التأثير البيولوجى لبذور الشيا وحب العزيز على أمراض القلب فى الفئران

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المستخلص:

يهدف هذا البحث إلى معرفة القيمة الغذائية لبذور الشيا وحب العزيز وكيفية حمايتهما من التأثيرات الضارة للكادميوم في الفئران المصابة بأمراض القلب. تم استخدام ٤٨ من فئران الألبينو تزن تقريباً ٢٠٠ ± ٥ جرام. بعد أسبوع من التكيف، تم تقسيمهم عشوائياً إلى ٨ مجموعات (٦ فئران) لكل مجموعة. المجموعة الأولى تغذت على الغذاء الأساسي كمجموعة ضابطة سالبة، بينما المجموعات من ٢ إلى ٨ تم حقنها بالكادميوم (٥ ملجم/كجم من وزن الجسم) على شكل كادميوم كلوريد. المجموعة الثانية تغذت على الغذاء الأساسي كمجموعة ضابطة موجبة. المجموعة الثالثة تغذت على الغذاء المدعم بـ ١٠٪ بذور الشيا. المجموعة الرابعة تغذت على الغذاء المدعم بـ ١٠٪ حب العزيز. المجموعة الخامسة تغذت على الغذاء المدعم بـ ٢٠٪ بذور الشيا. المجموعة السادسة تغذت على الغذاء المدعم بـ ٢٠٪ حب العزيز. المجموعة السابعة تغذت على الغذاء المدعم (١٠٪ بذور الشيا + ١٠٪ حب العزيز). المجموعة الثامنة تغذت على الغذاء المدعم (٢٠٪ بذور الشيا + ٢٠٪ حب العزيز) لمدة ٦ أسابيع. أوضحت النتائج أن معدل الزيادة في وزن الجسم زاد بطريقة معنوية في المجموعات التي تغذت على بذور الشيا وحب العزيز مقارنة بالمجموعة الضابطة الموجبة. بالنسبة لإنزيمات الكبد، كان هناك انخفاض ملحوظ في المجموعات التي تغذت على بذور الشيا وحب العزيز مقارنة بالمجموعة الضابطة الموجبة. أيضاً، كان هناك تحسن ملحوظ في وظائف الكلى ومستويات الدهون في المجموعات التي تغذت على بذور الشيا وحب العزيز مقارنة بالمجموعة الموجبة. نتيجة لذلك، توصي الدراسة بأن بذور الشيا وحب العزيز لهما تأثير إيجابي وفعال على أمراض القلب في الفئران.

الكلمات المفتاحية: بذور الشيا – مستويات الدهون – وظائف الكلى – إنزيمات الكبد – حب العزيز.

Biological Effect of Chia Seeds (*Salvia hispanica L.*) and Tiger Nut (*Cyperus esculentus L.*) on Cardiovascular Diseases in Rats

Abstract

This research examined the value of chia seeds and tiger nut in diets and how was it protected rats from the damaging effects of cadmium (Cd) in cardiovascular diseases. Forty-eight albino Sprague-Dawley strain rats, each weighed approximately 200±5 gm, were randomly assigned to eight primary groups. Basal diet has been administered to group 1, which act as the negative control, while groups from (2-8) were induction with cadmium (5mg/ kg/bwt/rat) as Cd chloride. Group 2 positive control (+ve). Group (3-8) were fed on basal diet that was supplemented with 10% chia seeds powder , 10% tiger nut powder , 20% chia seeds 20%, 20%tiger nut, chia seeds 10% +10% tiger nut & chia seeds 20% +20% tiger nut for 6 weeks. Results showed that, the groups have been treated with chia seeds and tiger nut experienced greater gains in body weight than the positive group. When equated with the group serving as the positive control, levels of the enzymes of liver reduced significantly in groups fed on diet supplemented with chia seeds and tiger nut. When rats were given a diet consisting of chia seeds and tiger nut powder levels of creatinine, uric acid and urea show considerable improvements. chia seeds and tiger nut powder had a positive influence on the cardiovascular disease in rats.

Key Words: Chia seeds - Lipid profile - Kidney function - Liver enzyme - Tiger nuts.

Introduction

In developed countries, cardiovascular diseases (CVD) are among the most prevalent causes of mortality. In comparison to high-income regions, the highest proportion of cases dying from cardiovascular diseases is recorded in middle- and low-income regions, with a total of more than 17.5 million deaths annually **(Aparicio *et al*., 2021 & Amini *et al*., 2021).** In the older age group, the phenomenon is most frequently observed; never less, there was an increasing tendency in the most economically productive areas **(Andersson and Vasan, 2018)**. In the coming years, the prevalence of cardiovascular disease may increase because of the population's aging. The frequency of (CVD) is enhanced by the growing incidence of illnesses such as diabetes and obesity, in addition to the conditions that are related to smoking **(Kondo *et al*., 2019).**

The association among socioeconomic status (SES) and (CVRFs) has been demonstrated in numerous investigations, and (CVD) continues to be one of the most prevalent causes of mortality all over the world (**Tao *et al.,* 2024**). Although there have been significant developments in the therapy of cardiovascular disease, it still one of the most frequent reasons of mortality worldwide. Both developed and developing countries continue to suffer from elevated rates of (CVD) mortality and morbidity **(Goswami *et al.,* 2021),** present a significant load on finance, world health, and society **(Timmis *et al.,* 2022; Townsend *et al.,* 2022).**

