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Abstract

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Keywords

Chía; Salvia hispanica ; Chemical Characteristic ; Rats; Lipids Profile ; Glucose.



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Abstract

The purpose of this study was to determine the major nutrients in chia seeds, the amino acid content of protein, the fatty acid content of extracted oil, and to investigate the effects of seeds consumption for 5 weeks on glucose and lipids levels in rat's blood. The results showed each hundred grams of chia seeds contains (3.76, 24.61, 35.75, 31.03, 21.12 and 4.66gm) moisture, protein, fat, total carbohydrates, crude fiber and ash respectively with 3.80mg of vitamin C. The result of amino acid analysis was indicates glutamic acid, asparagine, arginine from nonessential and tryptophan, phenylalanine from essential amino acids to be main components of seeds protein and represent of 3.264,3.300 ,2.550 and 1.858, 1.148gm/100gm sample, respectively. The analysis of fatty acid composition, allowed the quantification of twenty seven fatty acids. Unsaturated fatty acids and, in particular, linoleic and α -linolenic acids, were predominant in which there content were 18.233 and 50.641%, respectively, while palmitic acid was the major saturated fatty acid found in seeds and comprised 9.218% of total lipid. Also The effects of chia seeds on glucose levels and lipid profiles of rat serum were investigated, sugar, triglycerides and total cholesterol level in rats blood were significantly lower (P<0.05) and high density lipoprotein level was significantly higher (P < 0.05) in the group of rats fed on chia seeds compared with control group.

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Introduction

Chia (Salvia hispanica, L.) is an annual herbaceous flowering plant that goes to the genus salvia that contains over 900 species widespread throughout the world and belongs to the mint family, Lamiaceae [1], these seeds is native to central, southern Mexico and northern Guatemala, it is began to be used in human food in these regions around 3500 BC, but is not widely known in Europe and Middle East [2]. Muñoz et al [3] stated that the word —chia|| is a Spanish adaptation of chian or chien in its plural form, meaning —oily,|| which comes from Nahuatl, the language of the Aztecs.

The plant produces numerous small seeds with size ranging from 1 to 2mm, the shape is oval and varies in color from black, grey and black spotted to white seeds, which mature in autumn [4]. Coates and Ayerza [5] reported that chia seeds together with corn, beans, and amaranth, were important crops of the basic foods of several Central American civilizations, also it considered an alternative crop to diversify and stabilize the economy of many countries.

Nowadays, chia seeds are a potential source of nutrients for the food and animal feed industries, although chia is not a well-known food, its global production is increasing due to its healthy properties and popularity [6]. Chia seeds are being reintroduced to western diets in order to improve human health. In this respect, chia seeds have been investigated and recommended due to their high levels of proteins, antioxidants. These species are characterized by the highest total phenol and flavonoid contents [7].

The pervious results suggest that salvia species are healthy plant foods, phytochemicals, dietary fiber, vitamins and minerals and but particularly due to their oil content with the highest proportion of poly unsaturated fatty acids compared to other natural sources known that are useful as a dietary supplement, also Chia seed oil contains no cholesterol [8].

All these nutritional characteristics are very important parameters that promote the defense of consumers from some adverse conditions, such as protection against some cardiovascular diseases, hypercholesterolemia, hyperglycemia, low efficient intestine function and contribute to the trapping of free radicals and prevent some types of cancer ([9] and [2]), as is well known consumers are more aware of food and dietary issues and are monitoring and adjusting what they consume as they have become more proactive and diligent in improving their overall health through their daily diet [10]. In 2009, it was approved as novel food by the European Parliament and the European Council [11]. According to scientific opinion, whole or ground chia seeds do not cause any adverse allergenic, anti-nutritional, or toxic effects ([11] and [3]), thus, chia seeds and derived products are promising sources of food.

Chia seeds were used as a popular drink called —chia frescall (fresh chia), is still made in Mexico by soaking chia seeds with water or fruit juice are

consumed in some regions as refreshing drinks, and chia flour is used in a variety of different food preparations [3] .Ali et al [12] reviewed that there are many Commercial usage of chia seed in animal feed, food formulation and health supplement. It was used as a nutritional supplements in food preparation, to produces composite flour with corn flour or oat flour to increased total dietary fiber [13] as well as in bread [14] and as ingredient for sugar cookies containing chia–oat composites[15].

