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DOWN REGULATION OF LIPID ACCUMULATION IN RATS BY ANTIOXIDANT RICH SEED PLANTS SUPPLEMENTATION

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Abstract

Chia and quinoa seeds considered as a powerful medicinal plants and an excellent dietary source of bioactive constituents i.e. flavonoids and phenolic compounds and plenty of omega-3 and omega-6 fatty acids, I hypothesize that chia and quinoa seeds powder and extracts may have a role in lowering lipid accumulation in non-alcoholic fatty liver-induced by high fat high fructose diet. Thirty-six male rats were randomly divided into 6 dietary groups, containing 6 rats in each; normal control group, high fat high fructose diet (HFHFrD) control group, HFHFrD with CS powder 10 g/kg (BW), HFHFrD with QS powder10 g/kg (BW), HFHFrD with CS extract 50 mg/kg (BW), HFHFrD with QS extract 50 mg/kg (BW). After 8 weeks, it was revealed that the consumption of CS & QS extracts were found to normalize many indicators which were shifted to pathological values as a consequence of HFHFrD-induced nonalcoholic fatty liver disorders: there was a significant increase in HDL-C and a significant decrease in total cholesterol, TG, LDL-C and VLDL-C. In addition, level of lipids peroxidation (LP) was reduced as compared to HFHFrD normal group. Thus, these observations suggest that chia and quinoa seeds are potential agents on management of fat accumulation in nonalcoholic fatty liver rats.

Key words*: chia seed, fatty liver disease, flavonoids, lipid peroxidation, phenolic compounds, quinoa seed.*

INTRODUCTION

Unhealthy lifestyle *i.e*. raising the consumption of fast food, refined foods which rich in fructose and limited physical activity considered as risk factors that led to, metabolic syndrome which is associated with the development of obesity, type 2 diabetes, inflammatory and fatty liver diseases (Couturier et al., 2016). Non-alcoholic fatty liver disease (NAFLD) is a rising epidemic worldwide that leads to various liver disease complications such as cirrhosis, hepatocellular carcinoma and liver transplant (Riazi et al., 2019). Nowadays, managing and treatment of NAFLD became a challenge to scientists in different areas. There are various strategies for management of NAFLD include modulation of lifestyle by reduce body weight through diet and physical activity, increasing consumption of healthy foods which rich in antioxidants, lowering saturated fatty acids and refined foods intake (McCarthy & Rinella, 2012). Until now, there is no confirmed drug can be used to treat NAFLD; only some clinical suggestion must be followed for management of NAFLD (El-Abd et al., 2018). There is growing concerns in functional food which contains bioactive components that can provide a therapeutic solution for NAFLD (Albillos et al., 2020).

Plant seeds represents one of the most effective dietary approaches that possess health benefits besides their nutritional values. These seeds contain high levels of bioactive ingredients such as fibre, mineral, omega-3 fatty acids, phenolic compounds which have a key role in prevention and control of diseases. Bioactive compounds present in dietary supplementation possess various biological properties including, hypolipidemic, hypocholesterolemia, anti-inflammatory, anti-atherosclerotic and hepato-protective effects (Mohamed et al., 2019). Chia (*Salvia hispanica*) and quinoa (*Chenopodium quinoa* Willd) seeds are herbaceous plants which possess novel functional and biological activities (Goyat et al., 2018). Appreciably higher levels of nutrient constituents and bioactive compounds make the two plants ideal effective functional foods grains against various diseases. Chia and quinoa seeds used for medicinal purposes for thousands of years (Suri et al., 2016; Mohamed et al., 2019), and this owing to being rich on protein, polyphenols, vitamins, and minerals (Hernández-Ledesma, 2019). Polyphenols, including phenolic acids, flavonoids, and

tannins make up bioactive secondary plant metabolites that contribute to diverse physiological properties, including antimicrobial, antioxidant, anti-inflammatory, antitumor, and anti-carcinogenic effects (El-Abed et al., 2018; Mohamed et al., 2019).