Cadmium (Cd) is a type of heavy metal that get accumulated in the environment as a result of raising industrial utilization, resulting in health and occupational hazards. Humans are exposed to cadmium through their diet and water, and it involves bioaccumulation, which poses a threat to the well-being of humans **(El-Sharaky *et al*., 2007).** Several organs, such as the kidney heart, and liver are impacted by the accumulation of cadmium in its soluble form specially as cadmium chloride ($CdCl_2$). Cadmium directly induces hyperglycemia and hypertension, leading to harmful effects on gene transcription in the vascular endothelium. This, in turn, affects the cardiovascular system (**Edwards and Prozialeck, 2009&Gallagher and Meliker, 2010).** cd accumulates in the aortic wall, and it also increase the thickness of middle layer of vascular wall and myocardial infarction **(Messner *et al*., 2009 & Abu-Hayyeh *et al*., 2011).** Furthermore, heart has been affected due to direct myocardial structural damage **(Ferramola *et al*., 2012).** Cadmium exerts its harmful effects by generating free radicals and subsequently elevating levels of the reactive oxygen species (ROS), which in turn results in a lowered antioxidant level. The primary events that induce cadmium poisoning are oxidative stress apoptosis, DNA damage and progression of cell cycle **(Kim *et al.*, 2015).** Heart is a vital organ that is especially susceptible to oxidative stress, as it is more susceptible than the majority of remaining tissues. Cardiotoxic impacts of Cadmium are observed at concentration as low as 0.1 mm **(Beyersmann and Hartwig, 2008).**

Chia seeds are the ediable seeds of *salvia hispanica*. Essentially, it belongs to the mint family (Lamiaceae). The surface is characterized by gray coloration with white and black stain. The chia seeds are shaped like an egg. and have a width of approximately two ml (0.08 inches). Mexico, Northern, and Guatemala are the origins of Chia (*Salvia hispanica L.*), which was classified under the labiatae family **(Madaan *et al*., 2020).** Chia seeds are a viable source of phytochemicals that possess kaempferol chlorogenic acid, myricetin, quercetin, & caffeic acid. These compounds were believed to possess anti-cancer and anti-inflammatory characteristics, in addition a protection of liver and heart **(Ullah *et al.*, 2016&Melo *et al*., 2019).** Microchemicals and omega-3 fatty acids are present in small-sized chia seeds, which have immune-boosting properties. It reduces the swelling and sensitivity reactions by means of maintenance **(Lokhande *et al*., 2019).**

Among plant sources, chia seeds are known to have the greatest known concentration of α-linolenic acid (ALA) **(Ayerza and Coates, 2011).** A crucial fatty acid that is linked to specific physiological functions is (ALA). Per 100 grams, chia seeds contain as much as thirty-four to forty g of dietary fiber. This amount of dietary fiber is equivalent to the daily requirement of the American Dietetic Association and the EFSA following the consumption of approximately sixty- three to seventy- four grams of seeds (**Slavin, 2008).** Moreover, chia seeds are a high-quality protein source, containing nineteen to twenty-three grams of protein per 100 grams of seeds, which have protein content higher than most seeds that are usually consumed **(Sandoval-Oliveros & Paredes-López, 2013)**. Additionally, the nutritional value of the seed is improved by the presence of minerals like magnesium, potassium, and calcium, additionally to antioxidant compound and vitamins **(Marcinek and Krejpcio, 2017).**

The human body is aided in combating a variety of chronic diseases by the functional components found in agricultural foods, which is the reason why their growth is on the rise. Currently, chia seeds, which are edible seeds of the plant *salvia hispanica*, belong to the mint family. These seeds are made up of a variety of functional components, such as peptides, omega 3 fatty acid, polyphenol, anti-oxidant, fiber, mineral and vitamin. In addition, these seeds are an exceptional source of vegetable protein, ash, unsaturated fat, and carbohydrates. Chia seed components are beneficial in the therapy of cardiovascular illness by lowering cholesterol, platelet accumulation, oxidation & blood pressure, Nevertheless, pancreatic beta cells are protected from inflammation by polyphenols and antioxidants. These components offer protection against the damage of cell of a variety of body parts, which could be beneficial in the therapy of a many types of cancers, such as pancreatic, liver, colorectal, and breast **(Waseem *et al*., 2023).**

A sweet nut-like tuber known as the tiger nut (*Cyperus esculentus L*.) is edible and is most frequently found in Chile, Nigeria, Senegal, Ghana, and Spain **(De Castro *et al*., 2015 & Omeje *et al*., 2022).** It is available in three colors: yellow, brown, and black. Ghana is characterized by the prevalence of the black variety **(Asante *et al*., 2014).** It is grown for the consumption of both humans and livestock, and it is consumed either raw or baked, resulting in a variety of identities **(Bamishaiye *et al*., 2010)**. It is a crop that is sometimes regarded as a weed, despite its high potential for development, and is underutilized **(Roselló-Soto *et al*., 2018).** Tubers are a crucial part of the diets of the people of West Africa due to their affordability, year-round availability, and nutritional advantages **(Adejuyitan *et al*., 2009).**

