

Effect of some germinated seeds on nonalcoholic fatty liver disease in albino rats

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Abstract

This research aimed to study the effect of germinated pumpkin, lentil and quinoa seeds dried powder on nonalcoholic fatty liver disease (NAFLD) in male albino rats. Seeds of pumpkin, lentil and quinoa were germinated, oven dried and analyzed for their nutritive value of their macronutrients, mineral, phenols & flavonoids compounds contents. Sixty male Albino Rats were divided into 10 groups. Negative control group was fed on basal diet. The other groups were fed for four weeks on high fructose diet (HFD) to induce NAFLD. One of them is positive control group. After NALFD induction, the other eight groups were fed on basal diet mixed with different percentages of germinated seeds powders of pumpkin, lentil and quinoa and their mixtures. *Biochemical analyses* of the rats' serum was done at the beginning and at the end of the study for lipid parameters [Total cholesterol (TC) , triglycerides (TG) , HDL-C, LDL-C&VLDL-C] , Liver enzymes(AST& ALT), serum creatinine , blood urea & uric acid . Also, histopathological examination for liver tissues and analysis of the studied germinated seeds (GSs) were done. **Results** showed that using various germinated seeds powders in

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NAFLD rats led to significant improvement in lipid profile, liver enzymes, creatinine and uric acid especially in the group fed on 10% pumpkin, followed by groups 10% mix (pumpkin, lentil and quinoa) and 10% quinoa, respectively. These improvements happened in parallel with enhancement in liver tissues that were checked by microscopic examination. Compositional analysis of germinated seeds revealed increase their photochemical compounds' contents that may be beyond their favorable effects on the liver. Conclusion germinated pumpkin, lentil and quinoa seeds improve both liver functions and liver tissues of NAFLD rats.

Introduction

Liver function can be impaired and hepatocytes damaged upon exposure to drugs, alcohol, infections, or malnutrition (*Mroueh et al., 2004*).

Nonalcoholic fatty liver disease (NAFLD), besides increasing liver morbidity and mortality, is associated with development of atherosclerosis. Dyslipidemia and chronic inflammation are recognized to drive the development of NAFLD as well as atherosclerosis (*Armstrong et al., 2014*). However, foods rich in antioxidants have been proposed as a tool to prevent and cure liver damage (*Morisco et al., 2008*).

Sprouts' contents of nutrients; phytochemicals, vitamins, minerals, enzymes and amino acids are of almost importance for human health (*Finley, 2005*).

Sprouting is the practice of germinating seeds to be eaten raw or cooked. Sprouts can be germinated at home or produced industrially. They are a prominent ingredient of the raw food diet and common in Eastern Asian cuisine (*Chavan et al., 1989*).

Therefore, this study aimed to investigate the effect of germinated pumpkin, lentil and quinoa seeds on nonalcoholic fatty liver disease in albino rats.

Materials and Methods

Seeds preparation:

Pumpkin, lentil and quinoa seeds were germinated according to method of *Aslani et al., (2015)*, the seeds were soaked in water for 12 hours, and then the seeds were placed on a wet cotton tissue for 5 days. In these stages, temperature of environment was 25-30°C. After that, germinated seeds were oven dried at 40° C.

Diets:

The standard diet and high fructose diet (HFD) were prepared according to *Al-Okbi et al., (2015)*. After NAFLD induction, eight groups were fed balanced diet mixed with dried germinated seeds with different ratios to achieve treatment. Part of starch was substituted with those dried seeds with different percentages in balanced diet to make different experimental diets.

Determination of nutritive value:

Total protein of seeds powders was determined by the micro-kjeldahl method of *Jayaraman (1981)*. Lipid content of seeds powders was determined by the method of *Nicodimescu (1964)*.

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Fibers content of seeds powders was determined by the method of *Bligh and Dyer (1989)*.

Determination of phenols in germinated seeds were carried out by using high performance liquid chromatography (HPLC) as described by *Harborne, (2007) and Tiwari et al., (2011)*.

