drug used for treatment of cancer. Figure 1 illustrated the survival curve of HEPG2 cells which were exposed to various concentrations (5-50 µg mL^{-1}) of quinoa and safflower seeds powder for 48 h. Quinoa seeds powder was more efficient than safflower seeds powder where IC_{50} was 14.6 and 19.4 µg, respectively.

Protective effect of quinoa and safflower seeds against NAFLD

Effect of quinoa and safflower seeds on plasma and liver lipids: Plasma lipid concentrations in the different groups were shown in Table 5. NAFLD control rats exhibited significant increase in T-Ch, TG and LDL-Ch compared with normal control rats. In addition, significant elevations in total fat, T-Ch and TG in the liver tissues recorded by NAFLD control rats (Table 5) in comparison to normal control rats. The ratio of T-Ch/HDL-Ch elevated significantly in rats NAFLD control compared with normal rats, which means elevation of the risk of cardiovascular disease (CVD) that is associated with reduction of HDL-Ch plasma levels. Supplementation of high fructose diet (HFD) with 20% quinoa or safflower seeds powder improved plasma lipid profile and liver total fat, T-Ch and TG contents with variable degrees (Table 5). Feeding rats on HFD supplemented with quinoa or safflower seeds powder showed significant reduction of CVD risk as observed by reduction of the T-Ch/HDL-Ch ratio.

Effect of quinoa and safflower seeds on MDA, total protein and albumin: Plasma MDA levels as an indicator of lipid peroxidation elevated significantly in NAFLD control rats in comparison with normal control (Table 6). Plasma levels of MDA reduced significantly in rats fed on HFD supplemented with 20% quinoa or safflower seeds powder when compared with NAFLD control. Plasma total protein and albumin reduced significantly in NAFLD control rats. On the other hand, plasma total protein elevated in rats fed on HFD supplemented with 20% quinoa or safflower seeds.

Effect of quinoa and safflower seeds on liver and kidney functions: Increased plasma activities of AST and ALT were noticed in NAFLD control (Table 7). Treatment with quinoa and safflower seeds powder produced significant reduction in AST and ALT activities compared to NAFLD control rats. Significant elevations in kidney functions, plasma creatinine and urea, were recorded by NAFLD control (Table 7). Plasma uric acid elevated significantly in rats fed on HFD compared

Table 7: Liver and kidney functions of the different experimental groups

Groups	Uric acid (mg dL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	AST (U L^{-1})	ALT (U L^{-1})
Normal	1.41±0.036[a]	24.58±1.61[a]	0.61±0.01[a]	44.06±0.82[a]	19.77±0.59[a]
NAFLD control	2.25±0.112[b]	32.03±0.98[b]	0.75±0.03[b]	73.91±2.73[c]	46.60±2.53[c]
Quinoa seeds	1.49±0.064[a]	25.32±0.68[a]	0.64±0.03[a]	49.40±1.27[b]	24.86±0.63[b]
Safflower seeds	1.62±0.095[a]	26.15±0.57[a]	0.64±0.02[a]	50.36±0.94[b]	25.16±0.72[b]

In each row same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as mean values±standard error

Table 8: Nutritional parameters of the different experimental groups

Parameters	Normal	NAFLD control	Quinoa seeds	Safflower seeds
Initial body weight (g)	129.83±7.49[a]	129.83±5.37[a]	129.67±4.72[a]	129.67±2.50[a]
Final body weight (g)	193.33±7.48[b]	188.83±3.96[b]	174.00±4.55[a]	174.50±5.23[a]
Body weight gain (g)	63.50±5.02[b]	59.00±3.15[b]	44.33±3.09[a]	44.83±5.68[a]
Total food intake (g)	409.50±8.05[b]	395.00±6.68[b]	340.67±5.02[a]	411.33±2.99[b]
Feed efficiency ratio	0.16±0.01[b]	0.15±0.01[b]	0.13±0.01[b]	0.11±0.014[a]
Liver relative weight	2.83±0.13[a]	3.62±0.16[c]	3.23±0.06[b]	3.38±0.10[bc]

In each row same letter means non-significant difference while different letter means significant difference at 0.05 probability. The data are expressed as mean values±standard error

with normal rats. Feeding rats on high fructose diet supplemented with quinoa and safflower seeds powder attenuated the deterioration in liver and kidney functions with different degrees and reduced the elevation in plasma uric acid significantly compared with NAFLD control group.