Tiger nut contains a variety of nutrients that can be extensively examined. There are twenty- two point fourteen to forty- four-point ninety-two hundred percent lipids, three point twenty-eight to eight point forty- five hundred percent proteins, twenty- three point twenty-one-to-forty-eight-point twelve hundred percent carbohydrates, eight point twenty-six-to-fifteen-point forty-seven percent fibers, one point sixty-to-two-point sixty percent ashes in them **(Adel *et al*., 2015).** Furthermore, it is composed of substances that are bio active, including phenols, alkaloids, and organic acids **(Nina *et al*., 2019).** Edible oils that have high concentration of monounsaturated fatty acids are present in the tiger nut. The nutritional value of olive oil is comparable to that of tiger nut oil **(Rosselló-Soto *et al*.,2018).** Additionally, it is abundant in starch, a food ingredient that is both low cost and renewable (**Santos-Silveira and Francisco, 2020)**

Various oxidative stress-related signaling pathways could be regulated by oleaginous bean polysaccharide, which has been illustrated to prevent the production of free radicals, increase the antioxidant enzymes activity , and enhance capacity of antioxidant **(Hernández-Olivas *et al*., 2022).** The exceptional physiological activity of tiger nuts has also made them a popular addition to the human diet. The pleasant milk drink "horchata" is produced in Spain and other Mediterranean countries by crushing and rehydrating tubers **(Selma-Royo *et al.*, 2022).** They are appropriate for fermented milk drinks and snacks due to their sweetness and flavor, which promote the development of probiotics and decrease the duration of fermentation**(Kizzie-Hayford *et al.*, 2021).** The nutritional, rheological, culinary, and structural properties of egg pasta have been enhanced by the application of durum wheat semolina as an auxiliary. This has been documented **(Martín-Esparza *et al*., 2018&Martín-Esparza *et al.*, 2021).** The presented study attempts to investigate the healthy advantages of chia seeds and tiger nut in diets and how was it protected rats from cardiovascular diseases.

Materials and Methods

Materials:

Chia seeds and tiger nuts:

Dried tiger nuts and chia seeds were received From Harraz Comp., for Agriculture., Herbs, Seeds, and Medicinal Plants, Cairo, Egypt.

Diet and rats:

Laboratory Food Technology Research Institute, Agricultural Research Center Ministry of Agriculture and Population, Egypt, procured adult Albino rats of the Sprague Dawley strain, weighing 200 ± 5gm. At the El-Gomhouria Comp. for Trad. Chemicals and Drugs in Cairo, Egypt, all components of the basal diet (mineral constituents, choline chloride, D-L methionine, cellulose, vitamin and casein) have been obtained. In Cairo, Egypt, the local market was the source of soybean oil, maize starch, and sucrose.

Kits and chemicals for biochemical analysis:

All compounds utilized, including cadmium chloride, have been obtained from the El-Gomhouria Comp. for Trad. Chemicals & Drugs in Cairo, Egypt. Sigma-Aldrich Chemie GmbH, Egypt, supplied reagents for the biochemical analysis of total cholesterol, total lipids, & total glycerides in addition to liver functions (AST and ALT), kidney functions (BUN, Cr, & UA) & GSH and MDA.

Methods:

Preparation of powder and chia seeds and tiger nuts:

Chia seeds and dried tiger nut were cleaned and pulverized in a blender to obtain a fine powder.

Preparation basal diet with chia seeds and tiger nuts:

During the experimental period, the standard AIN-93 M basal diet has been provided to cover the requirement of nutrient of the rats, which has been prepared in accordance with the approved amounts of **Reeves *et***

***al*., (1993**). Chia seeds and tiger nuts have been incorporated into the basal diet at a rate of ten percent and fifteen percent, respectively, to create a complementary diet.

Route and doses of administration for induction of cardiovascular diseases:

Renugadevi and Prabu, (2010) described the methods for injecting 5 ml/kg of dissolved cadmium in saline at a ratio of 1: 1 (V: V) intraperitoneally (IP) every week to all the groups of rats, with the exception of the -ve control group (normal rats), in order to cause cardiovascular cell injury during the six-week experiment. Normal rats received IP injections of saline alone at similar doses.

Determination of nutritive value:

The several techniques established by **A.O.A.C**. **(2010)** have been utilized to assess the proximate composition of tiger nuts and chia seeds.

Biological experiment:

After an adaptation period, forty - eight Sprague-Dawley albino rats, weighing 200 ±5 g was randomly assigned into 2 groups (6 rats). The first group being nourished the basal diet as the negative control (-ve), and the second group 42 rats being subjected to cadmium (10mg/kg body weight) as Cd chloride ($CdCl_2$) and divided into 7 groups. Group two positive control (+ve). Group 3 fed on basal diet supplemented with10% chia seeds. Group 4 fed on basal diet addition to tiger nut 10%. Group 5 fed on basal diet supplemented with chia seeds 20%. Group 6 fed on basal diet supplemented with tiger nut 20%.%. Group 7 fed on basal diet supplemented with chia seeds 10% +10% tiger nut. Group 8 fed on basal diet supplemented with chia seeds 20% +20% tiger nut for 6 weeks.

The animals have been accommodated in the Agricultural Research Center's Animal House, Egypt, Giza, at a moderate humidity of 56% & a temperature of 25±2°C, under hygienic conditions. *Ad libitum* access to basic diet & water was provided.