The aim of this study was to determine some chemical Compositions of Salvia hispanica L. seeds available in Erbil market and know its nutritive value and to investigate the effect of chia supplementation on serum glucose, triglyceride, total cholesterol, high density lipoprotein, and low density lipoprotein contents when fed to rats.

Materials and methods

Materials

Chemicals—all chemicals used were of analytical grade and purchased locally.

Chia seeds— (Salvia hispanica L.) were purchased from Erbil local market, cleaned, packed in polypropylene bags and stored at 5 °C until further use and grounded using a grinder with cooling system before analysis.

Animals— white female rats with 5weeks age and weighing about (140-160)gm were obtained from animal house, Biological Department, Education College, Salahaddin University.

Diet—wheat flour, powder of Soy bean, sunflower oil, vitamins and minerals were purchased from Erbil local market.

Kits from (BLOLABO® Company, France) were used to determine serum glucose; triglycerides, Total cholesterol, HDL-C and LDL-C.

Methods

Chemical composition:

Sample of chia seeds was analyzed for chemical composition (moisture, protein, fat, ash and crude fiber) using the AOAC procedures[16]. The crude protein content (N \times 6.25) of the samples was estimated by the micro

Kjeldahl method, the crude fat was determined by extracting a known weight of powdered seeds with diethyl ether, using a Soxhlet apparatus, the ash content was determined by incineration at 600 °C for 3 h. to obtain carbon free white ashes. Total carbohydrates were calculated by difference and vitamin C content was analyzed using the spectrophotometric method according to that described by [17].

Amino acids:

Amino acids were determined using LC-MS HPLC analyzer (RMIT University- Melbourne, Victoria, Australia- School of Health and Biomedical Sciences College- Department of Medical Sciences). An Agilent 1200 LC-system coupled to an Agilent 6410 Electro spray Ionisation-Triple Quadrupole-MS was used for quantification experiments. Agilent Technologies 6890 gas chromatography using helium as the carrier gas and a flame ionization detector.

Fatty acids:

Fatty acids within the lipid extracts were derivitised to fatty acid methyl esters (FAME) using 14% boron trifluoride–methanol [18].FAMEs were analyzed on gas chromatography-mass spectrometry (GC-MS) chromatography (RMIT University- Melbourne, Victoria, Australia- School of Health and Biomedical Sciences College- Department of Medical Sciences).

Minerals:

The obtained ashes were dissolved in hydrochloric acid, filtered and analyzed in lab of Kurdistan Institution for Strategic Studies / Sulaymaniyah city , sodium and potassium, were measured by flame atomic absorption spectrophotometry using A A Analyst 700 (Perkin Elmer).While calcium, magnesium, iron, copper ,zinc and selenium were measured by inductive couple plasma optical emission spectrophotometry(ICP OES,Optima2100 DV).

Animals Experiment:

The experiment was carried out in Gerda rash field –Animal Resource Department -Agriculture College. Twelve White female rats with 3weeks age and weighing about (140-160)gm were divided randomly into two groups each of 6 rats and housed in stainless steel cages and maintained under laboratory controlled of temperature (25 ± 2) with a 12-hour light/dark. Water and food were available freely. The basal diet pellet used in experiment was formulated in which comprise from (the contains 55% wheat flour, 26% powder of Soy bean, 5% sunflower oil and 5% of vitamins and minerals). The first group (control) fed on basal diet and the second group (treatment) fed on treated with ground chia seeds at an amount of 15% of basal diet for five weeks, during that period, food consumption was recorded. At the end of the experimental period, after a 12-hour fast, body weights were recorded. Animals were anesthetized with ethyl ether and blood was obtained by heart puncture with the help of sterilized needle and sterilized syringe and transferred into the appropriate sterilized centrifuge tube, serum was separated after centrifugation at 1500 rpm for 15 minutes, serum glucose and lipid profiles were measured using colorimetric assay kits.