Consequently, the current work aimed to investigate the effect of chia and quinoa seeds powder and extracts on non-alcoholic fatty liver disease induced by high fat high fructose diet in rats.

MATERIALS AND METHODS

Chia (*Salvia hispanica*) and quinoa (*Chenopodium quinoa* Willd) seeds were obtained from Agriculture Research Centre, Giza, Egypt, Fructose was purchased from the International Company for Scientific and Medical Supplies, Cairo, Egypt; Kits used for the measurements of lipid profile were purchased from Diagnosticum Zrt, Budapest and those for measurements of TAC and LPO were obtained from Labor Diagnostika Nord GmbH and Co, Germany. All other chemicals were of analytical grade and thirty six male of Sprague-Dawley rats weighing 140-150 g were used. The animals were obtained from animal house of National Research Centre, Cairo, Egypt. The animals were acclimatized for 1week before dietary manipulation and were housed individually in metallic cages under laboratory healthy conditions.

Preparation of chia and quinoa seeds powder and extracts: chia and quinoa seeds were dried in an air circulated oven at 40°C and then reduced into powder type and stored in airtight containers and kept at 5-7°C till used. The extracts were prepared by using chia and quinoa seed powder (10 g), extracted in (500 ml) redistilled water (12 hrs.) and stored at ~ 2°C until used (Singh et al., 2001).

Proximate chemical analysis of chia and quinoa seeds powder: chia and quinoa seeds powder were sieved through 100-mesh sieve. The powder samples were analysed for water, protein, fat, dietary fibre, ash according to the method of (AOAC, 2012).

Assessment of fatty acids of chia and quinoa seeds: The fatty acid profile of ethanolic extract of chia and quinoa seed oils were determined by gas chromatography as described by (Aldai and Osoro, 2006)

Total phenolic content: chia and quinoa seeds powder were extracted with 80% ethanol twice according to the optimized extraction which described by (Carciochi et al., 2015). Total phenolics were determined using Folin-Ciocalteu UVPC spectrophotometer. The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g on dry weight basis.

Diets and Animals treatments: Two types of diets were used in this study: 1- basal diet was based on AIN-93 recommendations (Reeves et al., 1993). 2- HFHFrD, consisted of basal diet contain 20% fat (15% beef tallow + 5% corn oil) combined with fructose added in drinking water at a level of 13% w/v which is the concentration range reported for soft drinks (Light et al., 2009). Thirty six rats were divided into six groups, each of six rats. The first groups served as normal control healthy rats which received standard diet. The second named HFHFrD which were fed on high fat high fructose diet. Rats in groups three and four were fed on high fat high fructose diet supplemented with 10 g/kg (BW) chia or quinoa seeds powder, groups five and six were administrated 50 mg/kg (BW) chia or quinoa extracts. Body weight and feed intake were recorded weekly all the period of experiment. After 8 weeks, body weight changes and feed efficiency ratio were calculated according to the method of (Mohamed et al., 2019).

Blood samples were collected after 12 hours fasting at the end of the experiment (8 weeks). Using the retro-orbital by means of a micro capillary glass tubes, blood was collected into a dry clean centrifugal tube and left to clot in a water bath (37°C) at room temperature for half an hour. the blood was centrifuged for 10 minutes at 3000 rpm to separate the serum was carefully aspirated and transferred into clear quit fit plastic tubes and kept frozen at (-2°C) until analysis.

Biochemical analysis: alanine amino transferase (ALT) & aspartate amino transferase (AST) enzymes were measured according to the methods described by (Breuer, 1996). Total bilirubin and total protein were determined according to the methods described by (Henry, 1974; Gowenlock et al., 1988) respectively. Total cholesterol (TC), Triglycerides (TG) and high density lipoprotein

(HDL) were determined in serum according to the methods described by (Allain, 1974; Fassati & Prencipe, 1982; Burstein, 1970) respectively. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined according to the method of (Friedewald et al., 1972).