Effect of quinoa and safflower seeds on the nutritional parameters: Nutritional parameters of all the experimental groups were presented in Table 8. Non-significant changes were observed in final body weight, body weight gain and total food intake between NAFLD control and normal control rats. Final body weight and body weight gain of rats fed on HFD supplemented with 20% of quinoa seeds powder reduced significantly when compared with NAFLD group. Rats feeding on diet supplemented with safflower seeds powder showed significant reduction in feed efficiency ratio compared with other rats groups. Liver relative weight of NAFLD control increased significantly when compared with normal rats groups. Liver relative weight of rats fed on HFD supplemented with 20% of quinoa seeds powder reduced significantly when compared with NAFLD control rats or rats of HFD containing safflower seeds powder.

DISCUSSION

In the present research quinoa and safflower seeds powder showed anticancer activity against HEPG2 cancer cell line that may be imputed to the presence of phytic acid, phenolic compounds and polyunsaturated fatty acids (PUFAs) in these seeds. It was reported previously that phytic acid prevented and inhibited tumor development[42]. The anti-carcinogenic activity of phytic acid may be due to its suppression of forming of oxygen free radicals through prevention of the Fenton reaction in iron chelation[43]. Feruloylserotonin is bioactive component present in safflower seeds, which possess antibacterial, anti-inflammatory and free radical scavenging activities[20]. Choi *et al.*[44] clarified the cytoprotective activity of feruloylserotonin based on its ability to prevent oxidative stress-induced cell death, lipid peroxidation and phosphorylation of histone H2AX in H_2O_2 treated HEPG2 and HACAT cells.

Accumulation of fat is the implicit cause of NAFLD which induce disturbance in lipid metabolism in the liver[45]. NAFLD in many patients ultimately progresses to liver cirrhosis and hepatocellular carcinoma[46]. NAFLD is developed after hepatic steatosis and is associated with cellular stresses, such as oxidative stress, gut-derived lipopolysaccharide stress, etc.,[3,47]. High intake of fructose is one of the most important causes of NAFLD[48] since fructose increases fat synthesis and suppresses fat oxidation which stimulates fat accumulation in liver[49]. Fructose also induced elevation of oxidative stress and inflammation[50]. In the present research feeding rats on high fructose diet for four weeks proved induction of NAFLD, which characterized by accumulation of fat in liver tissue, dyslipidemia, elevation of liver function (AST and ALT) and elevated MDA in addition to reduction in plasma total protein and albumin. High fructose diet also elevated kidney dysfunction which may be used as an indicator of hepatorenal syndrome. Fan *et al.*[51] and Al-Okbi *et al.*[52] also found that rats feeding on diet rich in fructose showed renal damage which associated with inflammation and lipid accumulation. Glomerular hypertrophy has occurred after fructose intake for short (four weeks) or long-term[53,54]. Fructose intake induced glomerular hypertension and reduced renal blood flow which associated with preglomerular vascular disease[55]. In the present study HFD elevated uric acid significantly in rats.

Higher levels of uric acid due to fructose consumption were observed also in liver tissues[56]. Synthesis of uric acid from amino acid precursors is stimulated by high fructose diets, which elevates fasting serum uric acid levels[7].

Rats fed on HFD supplemented with quinoa and safflower powder as dietary supplements exhibited significant improvement in plasma lipid profile and liver fat in addition to the reduction in plasma MDA and uric acid. This improvement may be due to the affluent of quinoa and safflower seeds powder in polyunsaturated fatty acids, phenolic compounds and phytic acids. Intake of PUFAs is inversely associated with the incidence of heart disease via the reduction of plasma cholesterol and triacylglycerol[57]. In the present study quinoa seeds contained 7.1% oil; this result is coinciding with those recorded by Vega Galvez *et al.*[58] who found that oil content in quinoa ranged from 2-10%. Vitamin E presented in quinoa oil protects PUFAs from oxidation. Omega-3 and omega-6 play important roles in human health such as membrane function, immunity and brain development[59]. Safflower seeds contain phenolic compounds such as lignans, glucosides, flavonoids and serotonins[60]. Safflower seeds contain arctigenin, which is considered plant lignan and acts as precursor for the mammalian lignans[61]. Arctigenin displayed inhibition activity of TNF-α (inflammatory marker) production[62,63] and hepatoprotective effect in primary cultures of rat cells which may be due to its antioxidant activity[64,65]. It was reported that the main phenolic in quinoa seeds are flavonol glycosides[66]. Quercetin and kaempferol are the main flavonol glycosides in quinoa seeds which present at the level of approximately 839 μg g^{-1} dry weight[67]. Lutz *et al.*[68] identified isoflavones in quinoa seeds; genistein and daidzein presented by 0.05-0.41 and 0.7-2.05 mg/100 g, respectively. Quinoa and safflower seeds contain fibers as shown in the proximate analysis of both seeds. Satiety feeling, reduction of cholesterol and lipid absorption, management of the postprandial insulin level, catalyzing the turning endogenous cholesterol to bile acids as well as enhancement of the gut microbiota can be achieved via the consumption of dietary fiber[69]. Diet supplemented with quinoa seeds powder elevated plasma total protein and albumin similar to normal control rats. Quinoa is rich in high quality protein and amino acids especially lysine and methionine[14] to which it can be attributed the increments in both parameters.