Assessment of feed intake and body weight gain:

Depending on the total daily feed consumption of each group, Total feed consumption for each rat was determined. To assess the alteration in body weight, the rats' body weight was recorded at onset of the experimental period (IBW) & at the end of the experimental period (FBW). **Kratochvílova *et al*., (2002)** provided the following equations for estimating body weight gain (BWG) in that case.

$$BWG = FBW (g) - IBW (g)$$

The collection of blood samples:

All rats were forbidden from consuming food for approximately 12 hours following the conclusion of the experimental interval (six weeks). Each animal was subsequently sacrificed after being anaesthetized with diethyl ether. Blood was drawn into clean, dried centrifugation tubes using a 5 ml syringe at the time of heart puncture. The collected blood samples were allowed to clot at ambient temperature. Subsequently, serum has been separated by centrifugation at three thousand round per minute for fifteen minutes. Subsequently, the clear serum samples were transferred to a sealed Eppendorf pipe and stored in a deep freeze at minus twenty Celsius degree until they were required for biochemical examination

Biochemical Analysis:

The levels of alanine aminotransferase (ALT) & aspartate aminotransferase (AST) were calculated by utilizing the methods defined by **(Thomas, 1998). Henry (1974)** described the procedure used to measure serum creatinine (Cr). **Fossati *et al*., (1980)** provided the methodology used to measure serum uric acid. **Patton and Crouch, 1977** provided the methodology used to measure serum urea nitrogen. Both malondialdehyde (MDA) and glutathione levels in the blood were tested using established protocols (**Sinha, 1972 & Draper and Hadly, 1990), respectively. Meiattini, (1978), Fossati and Praneipe (1982), and Young (2001)** were used to calculate total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c). Very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) have been estimated as described by **Friadwald *et al*., (1972)** equations:

VLDL-c (mg/dl) = (Triglycerides /5)

LDL-c (mg/dl) =TC - (HDL-c + VLDL-c).

Atherogenic Index (AI) was estimated according to **Omonkhua *et al*., (2013)** as follow:

Atherogenic index (AI) = LDL-cholesterol/HDL-cholesterol

Statistical analysis:

SPSS application was used to examine the data. To determine whether there were statistically noteworthy variations among the groups, an ANOVA was performed **(SPSS, 1986).**

Animal ethics approval:

The experimental procedure for this research was permitted by the Institutional Animal Care & Utilization Committee (ARC-IACUC) at the Agricultural Research Center in Egypt (ARC /FHE/88/ 24) for animal care & experimentation.

Results and Discussion

The Chemical constitution of chia seeds and tiger nuts:

Table (1): Chemical composition of chia seeds and tiger nut (g per 100 g)

Nutrients		Chia Seeds	Tiger Nut
Nutrients (g)	**Proteins**	17.9	9.50
	Fats	32	36.41
	Carbohydrate	40.1	45.18
	Fiber	30.9	9.51
	Ash	4.5	5.20
Vitamins (mg)	**Vitamin E**	1.8	40.9
	Vitamin C	0.5	33.5
	Vitamin B_2	-	8.7
	Vitamin A	-	50.9
Minerals (mg)	**Zinc**	4.3	0.09
	Iron	7.5	0.60
	Potassium	410	240
	Magnesium	345	60.30
	Calcium	620	140
	Phosphorus	870	121
	Copper	0.9	0.01
	Sodium	10	235

Nutrition value of chia seeds and tiger nut is shown in Table (1). Results indicates that, seeds of chia rich in carbohydrates, fats, fiber and protein, vitamin E, vitamin C, phosphorus, calcium, magnesium, zinc and potassium. Additionally, it contains reasonable amount of ash, zinc, iron, sodium. However, low in copper, vitamin C, vitamin E concentration. Outcomes exposed that, tiger nut is an adequate source of carbohydrates, fats, vitamin C, vitamin E, Vitamin A**,** calcium, potassium, phosphorus, sodium, but low in fiber, protein, Vitamin B_2, zinc, iron & copper.

Although chia seeds are an exceptional source of a variety of essential minerals, they are an inadequate source of many vitamins.

calcium, phosphorus, copper, selenium, iron, magnesium, and manganese are abundant in these. Minerals, like vitamins, are essential to preserve health and facilitate the body's growth & development. The body utilizes minerals to perform a variety of functions, including the formation of strong bones and the transmission of impulses from neurons. Nevertheless, various minerals are utilized to regulate the heartbeat or synthesize hormones. **(Bailey *et al*., 2011).** Chia seeds are an exceptional source of a variety of essential minerals, but they aren't a reliable source of vitamins. These contain elevated concentrations of iron, calcium, phosphorus, manganese, selenium, copper, & magnesium. **(Da Silva *et al*., 2016**). Manganese, which is important for the process of ingestion, digestion, maturation, and progression, is abundant in whole grains and meals. **(Aschner and Dorman, 2006).** In general, it is found in foods that are great in protein, such as chia seeds. The health of bones and the maintenance of tissues are both enhanced by phosphorus. **(Takeda *et al*., 2012).**