Urea, uric acid and creatinine were determined according to (Patton and Crouch, 1977; Henry, 1974; Jaffe, 1980) respectively. Total antioxidant capacity (TAC) and lipid peroxidation (LPO) were determined in according to (Cao et al., 1993; Ohkawa et al., 1979).

The data statistical Analysis as the mean ±SD. Data for multiple variable comparisons was analysed by one-way analysis of variance (ANOVA). Duncan's test was used for the comparisons of significance between groups as a post hoc test according to the statistical package program (Armitage & Berry, 1987).

RESULTS AND DISCUSSIONS

Proximate composition of chia and quinoa seeds (dry basis %)

Table 1 summarized the chemical composition of chia and quinoa seeds powder. Chia seeds contained high amount of protein (16.5 g/100 g) and fat (30.7 g/100 g) than quinoa seeds. Meanwhile quinoa seeds were found to contain high content of carbohydrate (69.34 g/100 g) than chia seeds which recorded (42.1 g/100 g). The current results are in agreement with the finding of other authors (Vega-Galvez et al., 2010; Halaby et al., 2017**)** which have demonstrated that quinoa seeds are one of the best vegetal protein sources which provides a protein value similar to casein in milk and higher than those present in popular grains. Furthermore, da Silva et al. (2017) reported that chia seed as rich source of dietary fibre. This, unique nutritional composition of chia seed, such as dietary fibre, omega-3 and omega-6 fatty acid and antioxidant properties making its consumption as a perfect choice to increase satiety index and reduce the risk of various diseases (Muñoz et al., 2013; Aktaş & Levent, 2018).The alterations in chemical constituents and antioxidant values of plants depend on several factors such as different genotype, growing condition, agronomic practices employed, season, maturity, post-harvest and storage conditions (Navruz & Sanlier, 2016).

Table 1. Proximate chemical composition of quinoa and chia seeds

Nutrients	Contents	
	Quinoa	Chia
Water (g/100 g)	6.90±0.04	4.6±0.14
Crude Protein (g/100 g)	15.05±0.05	16.5±0.06
Crude Fat (g/100 g)	4.93±0.14	30.7±0.09
Dietary Fiber (g/100 g)	8.92±0.05	30.4±0.14
Ash (g/100 g)	3.78±0.06	4.8±0.04
Carbohydrates*	69.34±0.15	43.4±0.12

Data are expressed as mean ± SD, n = 3, *calculated by difference

Total phenolic and flavonoid contents

The results of the total phenolic and total flavonoid contents of chia and quinoa seed extracts are shown in Table 2.

Table 2. Total phenolic and flavonoid contents of chia and quinoa seeds

Particulars	Chia seeds	Quinoa seeds
Total phenolic (mg/100 g GAE)	35.32 ± 0.22	135.23± 2.28
Total flavonoids (mg/100 g QE)	40.29 ± 1.18	37.33± 1.33

Data are expressed as mean ± SD, n= 3, GAE: Gallic acid equivalents, QE: Quercetin equivalent

Total phenolic of quinoa seed was higher than chia seed (135.23 vs 35.32 mg/100 g GAE). These results are in agreement with Gordillo-Bastidas et al. (2016) they reported that quinoa is a more effective quality food due to its bioactive flavonoids. The total phenolic content of quinoa is 135.23 mg/100g GAE. Gordillo-Bastidas et al. (2016) & Halaby et al. (2017) confirmed these results as quinoa seeds contains more phenols than whole cereals.

The different ranges of total flavonoids and total phenolic contents from previous studies are probably owing to the different origins of quinoa seeds and its extract solvents.

Fatty acids profile

Regarding the fatty acid profile (Table 3), chia and quinoa seeds contained three important fatty acids including α-linolenic acid (C18:3), linoleic acid (C18:2) and oleic acid (C18:1) with the most marked levels. Therefore, the levels of unsaturated fatty acids were more than saturated fatty acids about eight times.