The non-significant change in body weight gain between NAFLD control rats and control normal rats is coincide with the work of Chang *et al.*[29] that demonstrated that the induction of fatty liver didn't change the body weight. Reduction of total food intake in the present study in rats feeding on diet supplemented with 20% of quinoa seeds powder indicated that these pseudo-cereals have awesome ability to decrease food intake and are able to keep on standard body weight upon regular consumption. The present results are coinciding with those obtained previously by Mithila and Khanum[70].

CONCLUSION

It was concluded that the dietary supplements, a hopeful area of research for discovery alternative medicine for treatment and demonstrated to protect from diseases such as NAFLD. Quinoa and safflower seeds as rich sources of phenolic compounds, polyunsaturated fatty acids and phytic acid possessed anti-cancer activity against HEPG2 cancer cell line and exhibited protective impact against NAFLD. So, these seeds especially quinoa seeds can be used as dietary supplements for weight loss and prevention from NAFLD and liver cancer.

SIGNIFICANCE STATEMENT

This study confirmed that quinoa and safflower powder as dietary supplement are good strategy for prevention of NAFLD in rats fed on high fructose diet. Both plants powder reduced weight gain, improve dyslipidemia, reduced lipid peroxidation and prevent hepatic lipid accumulation effectively. Quinoa and safflower powder were effective as anti-cancer against hepatic cancer in cell line technique using HEPG2 cell.

REFERENCES

1. Ganzetti, G., A. Campanati and A. Offidani, 2015. Non-alcoholic fatty liver disease and psoriasis: So far, so near. World J. Hepatol., 7: 315-326.
2. Takeuchi, M., J.I. Takino, A. Sakasai-Sakai, T. Takata and T. Ueda *et al.*, 2014. Involvement of the TAGE-RAGE system in non-alcoholic steatohepatitis: Novel treatment strategies. World J. Hepatol., 6: 880-893.
3. Sun, X., Y. Zhang and M. Xie, 2017. The role of peroxisome proliferator-activated receptor in the treatment of non-alcoholic fatty liver disease. Acta Pharm., 67: 1-13.
4. Bodzin, A.S. and R.W. Busuttil, 2015. Hepatocellular carcinoma: Advances in diagnosis, management and long term outcome. World J. Hepatol., 7: 1157-1167.
5. Lim, S., T.J. Oh and K.K. Koh, 2015. Mechanistic link between nonalcoholic fatty liver disease and cardiometabolic disorders. Int. J. Cardiol., 201: 408-412.
6. Harrison, S.A. and C.P. Day, 2007. Benefits of lifestyle modification in NAFLD. Gut, 56: 1760-1769.