Experimental design:

Adult male 60 albino rats of Sprague Dawley strain weighing 160 ± 10 g were kept individually in cages. The rats were divided into 10 groups each consisted of 6 rats. The first group was fed on basal diet as negative control. The other groups were fed for four weeks on HFD to induce NAFLD according to *Al-Okbi et al., (2015)*. Then these groups discontinued on HFD and were fed on basal diet. One group of them considered as a positive control. The other eight groups fed on balanced diet containing 5 & 10% (pumpkin seeds, lentil, quinoa and their combinations).

Biochemical analysis:

At the end of the experimental period, rats were fasted overnight, than the rats anaesthetized and sacrificed, and blood samples were collected and centrifuged to separate the serum to estimate some biochemical parameters i.e. total cholesterol (TC) (*Allain et al., 1974*), triglycerides (TG), (*Fredrickson et al., 1967*), High-density lipoprotein cholesterol (HDL-C), (*Stein, 1986*). Low density lipoprotein cholesterol (LDL-C) and Very Low-density lipoprotein cholesterol (VLDL-C) were calculated according to the equation of *Friedwald et al., (1972)*. Determination of glucose was carried out by enzymatic colorimetric method according to *Trinder (1969)*. Malondialdehyde (MDA) was done according to the method

of *Uchiyama and Mihara (1978)*. Aspartate amino transferases (AST) and alanine amino transferases (ALT) were measured according to the method described by *Tietz, (1995)*. Serum creatinine and serum uric acid were measured according to the method described by *Tietz, (1995)*. Serum urea was measured according to the method described by *Tietz (1990)*.

Histopathological examination:

Liver tissues were examined according to the method of *Drury and Wallington (1980)*.

Statistical analyses:

Data were analyzed by SPSS statistical package (*Armitage et al., 2002*).

Results and Discussion

Germinated seeds (GSs) compositional analysis for macronutrients and minerals as illustrated in Table (1) revealed the following:

Protein: Among the 3 types of the analyzed germinated seeds, the highest value of protein was recorded for germinated pumpkin seeds followed by lentil then quinoa. Protein percent increased in germinated pumpkin seeds when compared with raw seeds by (10.22%). While protein values were decreased in germinated lentil (-9.68%) and germinated quinoa (-18.18%) when compared with raw seeds.

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Fat: The highest value of fat was recorded for germinated pumpkin seeds followed by quinoa, then lentil. Fat content was reduced in germinated pumpkin seeds when compared with raw seeds by (-16.36%). Also fat value of lentil seeds was decreased (-19.13%) when seeds were germinated but percent of fat value increased (12.70%) in quinoa seeds via germinating process.

Carbohydrates: Quinoa germinated seeds had the highest value of carbohydrates among 3 types of seeds, followed by lentil seeds. Pumpkin seeds had the lowest value of carbohydrates. Although pumpkin record the greatest increase of carbohydrates (55.65%) by germination. While carbohydrates of germinated lentil and germinated quinoa decreased by (-4.74%) and (-0.72%), respectively.

Fiber: Lentil seeds had the most increasing amount of fibers when compared with pumpkin germinated seeds and quinoa germinated seeds. Germination process increased percent of fibers in pumpkin, lentil and quinoa by 53.08%, 46.37% and 44.40%, respectively.

EL-Adawy et al., (2003) reported that significant decreases were observed in total protein, fat and total carbohydrate contents with increased germination time in all legume seed flours, while non-protein nitrogen, ash and fiber contents significantly increased. The decrease in proteins, fat and carbohydrate contents could be attributed to their use as energy sources to start germination (*Panteleev, 1995*). The decrease in protein, fat and carbohydrate contents may have led to the apparent increase observed in the other chemical components (*EL-Adawy et al., 2003*). On the other hand, *Megat-Rusydi et al., (2011)* revealed that carbohydrate content was increased in germinated mung beans and kidney beans and

decreased significantly in germinated white, black, red and brown rice.

Compositional analysis of germinated pumpkin, lentil and quinoa seeds are shown in table (2) and revealed that:

Lentil GSs involved the greatest value of total Phenolic compounds mainly gallic acid, chlorogenic acid and benzoic acid.

Vanillic acid, caffeic acid, p-coumaric acid and ferulic acid were most abundant in quinoa seeds.