-Statistical analyses:

All data were analyzed by using CRD (Complete Randomize Design) by SAS (Statistical Analysis System, 2005), as per variance, significant differences among treatment at level (0.05) were determined by Duncan's multiple range tests [19].

3. Results and dissection

Chia seeds chemical composition

Table (1) shows the proximate chemical compositions of chia seeds, the seeds of chia contents (3.76, 24.61, 35.75, 31.03, 21.12and 4.66%) moisture, protein, fat, total carbohydrates, crude fiber and ash, respectively with 3.80mg\100gm of vitamin C.

Table (1) Proximal composition of chia seeds (%)

Component	Content%
Moisture	3.76±0.218
Protein	24.61±1.05
Fat	35.75±1.22

Carbohydrates	31.03±1.38
Crude Fiber	21.12±0.92
Ash	4.66±0.110
Vitamin C	3.80±0.095
(mg\100gm)	

Results are expressed as $(x \pm d)$, being x the average and d, the standard deviation

(n = 3).

Data revealed that the chia seeds were rich source of oil and protein making it an ideal energy source material. Its high content of dietary fiber is of importance, where in it acts as an intestinal regulator. These results except moisture content were similar with those of Martínez et al [20], they reported that the chemical composition of chia seeds were moisture 0.072, total protein 0.218, ash 0.044, carbohydrates 0.334 and 0.317 oil content (gm /gm seeds), but differ except in lipids content than that obtained by Iglesias-Puig and Haros [14], in which the seeds were contains 33.9 , 20.2 , 2.3 , 30.9 lipids, proteins ,ash and total dietary fiber (gm /100 gm seeds), The determined content of dietary fiber was lower than that reported by Segura-Campos et al [21] in raw chia flour (34.46%) with near values of other components. Chia contains slight quantities of vitamin C (total ascorbic acid) as shown in Table (1).However, it was about three times more than that recorded by U.S. Department of Agriculture [22].

Amino acid composition of chia seeds

Table (2) summarized the non-essential, essential and other non-common amino acids compositions of protein in chia seeds.

Table(2): Amino acids profile of chia seeds

Nonessenti	ial amino	Essential amino acids		Other amino acids	
acids (gm/	100gm seeds)	(gm/100gm seeds)		(gm/100gm seeds)	
Glu	3.300±0.288	Try	1.858 ± 0.102	Orn	0.053±0.005
Asn	3.264±0.317	Phe	1.148 ± 0.087	GABA	0.039 ± 0.00
Arg	2.550 ± 0.109	Val	0.465±0.034	ß-Ala	0.024±0.00
Asp	1.733±0.133	Lys	0.312±0.025	Cit	0.045±0.002
Gly	1.028 ± 0.091	Leu	0.222 ± 0.011	Tau	0.0087 ± 0.00
Tys	0.665 ± 0.041	Ilu	0.222±0.014		
Ala	0.465±0.034	Met	0.160 ± 0.009		
Ser	0.287±0.016	Thr	0.116 ± 0.007		
Cys	0.258±0.013				
His	0.321±0.019				
Pro	0.115±0.082				

Results are expressed as $(x \pm d)$, being x the average and d, the standard deviation

(n = 3).

The results in Table (2) indicates that nonessential amino acids were present in a various amount, among these amino acids glutamic acid and asparagine were the major and existent in a high level, also arginine present in appropriate level which has the good nutritive value especially for children. On the other hand tryptophan and phenyl alanine showed the highest values of all detected essential amino acids, but the sample had the lowest content of methionine and threonine. Also chia seeds contain traces of non-common amino acids including (ornithine, gamma amino butyric acid, beta-alanine, citruline and taurine) which are the intermediates products remains during protein metabolism. U.S. Department of Agriculture [22] illustrated that glutamic acid and arginine were the most abundance nonessential amino acids found in chia seeds. In contrarily to the results of the current study they reported that chia seeds were characterized by high quantity of leucine with a limited contains of methionine and threonine.