Due to its elevated concentration of dietary fibers, seeds have been utilized to improve blood glucose and lipid profiles and facilitate weight loss **(Toscano *et al*.,2015).** They are abundant in antioxidants, minerals, fatty acids, and proteins that are beneficial for the heart. **(Bays *et al*., 2022).** The primary reason for the increased nutritional content of chia seeds is predominantly due to their high concentration of mineral, high quality protein, dietary fiber and polyunsaturated fats **(Timilsena *et al*., 2016).** The insoluble portion comprise eighty-five to ninety-three percent of the 30-34 g of dietary fiber in chia seeds, while the soluble fraction accounts for (7to 15) percent. More dietary fiber is provided by chia seeds than by dried fruits and nuts. In its fatty acid composition, it contains an elevated concentration of polyunsaturated fatty acids, with ALA being particularly prevalent. It is also rich in plant protein. **(Grancieri *et al*., 2019).**

Results according to **Naeem and Youssef, (2022)** the raw tiger nut presented a higher carbohydrate content (53.72%), followed by fat content (23.4%), and moderate protein and crude fiber content (7.78% and 7.5%). Ash content was the lowest at 2.4%. **Sabah *et al*., (2019),** discovered that tiger nut tubers contained the greatest proportion of oil (30.01%) and carbohydrates (45.73%). Further, tiger nut tubers contained five-point eight percent protein, two point twenty- three percent ash, and fourteen-point eighty percent crude fiber. **El-Naggar, (2016)** studied that the fiber content was 6.5% and this result was similar to the results of this study's measurements. **Ayat and Akram, (2021)** reported that the chemical composition of raw tuber tiger nuts was 6.00% protein, 1.70%

ash, 4.30% crude fiber, and 58.23% total carbohydrates. Concerning minerals contents of the raw tiger nut, the results indicated that K showed the high amounts (262.10 mg/100g), followed by Ca (244.06 mg/100g), Na (163.24 mg/100g), then P (160.04 mg/100g) and finally Mg (101.01 mg/100g). However, the fewest value was that of Fe (3.31 mg/100g).

Table (2): Effect of chia seeds and tiger nut on body weight gain, feed intake & feed efficiency ratio on cardiovascular diseases in rats.

Parameters / Groups	**Body weight Gain** (%)	**Feed intake** (g/day/rat)	**Feed efficiency ratio**
Control (-Ve)	126.00±5.43[a]	9.97 ±0.39[a, b]	12.62 ±0.42[c]
Control (+Ve)	104.80±17.95[f]	6.20 ±0.53[c]	9.68 ±2.02[d, e]
Chia seeds (ten percent)	120.20±11.52[b]	9.91 ±0.46[a, b]	12.12 ±0.85[c]
Tiger nut (ten percent)	120.40±4.82[b]	10.88 ±0.55[a]	19.54 ±2.38[a]
Chia seeds (20%)	116.20±8.58[c]	10.74 ±0.40[a]	10.67 ±0.71[d]
Tiger nut (20%)	125.20±11.12[a, b]	8.83 ±0.57[b]	14.24 ±1.80[b]
Chia seeds (10%) + Tiger nut (ten percent)	112.80±15.43[d]	8.92 ±0.69[b]	12.80 ±2.69[c]
Chia seeds (20%) +Tiger nut (20%)	109.00±16.73[e]	8.06 ±0.38[b, c]	13.59 ±2.52[b, c]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

In Table (2), data demonstrated that the body weight gain percentage for the positive control group was significantly lower (P<0.05) than that of the negative control group (104.80±17.95% vs 126.00±5.43%). In comparison to the +control group, the BWG% of all groups that have been fed diets provided with chia seeds and tiger nut raised significantly. The group of rats that were treated with tiger nuts at a 20% level presented the greatest BWG %, with a mean value of 125.20±11.12%.

The outcomes demonstrated that the FI of the groups that were fed the examined materials at the various supplemented levels increased. The (+ve) control group demonstrated a decline in FI compared to the (-ve) control group. The mean value of FER was 9.68 ±2.02 in the (+ve) control group and 12.62 ±0.42 in the (-ve) control group, respectively, indicating a significant reduction. The FER of the groups that were fed on all of the tested materials didn't differ significantly from that of the negative control group, with the exception of the group of rats that were treated with tiger nut at a 20% concentration.

This result disagrees with **Da Silva *et al*., (2016)** who discovered that chia seeds decreased body weight compared to the group of animals that are fed the standard control diet. As well, these outcomes are in compliance with research by **Creus *et al*., (2016)** who found three months of chia eating led to a considerable increase in rat body weight. Also, these data are harmonious with **Montes Chani *et al*., (2018)** they confirmed that long-term (13-month) ingestion of black chia causes a considerable rise in rat body weight. According to **Da Silva *et al*., (2016)** discovered that the rats weight gain could be attributed to the reduced digestion of chia protein, among other causes

Table (3): Effect of chia seeds and tiger nut on serum liver enzymes on cardiovascular diseases in rats.

Parameters / Groups	ALT	AST
	(U/L)	
Control (-Ve)	28.29±2.81[e]	33.86±1.46[d]
Control (+Ve)	76.43±1.71[a]	103.71±5.99[a]
Chia seeds (10%)	34.71±1.38[c, d]	40.71±3.90[c]
Tiger nut (10%)	35.43±3.04[c]	45.86±3.71[b]
Chia seeds (20%)	33.29±3.14[d]	38.29±2.69[c, d]
Tiger nut (20%)	37.57±2.63[c]	41.29±4.88[c]
Chia seeds (10%) + Tiger nut (10%)	36.43±2.63[c]	39.71±2.69[c]
Chia seeds (20%) +Tiger nut (20%)	41.29±1.79[b]	45.00±2.16[b]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

The activities of serum AST and ALT were significantly raised in rats with (CVD) diseases (Table 3). In comparison to the +ve group, the aspartate aminotransferase and serum alanine aminotransferase levels were significantly reduced (P<0.05) when the cardiovascular diseases group was fed chia seeds and tiger nut separately or in combination. Nevertheless, the rats that have been fed chia seeds at a twenty percent concentration demonstrated the greatest decrease in liver enzymes in comparison to the other group.