The present study matches with those of **Fouad and Rehab, (2015)** which verified that total phenolic contents of dry plant material of un-germinated lentil seeds was significantly ($P \leq 0.05$) increased by germination process. In accordance with other work, vanillic acid was the most abundant of identified phenolic acids in quinoa sprouts (**Alvarez-Jubete et al., 2010**). While **Lee et al., (2017)** found that vanillic acid and caffeic acid were the least abundant of identified phenolic acids in lentil sprouts.

Amutha et al., (2014) reported that, the medicinal property of pumpkin seeds may be attributed to the presence of flavonoids and phenolic compounds which counteract the free radicals responsible for various health complications. Pumpkin seeds extract was found to be rich in quercetin which is the aglycone or sugarless form of rutin, the major bioflavonoid in the human diet.

Biochemical results:

Lipid profile of rats fed on different levels of dried powder of some germinated seeds and their mixtures illustrated in table (3). Feeding rats for 4 weeks on HFD induced NAFLD, including hyperlipidemia which expressed as increased TC, TG, LDL-C and

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deceased HDL-C. Dietary intervention by adding germinated seeds powder (pumpkin, lentil and quinoa) to the diet, had been attenuated and recovered lipid profile to some extent according to the type of each seed that had been used in this study. Treated rats with 10% pumpkin, was the most effective group as it significantly ($P < 0.5$) decrease TC, TG, LDL-C, and VLDL-C and also significantly increase of HDL more than any other treated groups, followed by the groups treated with 10% mix and 10% quinoa which did similar effects as group 10% pumpkin, with a little bit difference.

These results agreed with those reported by *Makni et al., (2010)* who stated that Pumpkin seed oil possess anti-inflammatory, hypolipidemic and antioxidant effect. Thus, this functional food might also reduce the progression of fatty liver to NASH (*Hyounjeong et al., 2007 & Park et al., 2010*). Additionally, betalain (which is naturally exists in quinoa seeds) potentially offers similar health benefits (*Wu, 2016*).

Serum glucose & malondialdehyde (MDA) of rats fed on different levels of dried powder of some germinated seeds and their mixtures presented in table (4).

The mean values of serum glucose and MDA increased significantly in the positive control group, as compared to the negative control group. On the other hand, all treated groups decreased significantly as compared to the positive control group. The best results were recorded for the group which treated with 10% pumpkin.

In this respect, *Al-Okbi et al., (2015)* stated a similar significant reduction in plasma glucose levels of rats fed on diet consisted of pumpkin seeds powder compared to High Fructose Diet (HFD) fed rats.

Mallick et al., (2017) stated that the lipid peroxidation level (MDA) is drastically increased in hyperlipidemic rats group and then significantly decreased after administration of different doses of extract of pumpkin seed for 30 days. Also, *Pasko et al., (2010)* reported that quinoa seeds can act as a moderate protective agent against potential of fructose-induced changes in rats by reducing lipid peroxidation.

Serum liver enzymes and Kidney functions of rats fed on different levels of dried powder of some germinated seeds and their mixtures illustrated in table (5). The mean value of serum liver enzymes including AST and ALT, also kidney functions (uric acid, creatinine and urea) increased significantly in positive control, as compared to the negative control group. Serum alanine amine transferase (ALT) decreased significantly ($p < 0.05$) in treated groups, as compared with positive control. The best result was recorded with the 10% pumpkin group, followed by 10% mix and 10% quinoa groups, and there were no significant differences between them. Similar results were obtained for serum aspartate amine transferase (AST).

Similar effects on ALT&AST were obtained by *Halaby et al., (2017)* for hypercholesterolemic rats fed on diet fortified with quinoa seeds powder. Also, *Al-Okbi et al., (2015)* reported that treatment by mixture of plant sources containing pumpkin seeds powder resulted

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in significant decrease in AST and ALT activity compared to the HFD-fed rats.

The mean values of uric acid of treated groups were significantly lower when compared to positive control group. Best result was recorded for mean value of 10% pumpkin group, followed by 10% mix group and 10% quinoa group. As regard creatinine, only two groups: 10% pumpkin and 10% mix were significantly lower than positive control group. As for urea, the results showed that the mean value of three groups: 10% pumpkin group, 10% lentil and 10% quinoa were the only significantly lowered groups than positive control group.