Table 3. Fatty acids composition of quinoa and chia seeds

Fatty acids (g/100 g)	Quinoa	Chia
C12:0	0.12±0.04	N.D.
C14:0	0.41±0.12	0.11 ±0.03
C16:0	9.15±1.32	0.14 ±0.05
C16:1	0.22±0.09	0.14 ±0.05
C18:0	0.84±0.12	0.74 ±0.14
C18:1 (ω-9)	6.01±1.15	8.4 ±2.05
C18:2 (ω-6)	20.63±3.30	22.91 ±3.94
C18:3 (ω-3)	58.94±2.59	54.76 ±4.72
C20:0	0.43±0.08	3.42 ±1.03
C22:0	0.78±0.14	N.D.
C22:1	1.36±0.20	0.65 ±0.20
SFA	11.95	4.55
MUFA	7.37	9.05
PUFA	79.57	77.67
ω-3/ω-6	2.86	2.39

Values reported as means _ SD of three replicate analyses (n = 3).
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids;
PUFA: polyunsaturated fatty acid, N.D.: Not detected

These results are in agreement with Yi et al. (2018) as they reported that chia seed contain high level of α-linolenic acid (ω-3) and consider chia as a good daily supplementation to decline triglyceride and alleviate high density lipoprotein concentrations. They also linked these benefits to α-linolenic acid in chia seeds. Furthermore, fatty acids profile of quinoa seeds provides good functional lipids rich in monounsaturated and polyunsaturated fats. The results were in harmony with those of Halaby et al. (2017) they showed that the unsaturated fatty acids content of quinoa is 70-89.4%.

Effect of chia and quinoa supplementation on final weight, weight gain, FER and liver ratio

The results of feed intake and body weight gain of HFHFrD-induced fatty liver rats treated with chia and quinoa seed powders and extracts with two doses each are shown in Table 4, revealed that HFHFrD group showed significant increase in, final weight, weight gain and FER compared to normal control (NC) group.

Table 4. Effects of chia and quinoa supplementation on nutritional indicators

Indicators	NC	HFHFrD	CS	QS	CSE	QSE
Initial weight (g)	142.05± 9.70 a	147.00± 6.16 a	148.05± 5.62 a	149.00± 3.09 a	147.25± 3.10 a	148.25± 3.10 a
Final weight (g)	251.75± 10.35 cd	297.00± 14.52 a	279.75± 12.30 a	269.00± 11.91 b	263.04± 11.11 bc	255.04± 10.41 c
Weight gain (g)	109.25± 10.06 c	149.50± 14.82 a	131.25± 13.89 a	120.00± 12.42 b	116.56± 11.92 c	107.56± 10.92 cd
Feed efficiency ratio(FER)	0.073± 0.001b	0.078± 0.002a	0.093± 0.001b	0.086± 0.002a	0.077± 0.001 b	0.071± 0.001 b
Liver Ratio (%)	4.30± 0.43 b	5.23± 0.38 a	4.26± 0.27 b	4.40± 0.42 ab	4.46± 0.20 ab	4.27± 0.19 b

Data represented as mean ± SD, n = 6, values in each raw having different superscript (a, b, c, d ,e) are significantly different at ($p<0.05$), NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract.

Effect of chia and quinoa supplementation on lipid profile and renal function parameters

Data in Table 5 showed the parameters of lipid profile of rats groups that were fed on HFHFrD with treatments of chai and quinoa seeds and extracts for 8 weeks. Significant differences of TC, TG, HDL-C, LDL-C and VLDL-C levels were observed between HFHFrD group and the normal control (NC) group. HFHFrD group show a significant rising in TC, TG, LDL-C and VLDL-C levels, meanwhile a significant decline in HDL-C level comparing with normal control (N.C) group. Similarly, Korish & Arafah (2013) and El-Abed et al., (2018) found significant alleviation in lipid profile and oxidative stress parameters in nonalcoholic fatty liver rats induced by high fat high fructose administration. Generally, all treated groups with chia and quinoa seed powder and extracts showed a significant reduction in TC, TG LDL-C and VLDL-C levels and a significant increase of HDL-C in comparison with HFHFrD group. High levels of LDL-cholesterol are directly linked with cardiovascular disease development in humans (Rasheed & Cummins, 2018). Consumption of chia seed has shown promising results in reducing lipids levels, since it has good levels of unsaturated omega-3 fatty acids and dietary fiber (da Silva et al., 2017). As, omega-3 fatty acids supplementation has a basic role in promoting some markers in liver and kidney of