7. Jensen, T., M.F. Abdelmalek, S. Sullivan, K.J. Nadeau and M. Green *et al*., 2018. Fructose and sugar: A major mediator of nonalcoholic fatty liver disease. J. Hepatol., 68: 1063-1075.
8. Rochlani, Y., N.V. Pothineni, S. Kovelamudi and J.L. Mehta, 2017. Metabolic syndrome: Pathophysiology, management and modulation by natural compounds. Ther. Adv. Cardiovasc. Dis., 11: 215-225.
9. Bahadoran, Z., M. Golzarand, P. Mirmiran, N. Saadati and F. Azizi, 2013. The association of dietary phytochemical index and cardiometabolic risk factors in adults: Tehran lipid and glucose study. J. Hum. Nutr. Dietet., 26: 145-153.
10. Hassani, F.V., K. Shirani and H. Hosseinzadeh, 2016. Rosemary (*Rosmarinus officinalis*) as a potential therapeutic plant in metabolic syndrome: A review. Naunyn-Schmiedeberg's Arch. Pharmacol., 389: 931-949.
11. Inada, A., P. Figueiredo, R. Santos-Eichler, K. Freitas, P. Hiane, A. Castro and R. Guimaraes, 2017. *Morinda citrifolia* Linn. (Noni) and its potential in obesity-related metabolic dysfunction. Nutrients, Vol. 9. 10.3390/nu9060540.
12. Pisonero-Vaquero, S., J. Gonzalez-Gallego, S. Sanchez-Campos and M.V. Garcia-Mediavilla, 2015. Flavonoids and related compounds in non-alcoholic fatty liver disease therapy. Curr. Med. Chem., 22: 2991-3012.
13. Delatorre-Herrera, J., 2003. Current use of quinoa in Chile. Food Rev. Int., 19: 155-165.
14. Ando, H., Y.C. Chen, H. Tang, M. Shimizu, K. Watanabe and T. Mitsunaga, 2002. Food components in fractions of quinoa seed. Food Sci. Technol. Res., 8: 80-84.
15. Graf, B.L., P. Rojas Silva, L.E. Rojo, J. Delatorre Herrera, M.E. Baldeon and I. Raskin, 2015. Innovations in health value and functional food development of quinoa (*Chenopodium quinoa* Willd.). Comprehen. Rev. Food Sci. Food Safety, 14: 431-445.
16. Woldemichael, G. and M. Wink, 2001. Identification and biological activities of triterpenoid saponins from *Chenopodium quinoa*. J. Agric. Food Chem., 49: 2327-2332.
17. Yao, Y., X. Yang, Z. Shi and G. Ren, 2014. Anti inflammatory activity of saponins from quinoa (*Chenopodium quinoa* Willd.) seeds in lipopolysaccharide stimulated RAW 264.7 macrophages cells. J. Food Sci., 79: H1018-H1023.
18. Gomez-Caravaca, A.M., A. Segura-Carretero, A. Fernandez-Gutierrez and M.F. Caboni, 2011. Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology. J. Agric. Food Chem., 59: 10815-10825.
19. Turgumbayeva, A.A., G.O. Ustenova, B.K. Yeskalieva, B.A. Ramazanova, K.D. Rahimov, H. Aisa and K.T. Juszkiewicz, 2018. Volatile oil composition of *Carthamus tinctorius* L. flowers grown in Kazakhstan. Ann. Agric. Environ. Med., 25: 87-89.
20. Cho, H., O. Kim, Y. Lee, L.J. Kang, C.N. Nguyen, A. Ishihara and H.E. Kim, 2017. Feruloylserotonin inhibits hydrogen peroxide-induced melanogenesis and apoptosis in B16F10 and SK-Mel-2 melanoma cells. Biochem. Biophys. Res. Commun., 491: 973-979.
21. Kumar, N. and C. Chaiyasut, 2015. Hair growth promoting activity of *Carthamus tinctorius* florets extract-loaded nanostructured lipid carriers. Int. J. Pharm. Pharm. Sci., 7: 252-257.
22. Rashed, M.M., M. Shallan, D.A. Mohamed, K. Fouda and L.M. Hanna, 2014. Biological evaluation of anti-androgenic effect of some plant foods. J. Food Nutr. Res., 2: 645-651.
23. Mohamed, D.A., M.M. Rashed, M. Shallan, K. Fouda and L.M. Hanna, 2016. Amelioration of benign prostate hyperplasia in rats through plant foods. Int. J. Pharmacogn. Phytochem. Res., 8: 2063-2070.
24. AOAC., 2012. Official Methods of Analysis of the Association of Official Analytical Chemists. 19th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
25. Carciochi, R.A., G.D. Manrique and K. Dimitrov, 2015. Optimization of antioxidant phenolic compounds extraction from quinoa (*Chenopodium quinoa*) seeds. J. Food Sci. Technol., 52: 4396-4404.
26. Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.
27. Gao, Y., C. Shang, M.A.S. Maroof, R.M. Biyashev and E.A. Grabau *et al*., 2007. A modified colorimetric method for phytic acid analysis in soybean. Crop Sci., 47: 1797-1803.
28. Cordero, C.P., S.J. Morantes, A. Paez, J. Rincon and F.A. Aristizabal, 2009. Cytotoxicity of withanolides isolated from *Acnistus arborescens*. Fitoterapia, 80: 364-368.
29. Chang, W.C., H. Jia, W. Aw, K. Saito, S. Hasegawa and H. Kato, 2014. Beneficial effects of soluble dietary Jerusalem artichoke (*Helianthus tuberosus*) in the prevention of the onset of type 2 diabetes and non-alcoholic fatty liver disease in high-fructose diet-fed rats. Br. J. Nutr., 112: 709-717.
30. Watson, D., 1960. A simple method for the determination of serum cholesterol. Clin. Chem. Acta, 5: 637-642.
31. Burstein, M., H.R. Scholnick and R. Morfin, 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11: 583-595.
32. Schriewer, H., U. Kohnert and G. Assmann, 1984. Determination of LDL cholesterol and LDL apolipoprotein B following precipitation of VLDL in blood serum with phosphotungstic acid/$MgCl_2$. J. Clin. Chem. Clin. Biochem., 22: 35-40.
33. Megraw, R.E., D.E. Dunn and H.G. Biggs, 1979. Manual and continuous-flow colorimetry of triacylglycerols by a fully enzymatic method. Clin. Chem., 25: 273-278.