Similarly, *Jensen et al., (2018)* showed that fructose increases uric acid levels in the liver and stimulates the synthesis of uric acid from amino acid precursors. In addition, a meta-analysis found a dose-dependent rise in the incidence of NAFLD by 3% for every 1 mg/dl increase in serum uric acid, even after accounting for metabolic syndrome and other lifestyle factors (*Liu et al., 2015*).

Control rats fed on high fructose diet showed significant elevation in plasma levels of creatinine and urea as indicators of kidney function while feeding them on diet containing powder of pumpkin seeds (*Al-Okbi et al., 2015*) and quinoa seeds (*Halaby et al., 2017*) showed significant reduction in plasma levels of both creatinine and urea indicating improvement in kidney function.

Histopathological results:

Microscopically, liver of rats from negative control group revealed the normal histological structure of hepatic lobule (Picture

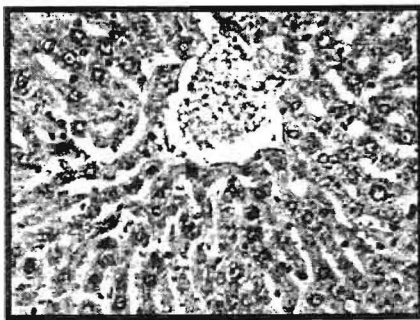
1). Meanwhile, liver of rat from positive control group showed macrovesicular steatosis of hepatocytes and mononuclear inflammatory cells infiltration (Picture 2). Also, positive control group showed macrovesicular steatosis of hepatocytes and congestion of central veins (Picture 3). Liver of rat from 5% pumpkin group showed small vacuoles in the cytoplasm of hepatocytes (Picture 4). Liver of rat from 5% lentil group showed macrovesicular steatosis of hepatocytes (Picture 5). Liver of rat from 5% quinoa group showed fatty change of hepatocytes and congestion of hepatic sinusoids (Picture 6). Liver of rat from 10% pumpkin showed no histopathological changes (Picture 7). Liver of rat from 10% lentil group showed macrovesicular steatosis of small focal hepatocytes associated with oval cells proliferation (Picture 8). Liver of rat from 10% quinoa group showed macrovesicular steatosis of focal hepatocytes (Picture 9). Liver of rat from 5% mix showing microvesicular steatosis of hepatocytes (Picture 10). Liver of rat from 10% mix group showing slight activation of Kupffer cells (Picture 11).

These findings agreed with those of **Mallick et al., (2017)** who found that the histopathological analysis showed disarrangement of hepatic cell and central vein of liver in untreated group (hyperlipidemic rats). After oral co administration of pumpkin seed extract at the doses of 50mg and 75mg /kg body weight/day shows the better protective action with well arranged hepatic cell and normal central vein than the untreated groups. However, these findings disagreed with those of **Morrison et al., (2015)** who revealed that microvesicular hepatosteatorosis was not reduced in mice fed a Western type of diet containing 9% refined pumpkin seed oil or 9% virgin pumpkin seed oil for 20 weeks.

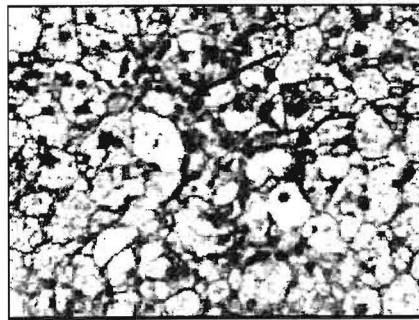
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Conclusion & Recommendation

Germination of pumpkin, lentil and quinoa seeds increase their phytochemical properties; that may be a direct cause of improving liver functions and dyslipidemia, in addition to, enhancing liver tissues of NAFLD rats. Germinated pumpkin seeds have the most favorable effects, followed by a mixture of the three types of germinated seeds. Researches on human with fatty liver disease are recommended to ameliorate the liver tissues and functions by using such natural germinated food items.