The level of liver function enzymes (ALT, AST and total bilirubin) was significantly increased as a result of cadmium intoxication, while the concentration of albumin & total proteins was reduced. In general, hepatic function enzymes are considered to be reliable indicators of liver function (**Nili-Ahmadabadi *et al*., 2018).** The hepatic tissues' disordered state and concurrent hepatic function are demonstrated by the elevated levels of these enzymes **(Kandemir *et al*., 2020).** Function of cell organelles (endoplasmic reticulum and mitochondria) and DNA is impacted by the over-production of reactive oxygen species, which subsequently impacts protein formation. necrotic lesion in the liver tissues and the hepatic functions that result from the aforementioned activities lead to a decline in the concentrations of total proteins & albumin. **(Dwivedi *et al*., 2015).** Additionally, Hepatocytes plasma membranes are impaired as a consequence of exposure to cadmium., which results in the excessive release of AST, ALP & ALT into the bloodstream. This is due to lipid peroxidation in cells **(Abdeen *et al*., 2017 & Nagai *et al*., 2016).**

Marineli *et al*., (2015) stated that the consumption of white chia seeds was effective in lowering aspartate aminotransferase levels, which is consistent with these findings. Additionally enhances liver damage. Also, these findings are consistent with the findings of in comparison to the rats that were fed chia seed, **Alamri, (2019)** discovered that the concentration AST & ALT were significantly greater in the positive & negative control groups**.** The hepatoprotective effects of chia may be linked to its great concentration of phenolic compounds, fiber, protein & α linolenic acid (omega-3), regarding **Fernandez-Martinez *et al*., (2019)**.

Table (4): Effect of chia seeds and tiger nut on serum kidney functions on cardiovascular diseases in rats.

Parameters / Groups	Urea	Creatinine	Uric acid
	mg/dl		
Control (-Ve)	34.00 ±2.82^{c}	0.71 ± 0.27^{b}	4.23±0.26^{b}
Control (+Ve)	41.14 ± 3.43^{a}	1.20 ± 0.19^{a}	5.54±0.35^{a}
Chia seeds (10%)	37.14 ± 2.34^{b}	0 .78± 0.04^{b}	4.27±0.28^{b}
Tiger nut (10%)	35.57 ±3.30^{b}	0.78 ± 0.09^{b}	4.14±0.23^{b}
Chia seeds (20%)	34.43 ± 2.87$^{b, c}$	0.75 ±0.06^{b}	4.69±0.21^{b}
Tiger nut (20%)	32.57 ±2.22^{c}	0.80 ±0.04^{b}	4.14±0.45^{b}
Chia seeds (10%) + Tiger nut (10%)	36.43 ± 2.76^{b}	0.79 ± 0.06^{b}	4.33±0.40^{b}
Chia seeds (20%) +Tiger nut (20%)	35.29 ± 4.30^{b}	0.75 ±0.07^{b}	4.07 ± 0.30^{b}

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Table (4) demonstrated effect that chia seeds and tiger nut on kidney functioning as measured by serum concentrations of (urea nitrogen, creatinine, & uric acid). Rats injected with CVD reported an increase in serum urea nitrogen concentration, with a mean value of 41.14±3.43 mg/dl. This was compared to the (–ve) control group, which had level that was 34.00±2.82 mg/dl. Groups of rats which treated with chia seeds and tiger nut had a reduction in urea concentration in comparison with the positive control group.

Table (4) relative to the negative control group, which had a creatinine concentration that was 0.71±0.27 mg/dl had reduction compare to (+ve) group, which had been exposed to cadmium, with an average value of 1.20±0.19 mg/dl that is higher than that of the normal control group. In comparison to the (+ve) control group, the groups of rats who have been nourished on chia seeds and tiger nut at whatever amount of intake demonstrated a lower concentration of creatinine in their serum.

In addition, Table (4) exhibited the reduction of uric acid in tested samples compared with positive control group. Compared to control group, rats that supplemented their diet with chia seeds and tiger nut exhibited lower serum uric acid activity at all levels. Group 8 administered chia seeds and tiger nut in combination at level 20% had the lowest serum uric acid activity, with an average value of 4.04±0.30 mg/dl.

Our results are in agreement with **Mostafa, (2021)** who found that indicated that the management with chia seeds at varying concentrations led to a significant reduction in urea, creatinine & uric acid levels in comparison with the (+ve) control group. Additionally, **Fayez *et al*., 2014** stated that Omega-three fatty acids exhibit a prophylactic effect against renal dysfunction in rats.

Table (5): Effect of chia seeds and tiger nut on serum lipid profile on cardiovascular diseases in rats.