rats (Valenzuela et al., 2014; Mañán et al., 2018). Furthermore, these results are in parallel with de Souza Ferreira et al. (2015) who observed a significant enhancement in lipid profile on rats fed a sucrose-rich diet in a long-term which treated with chia seed compared to a sucrose-rich diet group. In addition, it has been indicated that the protein of chia has a key role in blocking markers of cholesterol synthesis (Coelho et al., 2018). In addition, Kumar et al. (2016) due the effect of chia seed to bioactive dietary elements which responsible for its therapeutic properties. In another study of Berti et al. (2005) they evaluate the safety of quinoa seed consumption of 50 g quinoa/day for 6 weeks to celiac patients and reported a reduction in TG, and total, LDL cholesterol. Moreover, Foucault et al. (2011) demonstrated consumption of quinoa caused a reduction in triglycerides (TG) and total and low density lipoproteins (LDL) cholesterol levels in rats fed enriched-fructose diet, declined the negative impact of fructose on high density lipoproteins (HDL). Also, Zevallos et al. (2014) and Halaby et al. (2017) as they reported that high cholesterol diet fortified with quinoa seed powder at 40% improved levels of cholesterol and triglycerides levels.

Table 5 indicated that fructose group showed a significant increase in serum urea and creatinine concentration as compared to that in the control group.

Table 5. Effects of chia and quinoa supplementation on lipid profile and renal parameters.

Indicators	NC	HFHFrD	CS	QS	CSE	QSE
T C mg/dl	63.80± 7.34 f	107.55± 12.75 a	84.31± 7.43 d	80.02± 6.41 d	70.02± 7.22 e	68.02± 6.92 e
TG mg/dl	75.77± 9.49 e	119.38± 11.61 a	97.07± 10.21 b	89.51± 7.53 c	87.51± 7.53 c	83.51± 7.53 c
HDL-c mg/dl	39.57± 3.71 a	26.15± 2.43 e	32.02± 1.75 d	34.12± 2.42 c	38.12± 2.74 a	38.12± 2.74 a
LDL-c mg/dl	22.17± 2.04 e	40.30± 3.92 a	30.32± 2.66 c	22.77± 2.88 e	22.97± 2.88 e	22.53± 2.88 e
VLDL-c mg/dl	15.15± 1.90 d	24.68± 2.32 a	19.41± 2.04 b	17.50± 1.51 c	17.50± 1.51 c	16.50± 1.51 c
Creatinine mg/dl	0.85± 0.02 d	2.74± 0.21 a	1.94± 0.11 b	0.88± 0.12 d	0.94± 0.04 c	0.70± 0.15 e
Uric acid mg/dl	2.12± 0.33 c	4.44± 0.33 a	3.08± 0.19 b	3.35± 0.45 b	2.44± 0.28 c	2.18± 0.19 c
Urea mg/dl	24.80± 1.26 d	46.10± 3.08 a	27.85± 2.17 c	24.05± 1.75 d	24.75± 1.19 d	27.85± 2.17 c

Data represented as mean ± SD, n = 6, values in each raw having different superscript (a, b, c, d ,e) are significantly different at ($p<0.05$),TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density Lipoprotein Cholesterol, VLDL-C: very Low density Lipoprotein Cholesterol, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract.

Quinoa seed (QS) group showed no significant changes in serum creatinine and urea concentration as compared to normal control (NC) group. Consumption of chia or quinoa extracts with high fat high fructose diet showed no significant difference in serum urea and creatinine concentration as compared to N.C group. Fructose is popular food ingredient and has potential influence to alleviate oxidative stress. Study finding showed that high fat high fructose diet administration in HFHFrD control group showed a significant increase of creatinine, uric acid and urea concentration as compared to control group.