Fatty acid composition of chia seeds

The results of the fatty acids (FA) analysis of chia seeds oil (Figure 1) showed that the chia seeds FAs were highly unsaturated, with their main components being α - linolenic acid (ALA, C18:3n–3; 50.604gm/100gm of the total FA) and linoleic acid (LA, C18:2n–6; 18.215 gm/100gm of the total FA) and fatty acids ranked in the following order of abundance: α -linolenic acid (C18:3) > linoleic acid (C18:2) > palmitic acid (C16:0) > oleic acid (C18:1c)

> <u>elaidic acid</u> (C18:1t) > stearic acid (C18:0).Therefore, the incorporation of chia seed oil into the diet would be very beneficial due to the high content of PUFAs specially essential fatty acids, it have been well documented to provide numerous health benefits and Chia seeds with high in linolenic, a fatty acid which helps the body to absorb fat-soluble vitamins A, D, E and K. Omega-3's work to protect the heart by lowering blood pressure, bad cholesterol, and inflammation [23] and [24].

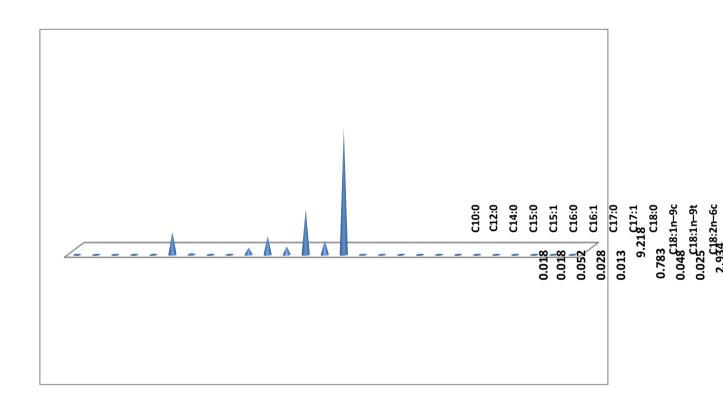


Figure (1) Fatty acids composition of chia seeds oil (Data are expressed as gm/100 gm of total fatty acid).

Although the results were in agreement with findings reported in generative for the studies ([25]; [26] and [22]) that the main FA was α -linolenic acid but lower content of this acid was obtained in the present study. On the other hand, FAs such as pentadecanoic, heptadecanoic acid, arachidonic, docosanoic acid and docosahexaenoic were registered here and not in the aforementioned studies.

There are many factors that may cause variations in the concentration of the fatty acids in chia seeds. One of them is the cultivation area of the plant itself (geographic location of the crop) [22]. According to Ali et al [12], differences in the environment, climate changes, availabilities of nutrient, year of cultivation, or soil conditions play crucial roles in the variation.

Minerals contains of chia seeds

Calcium, magnesium, potassium, sodium, iron, zinc, copper and selenium were detected in the chia seeds, being calcium and magnesium in the highest proportion, which are needed for strong bones and a healthy nervous system as shown in Table (3).

Table (3) Mineral contents of chia seeds

Element	Content	
	(mg/100gm)	
Са	749.42±28.3	
Mg	164.34±6.73	
K	65.73±2.90	
Na	4.58±0.182	
Fe	5.81±0.351	
Zn	3.16±0.129	
Cu	1.834±0.092	
Se	0.206±0.033	

Results are expressed as $(x \pm d)$, being x the average and d, the standard deviation

(n = 3).

These results are consistent with the findings of Capitani et al [27], when they determined the minerals in some chia byproducts and this concentration was higher than that illustrated for hard wheat, soft wheat, millet, rice and sorghum by Ragaee et al [28]. Since chia seeds contain a high level of calcium it is useful to be used as a substitute for milk for people who suffer from allergies against lactose and milk proteins.

The less contains of sodium and the high contains of potassium in chia seeds conferred that this seeds are important to those people with high blood pressure. Other minerals that are found in chia seeds include zinc , iron and copper and selenium, but the values zinc , iron and copper were lower than that of chia meals that reported by [27].