Parameters / Groups	TC	TG	HDL-c	LDL-c	VLDL-c
	(mg/dl)				
Control (-Ve)	120.43±7.45[e]	114.43±7.39[d]	40.14±2.41[a]	71.37±19.27[b]	22.89±1.47[d]
Control (+Ve)	232.14±6.81[a]	194.71±4.88[a]	32.29±1.79[d]	158.91±13.07[a]	38.94±0.97[a]
Chia seeds (10%)	131.43±3.59[c]	118.29±3.200[c]	40.43±3.95[a]	63.77±7.01[d]	23.66±0.64[c]
Tiger nut (10%)	127.57±5.22[d]	120.00±3.559[c]	35.29±2.05[c]	68.29±5.97[c]	24.00±0.71[c]
Chia seeds (20%)	129.14±2.41[d]	119.29±3.03[c]	39.71±3.09[a]	65.57±2.96[c, d]	23.86±0.60[c]

Tiger nut (20%)	131.86±4.10[c]	136.71±6.99[b]	34.86±3.23[c]	69.66±4.49[c]	27.34±1.39[b]
Chia seeds (10%) + Tiger nut (10%)	132.86±4.63[c]	114.14±3.97[d]	38.86±2.41[b]	71.17±6.91[b]	22.83±0.79[d]
Chia seeds (20%) + Tiger nut (20%)	137.00±11.0[b]	117.43±3.50[c]	38.14±2.11[b]	71.60±9.37[b]	25.83±2.40[b,c]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

The level of TC, TG, LDL-C, & VLDL-C in the (+ve) control group demonstrates a significant elevation as illustrated in table (5). Nevertheless, the serum HDL-C level has been significantly reduced compared to the control normal group. The mean values of the serum lipid profile were significantly reduced in diets that were mixed with tiger nut and chia seeds, either separately or in combination. Nevertheless, the serum HDL-C level was significantly elevated in comparison to the other tested group, the rats that have been fed either the 2 analyzed samples separately or the mixture of chia seeds and tiger nuts showed the most beneficial impact on their lipid profile.

(Silva *et al*., 2016) additionally assessed the impact of chia seed consumption on lipid and carbohydrate metabolism. The researchers discovered that the blood concentrations of VLDL, TG, TC & LDL were lower in the groups of animals that received chia seeds & flour than in the control group. In addition, the concentration of high-density lipoproteins has been observed to be higher.

These results are conformed with **Toscano *et al***., **(2015)** stated that overweight and obese people that expended 35 g of chia flour every day had their total cholesterol and their HDL-c levels rise (34) while VLDL-c levels decreased. These are conformed with results **Da Silva *et al*., (2016)** reported that wistar rats that received nutrition both conventional and thermally treated chia exhibited a higher
HDL-c levels, as well as greater intestinal muscle layer hypertrophy and protein digestibility.

The effect of chia seed oil consumption on the blood lipid profile was investigate an experiment performed by (**Sierra *et al*., 2015).** They demonstrated that the consumption of feed containing ten percent chia seed oil resulted in a decrease in TC, TG. Additionally, the concentration of LDL elevated. Nevertheless, it is essential to state that statistically significant variables were only observed in the triglesrides group.

The development of TG can be reduced by chia seeds, which have essential substances for example protein, omega-3 fatty acid high & fiber. In certain investigations, its advantages were revealed; nonetheless, the results are ambiguous **(Enes *et al*., 2020)**. many investigations have illustrated that chia seeds can reduce certain risk factors, such as insulin resistance stomach fat, and fatty oils, through experimentation and observation. Additionally, they have the capacity to enhance the amount of "great" HDL cholesterol. In addition to being an exceptional source of omega-3 fatty acids, they are also rich in antioxidants & supply iron, calcium & fiber **(Ayerza and Coates, 2007).** Chia seed oil supplementation altered the lipid profile of the adipose tissue and liver, leading to a decrease of hepatic cholesterol leptin and triglycerides **(Citelli *et al*., 2016).**

The consumption of chia seeds has been demonstrated to lower serum cholesterol levels because of the high concentrations of unsaturated omega-3 fatty acids and dietary fiber **(Megha *et al*., 2022).** The therapy of dyslipidemia, a metabolic disorder marked by an abnormally elevated cholesterol concentration in the blood, has been reported to be effective with chia seeds. It was recently illustrated that chia bioactive peptides can prevent production of critical indicators of cholesterol, including 3-hydroxy-3methylglutaryl coenzyme A reductase. **(Coelho *et al.*, 2018).**

Omega-3 fatty acids are abundant in chia seeds. It contains essential fatty acids, like alpha-linolenic acid and oelic acids. These essential fatty acids are changed to DHA & EPA in the human body following their ingestion. **(Jin *et al.,* 2012).** The level of high-density lipoprotein cholesterol, which is the "acceptable" cholesterol that protects against strokes & coronary failures, can be elevated by omega-3 unsaturated fatty acids. Research has demonstrated that chia seeds, especially chia seeds that have been grinded, can increase blood concentration of eicosapentaenoic acid & alpha-linolenic acid (**Nieman *et al*., 2012).**

Table (6): Effect of chia seeds and tiger nut on serum malondialdehyde and glutathione on cardiovascular diseases in rats.