These results come in parallel with the finding that suggest that increase of fructose consumption consider as key factor of metabolic syndrome and consequently to increase chronic renal disease (Abdel-Kaw et al., 2016).

These finding was in harmony with Halaby et al. (2017) they reported that quinoa seed fortified diet can enhancing creatinine, uric acid and urea, levels. The results of improvement in renal function may be linked with bioactive components that promote the biological functions by their antioxidants activities (Abderrahim et al., 2015).

Effect of chia and quinoa supplementation on liver function activities

With regard to liver activities (Figures 1, 2 and 3), AST, ALT and total bilirubin levels were significantly alleviated in the untreated fatty liver (HFHFrD) group compared to the normal control (NC), CS, QS, CSE and QSE groups. On the other hand, total portein significantly decreased in the HFHFrD group compared to the NC, CS, QS, CSE and QSE groups which was in agreement with (Charlton et al., 2011; Korish & Arafah, 2013; El-Abd et al., 2018).

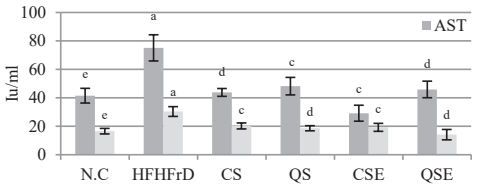

Figure 1. Changes of AST and ALT activities on different rats groups. Data represented as mean ± SD, n = 6, values in each bar having different superscript (a, b, c, d ,e) are significantly different at ($p<0.05$), AST: Aspartate aminotransferase, ALT: alanine aminotransferase, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract

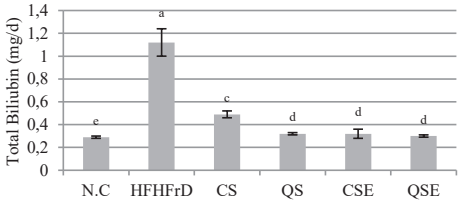

Figure 2. Changes of total bilirubin activity on different rats groups. Data represented as mean ± SD, n = 6, values in each bar having different superscript (a, b, c, d ,e) are significantly different at $p<0.05$, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract

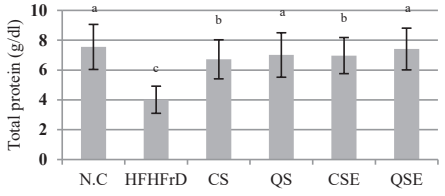

Figure 3. Changes of total bilirubin activity on different rats groups. Data represented as mean ± SD, n = 6, values in each bar having different superscript (a, b, c, d ,e) are significantly different at $p<0.05$, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract

As can be seen, the activities of AST, ALT and total bilirubin decreased with treating of chia and quinoa suppementation. treatment of HFHFrD rats with CS, QS, CSE and QSE decreased AST enzyme by about (41.69%, 35.82%, 61.14% and 38.89%), ALT by about (33.42%, 38.72%, 36.61% and 53.53%) and total bilirubin by about (56.25%, 71.42%, 71.42% and 73.21%) and caused an increase in total portein by about (67.58%, 74.81%, 73.81% and 84.78%) comparing with HFHFrD untreated group. From these results, it could be conculded that, treating HFHFrD rats with chia and quinoa supplementation improved liver enzymes activities.

In addition, Saxena et al. (2017) confirmed these results as quinoa seed powder have a positive effect in improvement of liver enzymes and oxidative stress markers and considered quinoa seed as hepatoprotective agent.

Oxidative stress parameters

The alterations occurring in total antioxidant capacity in different groups of rats are shown in (Figure 4).