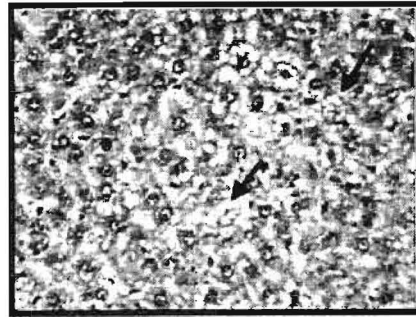
Picture (1)



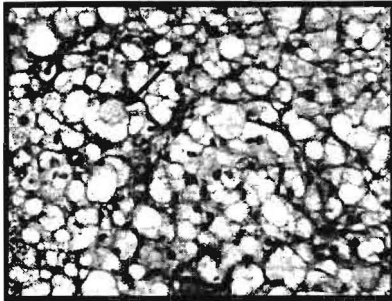
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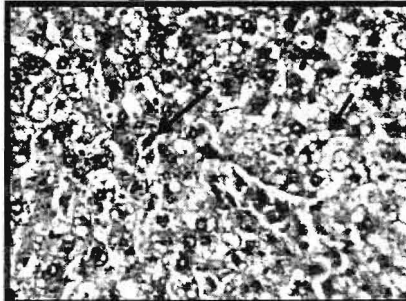
Picture (3)



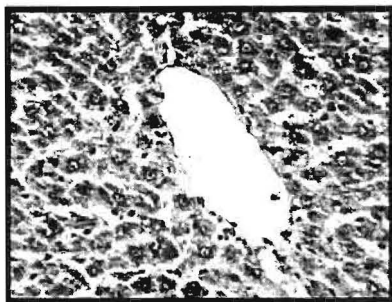
Picture (4)



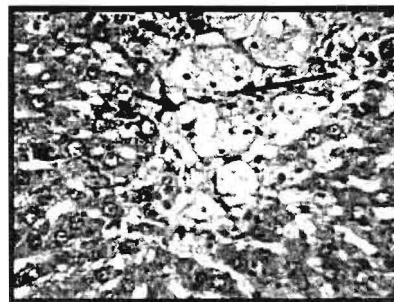
Picture (5)



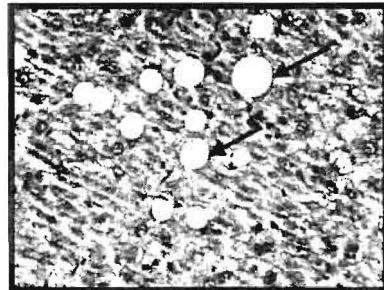
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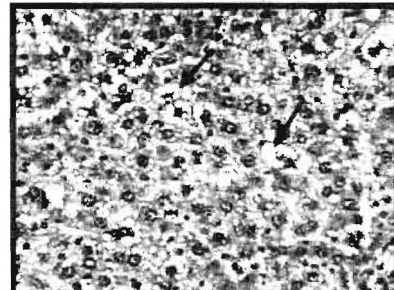
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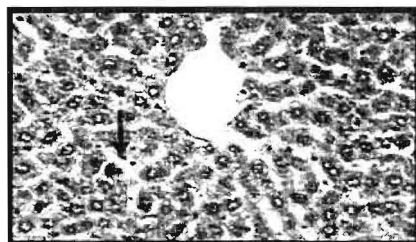
Picture (8)



Picture (9)



Picture (10)



Picture (11)

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Table (1): Compositional analysis of pumpkin, lentil and quinoa seeds per 100 g (pre and post germination)

Nutrient	Pumpkin			Lentil			Quinoa		
	Pre	Post	% of change	Pre	Post	% of change	Pre	Post	% of change
protein (g)	30.42	33.53	10.22	31.41	28.37	-9.68	15.8	13.5	-18.18
Fat (g)	50.49	42.23	-16.36	1.15	0.93	-19.13	7.1	7.1	12.70
Carbohydrates(g)	11.41	17.76	55.65	56.53	53.85	-4.74	65.5	68.5	-0.72
Fiber (g)	2.60	3.98	53.08	6.75	9.88	46.37	2.5	3.61	44.40

Table (2): Phytochemical analysis of germinated pumpkin, lentil and quinoa seeds

Compound	Pumpkin (mg/100g)	Lentil (mg/100g)	Quinoa (mg/100g)
Polyphenols			
Benzoic acid	9.25	25.35	11.05
Gallic acid	1.14	302.41	1.77
Vanillic acid	0.56	0.04	8.54
Chlorogenic acid	1.05	5.81	3.68
Caffeic acid	0.13	0.06	0.53
p-Coumaric acid	0.12	0.30	2.97
Ferulic acid	2.63	0.23	3.61
Total	14.88	334.2	32.15