34. Reinhold, J.G., 1953. Manual Determination of Serum Total Protein, Albumin and Globulin Fractions by Biuret Method. In: Standard Methods in Clinical Chemistry, Reiner, M. (Ed.). Academic Press, New York, pp: 88-97.
35. Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.
36. Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta, 90: 37-43.
37. Watts, R.W.E., 1974. Determination of uric acid in blood and in urine. Ann. Clin. Biochem., 11: 103-111.
38. Reitman, S. and S. Frankel, 1957. Colorimetric methods for aspartate and alanine aminotransferase. Am. J. Clin. Pathol., 28: 55-60.
39. Owen, J.A., B. Iggo, F.J. Scandrett and C.P. Stewart, 1954. The determination of creatinine in plasma or serum and in urine; a critical examination. Biochem. J., 58: 426-437.
40. Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159.
41. Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226: 497-509.
42. Vucenik, I. and A.M. Shamsuddin, 2006. Protection against cancer by dietary IP6 and inositol. Nutr. Cancer, 55: 109-125.
43. Silva, E.O. and A.P.F. Bracarense, 2016. Phytic acid: From antinutritional to multiple protection factor of organic systems. J. Food Sci., 81: R1357-R1362.
44. Choi, J.Y., H. Kim, Y.J. Choi, A. Ishihara, K. Back and S.G. Lee, 2010. Cytoprotective activities of hydroxycinnamic acid amides of serotonin against oxidative stress-induced damage in HepG2 and HaCaT cells. Fitoterapia, 81: 1134-1141.
45. Hwang, J.T., E.J. Shin, M.Y. Chung, J.H. Park, S. Chung and H.K. Choi, 2018. Ethanol extract of *Allium fistulosum* inhibits development of non-alcoholic fatty liver disease. Nutr. Res. Pract., 12: 110-117.
46. Wang, N., R. Kong, H. Luo, X. Xu and J. Lu, 2017. Peroxisome proliferator-activated receptors associated with nonalcoholic fatty liver disease. PPAR Res., Vol. 2017. 10.1155/2017/6561701.
47. Papandreou, D. and E. Andreou, 2015. Role of diet on non-alcoholic fatty liver disease: An updated narrative review. World J. Hepatol., 7: 575-582.
48. Ter Horst, K. and M. Serlie, 2017. Fructose consumption, lipogenesis and non-alcoholic fatty liver disease. Nutrients, Vol. 9. 10.3390/nu9090981.
49. Ackerman, Z., M. Oron-Herman, M. Grozovski, T. Rosenthal, O. Poppo, G. Link and B.A. Sela, 2005. Fructose-induced fatty liver disease: Hepatic effects of blood pressure and plasma triglyceride reduction. Hypertension, 45: 1012-1018.
50. Koteish, A. and A.M. Diehl, 2002. Animal models of steatohepatitis. Best Prac. Res. Clin. Gastroenterol., 16: 679-690.
51. Fan, C.Y., M.X. Wang, C.X. Ge, X. Wang, J.M. Li and L.D. Kong, 2014. Betaine supplementation protects against high-fructose-induced renal injury in rats. J. Nutr. Biochem., 25: 353-362.
52. Al-Okbi, S.Y., D.A. Mohamed, T.E. Hamed and R.S.H. Esmail, 2014. Rice bran oil and pumpkin seed oil alleviate oxidative injury and fatty liver in rats fed high fructose diet. Pol. J. Food Nutr. Sci., 64: 127-133.
53. Cosenzi, A., E. Bernobich, N. Plazzotta, P. Seculin, G. Odoni and G. Bellini, 1999. Lacidipine reduces high blood pressure and the target organ damage induced by high fructose diet in rats. J. Hyperten., 17: 965-971.
54. Oudot, C., A.D. Lajoix, B. Jover and C. Rugale, 2013. Dietary sodium restriction prevents kidney damage in high fructose-fed rats. Kidney Int., 83: 674-683.
55. Sanchez-Lozada, L.G., E. Tapia, P. Bautista-Garcia, V. Soto and C. Avila-Casado *et al*., 2008. Effects of febuxostat on metabolic and renal alterations in rats with fructose-induced metabolic syndrome. Am. J. Physiol.-Renal Physiol., 294: F710-F718.
56. Lanaspa, M.A., L.G. Sanchez-Lozada, Y.J. Choi, C. Cicerchi and M. Kanbay *et al*., 2012. Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: Potential role in fructose-dependent and-independent fatty liver. J. Biol. Chem., 287: 40732-40744.
57. Vijaimohan, K., M. Jainu, K.E. Sabitha, S. Subramaniyam, C. Anandhan and S.C.S, Devi, 2006. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rat. Life Sci., 79: 448-454.
58. Vega Galvez, A., M. Miranda, J. Vergara, E. Uribe, L. Puente and E.A. Martinez, 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: A review. J. Sci. Food Agric., 90: 2541-2547.
59. Tang, Y., X. Li, P.X. Chen, B. Zhang and M. Hernandez *et al*., 2015. Characterisation of fatty acid, carotenoid, tocopherol/tocotrienol compositions and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. Food Chem., 174: 502-508.
60. Kim, E.O., J.H. Oh, S.K. Lee, J.Y. Lee and S.W. Choi, 2007. Antioxidant properties and quangification of phenolic compounds from safflower (*Carthamus tinctorius* L.) seeds. Food Sci. Biotechnol., 16: 71-77.
61. Penalvo, J.L., S.M. Heinonen, A.M. Aura and H. Adlercreutz, 2005. Dietary sesamin is converted to enterolactone in humans. J. Nutr., 135: 1056-1062.
62. Cho, J.Y., J. Park, P.S. Kim, E.S. Yoo, K.U. Baik and M.H. Park, 2001. Savinin, a lignan from *Pterocarpus santalinus* inhibits tumor necrosis factor-α production and T cell proliferation. Biol. Pharm. Bull., 24: 167-171.