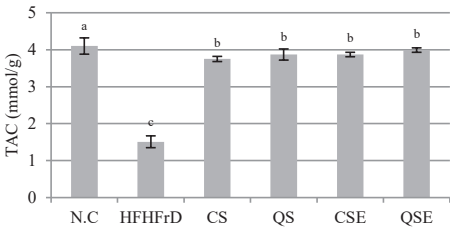

Figure 4. Changes of TAC (Total Antioxidant capacity) concentration on different rats groups. Data represented as mean ± SD, n = 6, values in each bar having different superscript (a, b, c) are significantly different at $p<0.05$, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract

The total antioxidant capacity concentration is decreased significantly ($p<0.05$) in HFHFrD group compared to normal control (NC) group. Treatment of HFHFrD rats with CS, QS, CSE and QSE had a very high significant influence in total antioxidant capacity ($p<0.05$) comparing with HFHFrD (untreated) rats. It can observed that high fat and high fructose induced depletion in the TAC concentration from 4.10 mmol in normal control (NC) group to 1.51 mmol (HFHFrD group) ($p<0.05$). There was a highly significant increase in TAC level at all supplemented groups in comparison to control (HFHFrD) group. These results

confirmed by the finding of Foucault et al. (2011) who indicated the protective effects of quinoa seed consumption against oxidative stress by enhancing the antioxidant capacity concentration and declining lipid peroxidation level in plasma and tissues of rats. It is well established that these supplements play a basic role as an indirect antioxidant and alleviate TAC level. Hence, it seems that treatment with chia and quinoa seeds powder and extracts supplements may decline the damage HFHFrD-induced nonalcoholic fatty liver in rats via enhancing antioxidant activity (Mohamed et al., 2019). High fat high fructose diet-induced significant increase in lipid peroxidation activity in liver (Figure 5) shows the concentration of LPO in all groups.

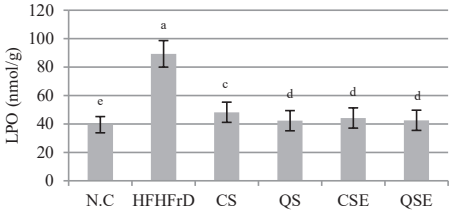

Figure 5. Changes of LPO activity on different rats groups. Data represented as mean ± SD, n = 6, values in each bar having different superscript (a, b, c, d ,e) are significantly different at $p<0.05$, NC: Normal Control, HFHFrD: High fat high fructose diet, CS: Chia seed, QS: Quinoa seed, CSE: Chia seed extract, QSE: Quinoa seed extract

After 8 consecutive weeks significant increase in LPO concentration was observed in untreated fatty liver (HFHFrD) group (89.31± 9.33 nmol/g) compared to N.C group (39.46±5.71 nmol). These results in the same line of (Crescenzo et al., 2018) who indicated that lipid oxidative damage induced by fructose rich diet that cause metabolic liver impairment. Hence, the LPO concentration decreased from 89.31 nmol for untreated (HFHFrD) group to 42.28 and 42.56 nmol in QS and QSE groups. Induction fatty liver by high fat high frcutose diet caused highly allevation in lipid peroxidation activity in liver. HFHFrD group exhibited a significant elevation of oxidative stress indicators (TAC & LPO) in addition to significant decline of the serum level of TAC in comparison with the NC group. These results in parallel with those studies of El-Abed et al. (2018) as they related this reduction to high fat diet consumption that alleviate free radicals which caused oxidative stress that plays avital role in the progression of NAFLD. Additionally, reactive oxygen species production activate lipid peroxides, thus caused the hepatic membranes damage (Li et al., 2014). In addition, Serviddio et al. (2013) reported that the increase in lipid peroxidation and the reduction antioxidants status have been observed in NAFLD patients and animals models.

CONCLUSIONS

It was concluded that dietary supplementation, is an alternative research area for the discovery of new functional ingredients for the control and management of fatty liver disease by diminishing lipids accumulation. Chia and quinoa seeds are considered as rich sources of bioactive compounds such as phenols, flavonoids, omega-3 & omega-6 constituents which abolished lipids accumulation and possess a protective influence against fatty liver disease. So, these herbal seeds can be used as functional dietary supplements in course of fatty liver disease through reducing lipid accumulation and enhancing lipids parameters, liver and renal functions.

ETHICS STATEMENT

The study experiment was performed in accordance with laws and regulation of the Medical Research Ethics Committee of National Research Center.

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