Table (3): Lipid profile of rats fed on different levels of dried powder of some germinated seeds and their mixtures

Groups	T.C	T.G	HDL	LDL	VLDL
(-ve)	144.43± 6.76 ^g	93.87± 5.68 ^g	54.13± 4.78 ^a	71.52± 10.50 ^o	18.77± 1.14 ^g
(+ve)	268.19± 47.41 ^a	207.56± 28.76 ^a	29.84± 2.68 ^d	196.83± 44.69 ^a	41.51± 3.75 ^a
5% Pumpkin	185.67± 10.78 ^c	156.68± 9.81 ^c	41.13± 3.36 ^b	113.21± 12.13 ^c	31.34± 1.96 ^c
5% Lentil	199.76± 9.44 ^b	163.16± 11.69 ^c	39.36± 6.67 ^b	127.76± 13.69 ^b	32.63± 2.34 ^c
5% Quinoa	188.17± 10.88 ^c	187.02± 11.35 ^b	37.08± 3.32 ^c	113.68± 12.66 ^c	37.40± 2.27 ^b
10%Pumpkin	154.66± 9.23 ^f	106.87±9.57 ^f	51.15± 4.00 ^a	82.14± 10.24 ^d	21.38± 1.91 ^f
10% Lentil	180.77± 7.54 ^c	148.61± 8.77 ^{c,d}	43.93± 4.38 ^b	107.12± 10.08 ^c	29.72± 1.75 ^{c,d}
10% Quinoa	170.65± 4.25 ^d	137.93± 11.58 ^d	49.14± 6.29 ^a	93.92± 8.07 ^d	27.59± 2.32 ^d
5% Mix	177.14± 9.68 ^c	141.24± 13.44 ^d	45.12± 4.29 ^b	103.78± 9.27 ^c	28.25± 2.69 ^d
10% Mix	165.79± 4.49 ^e	130.79± 6.68 ^e	50.32± 5.16 ^a	89.31± 10.34 ^d	26.16± 1.34 ^e

- Values were represented as mean ± SD. Means with different superscript letters are significantly different at p < 0.05.

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Table (4): Serum glucose & MDA of rats fed on different levels of dried powder of some germinated seeds and their mixtures

Groups	Parameter	Sr. glucose (mg/dl) Mean±SD	Sr. MDA Mean±SD
	(-ve)	87.00±7.13 ^d	3.53±0.19 ^b
	(+ve)	136.00±18.54 ^a	4.12±0.26 ^a
	5% Pumpkin	111.00±7.27 ^b	3.05±0.16 ^c
	5% Lentil	112.00±5.97 ^b	3.66±0.15 ^b
	5% Quinoa	116.17±11.84 ^b	3.78±0.25 ^b
	10% Pumpkin	93.00±6.59 ^{c,d}	2.38±0.31 ^a
	10% Lentil	110.00±8.76 ^b	3.15±0.20 ^c
	10% Quinoa	100.50±6.12 ^c	2.89±0.18 ^{c,d}
	5% Mix	107.00±6.45 ^b	3.04±0.24 ^c
	10% Mix	99.00±6.72 ^c	2.67±0.17 ^d

- Values were represented as mean ± SD. Means with different superscript letters are significantly

Table (5): Serum liver enzymes and Kidney functions of rats fed on different levels of dried powder of some germinated seeds and their mixtures