Animal's tests

The result of the present study showed that rats fed chia seeds for 5-weeks gained significantly ($P \le 0.05$) less body weight than control, although the

amount of food intake was significantly greater in that group as shows in Table (4). This behavior's may be attributed to high contains of these seeds of the dietary fiber and its diet palatability.

These results indicates that consuming of the seeds may have an effectiveness in promoting weight loss and altering disease risk factors of overweight people.

Table (4) the food intake and Weight gain of rats

	Food intake (gm /day)	Weight gain (gm)
Control	13.99±0.42 b	43.57±1.04 a
Treatment	14.68±0.33 a	39.37±0.70 b

The rat blood tests in Figure (2) showed that rats fed with chia characterized by significantly ($P \le 0.05$) low blood sugar levels compared to control group, due to high contains of dietary fibers in chia which have a grate capacity to absorb several times its weight of water, which helps provide a feeling of fullness or increase satisfaction after eating, leading to reduces the digestion time of carbohydrates, as well as to decrease the time of nutrient absorption, assisting in the control of blood sugar levels and a more stable release of insulin. ([29]; [30]; [27] and [3]).

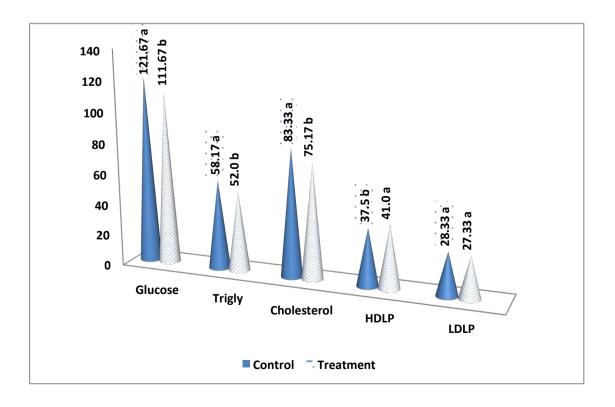


Figure (2) the blood tests of rats

Among rats fed with chia, it was observed that serum triglycerides, totalcholesterol level were significantly lower with non-significantly reduction LDLcholesterol of than in rats of control as shows in Figure (1). This may be attributed to the high polyunsaturated fatty acid content of this seeds principally omega-3 fatty acids [31], since it is known that consumption of these fatty acids may increase nitric oxide synthesis [32]. Nitric oxide decreases serum total cholesterol, triglycerides and LDL-cholesterol levels ([33] and [34]). Furthermore, polyunsaturated fatty acids may decrease the activity of the hepatic enzyme HMG-CoA, which is the major regulatory enzyme for cholesterol biosynthesis [35].

However, HDL-cholesterol was significantly ($P \le 0.05$) higher in the groups fed with chia than in the control-feed group. Previous studies Sanders et al [36] have reported an increase in HDL-cholesterol with polyunsaturated fat consumption. Another factor that may have contributed to the high HDLcholesterol level in these groups is the relatively high arginine contents in the seeds. Arginine is a precursor of nitric oxide which increases HDL-cholesterol concentration in serum ([37] and [38]). Furthermore, Nitric oxide is a beneficial agent in endothelium which may increase HDL-cholesterol concentration in serum [33].

Conclusions and Recommendations:.

In conclusion, the current study showed that chia seed is a good source of protein, oil, fiber, fatty acids such as pentadecanoic, arachidonic and docosahexaenoic were registered in chia oil and also contain more than 50% as α -linolenic acid and more than 18% as linoleic acid and high level of calcium. The results confirm that chia seed is the vegetable source with the highest content of fiber and essential fatty acids. From a physiological point of view, chia seed is a potentially interesting food ingredient due to its health benefits. Also chia seed has a beneficial effect on rats as it caused a significant reduction in serum concentrations of glucose, triglyceride and increased the serum levels of HDL-C. This seed could thus be beneficial for human consumption, for reducing cardiovascular risk, diabetic and osteomalasia.

For all these reasons it is preferable to plant these seeds in the Kurdistan Region and Iraq. It also advises the consumption of these seeds because of their importance to human health.

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