Parameters / Groups	MDA	GSH
	(ng/ml)	
Control (-Ve)	63.00±6.37[c]	4.33±0.55[a]
Control (+Ve)	144.29±6.92[a]	2.60±0.46[c]
Chia seeds (10%)	69.86±2.91	4.87±0.37[a]
Tiger nut (10%)	75.14±3.28[b]	3.90±0.39[b]
Chia seeds (20%)	73.57±2.07[b]	4.89±0.38[a]

Tiger nut (20%)	74.43±2.99[b]	3.59±0.47[b]
Chia seeds (10%) + Tiger nut (10%)	74.43±3.59[b]	3.89±0.68[b]
Chia seeds (20%) +Tiger nut (20%)	70.57±2.99[b]	4.57±0.43[a]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Outcomes in Table (6) explain the effect that varying doses of chia seeds and tiger nut had on serum levels of antioxidant activity like malonaldehyde & glutathione. Results indicate that there has been a significant elevation the concentration of serum glutathione in the negative control group in comparison with to the positive control group, with a mean value of 4.33±0.55 (ng/ml) and 2.60±0.46 (ng/ml). On one hand, the results showed that the malondahyde concentrations in serum increased substantially when contrasted with the (+ve) control. Conversely, the findings show that the level of malondialdehyde was much higher in the group that served as the positive control as compared to the healthy group. When compared with the group serving as the (+ve) control, the serum malondialdehyde levels in groups fed tested samples were shown to have reduced significantly. Additionally, the greatest value of MDA was found in group 8, which was fed on 20% tiger nut +20% chia seeds.

Marineli *et al*., (2015) carried out an experiment in which obese rats have been nourished chia seed oil or chia seeds at 133 & forty grams/kilogram diets, correspondingly, for six or twelve weeks. In contrast to the group of animals that were fed a high-fructose diet without chia supplementation, the activity of antioxidant enzymes in blood, including catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) elevated statistically significantly among animals that consumed oil or seeds. Following the ingestion of both chia oil and chia seeds in the same investigation, a reduction in blood concentrations of lipid peroxidation biomarkers, such as malondialdehyde was detected.

Furthermore, **Amelia *et al*., (2020)** it was discovered that vitamins such as E and C flavonoids, and polyphenols have significant free radical clearance molecules and antioxidant properties. **Ademosun and Oboh, (2015)** in vitro, the rat pancreas demonstrated the ability to scavenge radicals and the inhibition of lipid peroxidation and malondialdehyde formation. Furthermore, previous research indicated that the tiger nut may be beneficial in the decrease inflammation & oxidative stress in the liver **(Achoribo and Ong, 2017).** **Iboyi *et al*., (2021)** established that tiger nuts are beneficial in human body protection from a variety of disorders due to their elevated levels of oleic acid, antioxidants & vitamin

E. **Ezeh *et al*., (2014)** show that tiger nuts were also identified as containing phenolic compounds, as well as minerals like magnesium, calcium, vitamins C and E and potassium. Furthermore, **Ogunlade *et al*., (1997**) indicated that tiger nuts have protective nutrients due to their adequate levels of magnesium, copper, zinc, and iron, as well as vitamins E and C. Immunity is significantly influenced by zinc. Copper is involved in the metabolism of iron and interacts with numerous antioxidant enzymes, especially those that are involved in protein metabolism. Vitamin E, as reported by **Szymańska *et al*., (2020)** it is a potent antioxidant that is responsible for the decrease of lipid peroxidation, a process that can result in the destruction of DNA, proteins, and cell membranes in the body.

Table (7): Effect of chia seeds and tiger nut on atherogenic index on cardiovascular diseases in rats.

Parameters / Groups	**AI (mg/dl)**
Control (-Ve)	1.78±0.18[b]
Control (+Ve)	4.92±0.08[a]
Chia seeds (10%)	1.67±0.07[b]
Tiger nut (10%)	1.93±0.12[b]
Chia seeds (20%)	1.65±0.07[b]
Tiger nut (20%)	1.99±0.07[b]
Chia seeds (10%) + Tiger nut (10%)	1.83 ±0.17[b]
Chia seeds (20%) +Tiger nut (20%)	1.87±0 .19[b]

*Values are expressed as means ±SE.
*Values at the same column with different letters are significantly different at P<0.05.

Data presented in table seven impact of chia seeds & tiger nut as powder on atherogenic index (AI) levels of cardiovascular diseases rats. It may be detected that the average value of AI in the control positive group is higher than that in the control negative group 4.92±0.08 & 1.78±0.18 mg/dl, respectively, presenting significant distinctions among them. All groups of rats fed on chia seeds powder and tiger nut exposed significant reduction in average values comparing to control positive group. Numerically Group 5 received the most beneficial therapy. (chia seeds 20%) considering serum (AI).

Research has demonstrated that the atherogenic index is a reliable indicator of the risk of CVD and an effective method for monitoring the efficacy of lipid-lowering therapies. This is due to the fact that the low-density lipoprotein /high density lipoprotein ratio is considered to be more prognostic than either low density lipoprotein or high-density lipoprotein alone **(Onunogbo *et al*., 2012).**

Conclusion:

In summary, our findings indicated that the feeding of chia seeds and tiger nut powder to experimental animals resulted in a significant increase in HDL-c and body weight gain. However, the antioxidant enzyme levels and serum lipid profile had a significant ($P \leq 0.05$) reduction. Additionally, a highly significant ($p \leq 0.05$) enhancement in kidney functions and liver enzymes has been detected in compared to the positive control group, which is indicative of the potent therapeutic effect of consuming tiger nut powder and chia seeds for the therapy of CVD in rats.

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