Parameters Groups	ALT (U/L)	AST (U/L)	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
(-ve)	28.81±	26.38±	3.62±	0.74±	29.60±
	4.33 ^g	2.85 ^h	0.22 ^e	0.06 ^c	4.72 ^c
(+ve)	91.71±	85.78±	6.22±	1.16±	39.08±
	4.91 ^a	3.15 ^a	0.25 ^a	0.21 ^a	3.46 ^a
5% Pumpkin	59.25±	57.28±	5.52±	0.98±	38.57±
	5.81 ^c	6.04 ^c	0.32 ^b	0.12 ^{a,b}	4.25 ^a
5% Lentil	76.59±	69.86±	5.59±	1.08±	36.89±
	8.18 ^b	2.99 ^b	0.14 ^b	0.18 ^a	4.60 ^a
5% Quinoa	65.04±	61.93±	6.08±	1.03±	38.21±
	4.58 ^c	5.53 ^c	0.18 ^a	0.17 ^a	4.87 ^a
10% Pumpkin	35.83±	31.05±	4.45±	0.83±	33.13±
	3.18 ^f	3.44 ^g	0.21 ^d	0.09 ^b	2.93 ^b
10% Lentil	52.11±	50.42±	5.27±	0.99±	35.78±
	4.33 ^d	4.58 ^d	0.38 ^b	0.20 ^{a,b}	0.57 ^b
10% Quinoa	41.16±	40.11±	4.89±	0.92±	35.03±
	2.51 ^e	2.90 ^e	0.26 ^c	0.12 ^{a,b}	3.97 ^b
5% Mix	47.06±	47.09±	5.13±	0.97±	36.42±
	5.61 ^d	4.26 ^d	0.15 ^b	0.18 ^{a,b}	2.69 ^a
10% Mix	39.13±	36.12±	4.76±	0.87±	36.15±
	3.25 ^e	2.20 ^f	0.09 ^c	0.08 ^b	2.16 ^{a,b}

- Values were represented as mean ± SD. Means with different superscript letters are significantly different at p < 0.05.

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تأثير إنبات بعض البذور على الفئران البيضاء المصابة بالكبد الدهني

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الملخص العربي

يهدف هذا البحث الي دراسة تأثير البذور المنبتة والمجففة لبذور القرع والعس والكينوا علي الفئران المصابة بالكبد الدهني غير الكحولي. تم تجهيز البذور وانباتها ثم تجفيفا وتحليلها لمعرفة قيمتها الغذائية ومحتواها من المركبات الفينولية والفلافونات. تم عمل الدراسة علي ٦٠ فأر ثم قسمت الفئران الي ١٠ مجموعات. المجموعة الضابطة السالبة تغذت النظام الغذائي القياسي. المجموعات الأخرى تغذت لمدة أربعة أسابيع علي نظام غذائي عالي المحتوي في الفركتوز بغرض اصابة الفئران بالكبد الدهني غير الكحولي. ثم تم إيقاف التغذية علي الغذاء العالي الفركتوز وأكملت المجموعات تغذيتها علي النظام الغذائي القياسي ومجموعة واحدة منهم تم اتخاذها كمجموعة ضابطة موجبة، والمجموعات الثمانية الأخرى بعد اصابتها بالمرض، تم تغذيتها علي النظام الغذائي القياسي مع اضافة نسب مختلفة البذور المنبتة المجففة للقرع والعس والكينوا وخلطاتهم. تم عمل التحاليل المعملية للدم في بداية ونهاية التجربة وذلك بقياس مؤشرات مختلفة مثل دهون الدم، وانزيمات الكبد، ووظائف الكلي، وكذلك تم فحص أنسجة الكبد تحت المجهر. أظهرت النتائج أن استخدام مجفف البذور المنبتة في علاج الكبد الدهني للفئران أدى الي تحسن معنوي في صورة دهون الدم وانزيمات الكبد وبخاصة المجموعة التي تغذت علي بذور القرع المنبتة بنسبة ١٠% ويليها المجموعة التي تغذت علي خليط البذور المنبتة بنسبة ١٠% ، و المجموعة التي تغذت علي بذور الكينوا المنبتة بنسبة ١٠% ، علي التوالي. أيضا تحسنت قيم الكرياتينين وحمض اليوريك في مجموعتي الفئران التي تغذت علي بذور القرع المنبتة بنسبة ١٠% ، وتلك التي تغذت علي بذور الكينوا المنبتة بنسبة ١٠%، علي التوالي. هذه التغيرات الايجابية حدثت بشكل متوازي مع التحسن الذي حدث في خلايا الكبد التي تم فحصها تحت المجهر. كشفت تحاليل هذه البذور المنبتة أنها تحتوي علي كميات من مركبات الفيتوكيميكال والتي مكنت خلايا الكبد من استعادة وظائفها.