63. Cosentino, M., F. Marino, R.C. Maio, M.G. Delle Canne, M. Luzzani, S. Paracchini and S. Lecchini, 2010. Immunomodulatory activity of the lignan 7-hydroxymatairesinol potassium acetate (HMR/lignin^{TM}) extracted from the heartwood of Norway spruce (*Picea abies*). Int. Immunopharmacol., 10: 339-343.
64. Jang, Y.P., S.R. Kim and Y.C. Kim, 2001. Neuroprotective dibenzylbutyrolactone lignans of *Torreya nucifera*. Planta Med., 67: 470-472.
65. Kim, S.H., Y.P. Jang, S.H. Sung, C.J. Kim, J.W. Kim and Y.C. Kim, 2003. Hepatoprotective dibenzylbutyrolactone lignans of *Torreya nucifera* against CCl_4-induced toxicity in primary cultured rat hepatocytes. Biol. Pharm. Bull., 26: 1202-1205.
66. Gomez-Caravaca, A.M., G. Iafelice, V. Verardo, E. Marconi and M.F. Caboni, 2014. Influence of pearling process on phenolic and saponin content in quinoa (*Chenopodium quinoa* Willd). Food Chem., 157: 174-178.
67. Hirose, Y., T. Fujita, T. Ishii and N. Ueno, 2010. Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan. Food Chem., 119: 1300-1306.
68. Lutz, M., A. Martinez and E.A. Martinez, 2013. Daidzein and Genistein contents in seeds of quinoa (*Chenopodium quinoa* Willd.) from local ecotypes grown in arid Chile. Indust. Crops Prod., 49: 117-121.
69. De Carvalho, F.G., P.P. Ovidio, G.J. Padovan, A.A. Jordao, Jr., J.S. Marchini and A.M. Navarro, 2014. Metabolic parameters of postmenopausal women after quinoa or corn flakes intake-A prospective and double-blind study. Int. J. Food Sci. Nutr., 65: 380-385.
70. Mithila, M.V. and F. Khanum, 2015. Effectual comparison of quinoa and amaranth supplemented diets in controlling appetite; a biochemical study in rats. J. Food Sci. Technol., 52: 6735-6741.