Efficacy of Chia Seeds on Eliminating Repeatedly Heated Oil Hazards of Male Rats

Haggag M. Hamdy, Maysa M. El-Mallah, Amal M. Ebeed

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Cairo, Egypt.

Abstract

This research aimed to study the efficacy of chia seeds powder (CSP) on eliminating repeatedly heated oil hazards of male rats. Chemical constituents, fatty acid contents, total phenolic, total flavonoid and phenolic components of chia seeds were estimated. Thirty-five male albino rats classified into 5 groups. Group 1 fed on basal diet for 8 weeks. Group 2 served as +ve control and fed basal diet containing 4% repeatedly heated sunflower oil in basal diet constituents for 8 weeks. Groups 3-5 as the same of group 2 and fed 2, 4 and 6% chia seeds powder, respectively. Feed intake, body weight gain and feed efficiency ratio were recorded. Serum liver functions, lipid fraction, kidney functions, malondialdehyde and catalase were determined. Results indicated that CSP contained high amounts of fat, fibers, protein, linolenic acid, γ-linolenic acid, total phenols, total flavonoids and quercetin. Body weight % of 5th group was higher among all groups followed by 3^{ed} group. Whereas, feed efficiency ratio of 3^{ed} group had the highest mean values followed by 5th group. Remarkable improvement was observed in all groups fed CSP of all tested biochemical parameters. Rats fed 6% CSP showed significant improvement compared with +ve and other tested groups followed by group 4 that fed 4% CSP. According to all above findings CSP may be beneficial in the reduction of harmful side effects of re-used oil. So CSP may be useful for human beings.

Key Words

Chia seeds, re-used oil, liver and kidney functions, lipid fraction, oxidative and anti-oxidative markers

Introduction

Oils are one of the main components of a balanced diet in human health and its proper usage will protect and prevents against certain diseases. The edible oils are a source of energy. Oil contains essential fatty acids (FAs) and it acts as a carrier of fat-soluble vitamins**.** Last few decades, fast food consumption has been increasing throughout the world, because of its low cost, desirable taste, convenience and quick preparation methods. Definitely, the consumption of fast food is directly associated with cardio metabolic risk factors and the reason that fast foods are usually prepared by deep-frying that leads to a production of number of toxic and harmful substances in the food **Ghobadi *et al*., (2018).**

In the last days, fast food consumption has become increasingly popular throughout the world. Low cost, desirable taste, convenience, and quick preparation are among reasons that people show tendency towards fast food consumption. Fast foods are generally prepared from processed meats, which have low nutrient content and high in energy, total fat, trans fatty acids, and salt. Accordingly, consumption of fast foods is positively associated with cardiometabolic risk factors. In addition to unhealthy components, fast foods are usually prepared by deep-frying, a cooking technique during which a number of toxic and harmful substances are produced in the food **Altamimi *et al.*, (2022).**

The prolong use of edible oil for frying repeatedly leads to the formation of TPC (Total Polar Compound) that reduces the quality of the oil and become unhealthy for human consumption. Considerably, the nutritional and physicochemical properties of cooking oils are affected due to reheating. There is also a chance of occurrence of numerous chemical reactions such as oxidation, hydrolysis, and polymerization that lead to production of oxidized fats, Trans fatty acids, hydrolyzed fats, sterol derivatives, polymers, polar compounds, acrylamide, and heterocyclic compounds **Park and Jim, (2016).** This leads to obesity, chest pain, stomach ache, indigestion and even heart diseases as high consumption of Trans fats increases the levels of LDL cholesterol in the body.

Chia (*Salvia hispanica*), derived from the Nahuati word "chian" which means oily, the chia seeds are obtained from the chia plants belong to the family *Lamiaceae* scientifically named *Salvia hispanica* and is of high medicinal value. This annual herbaceous plant grows up to a height of 1 m. The leaves of the plants are serrated and arranged oppositely of 4–8 cm length. The plants bore white or blue flowers that are bisexual, 3–4 mm in size, growing in whorls at shoot tips. After blooming, chia form round fruits, containing many tiny oval seeds of 2 mm length and 1 mm in width. These seeds surfaces are smooth, shiny, black/brown in color. Chia seeds are of high nutritive value by the presence of high contents of dietary fiber approximately of about 30–34 g in which the insoluble fraction (IDF) accounts for approximately 85–93% and soluble dietary fiber (SDF) is approximately 7–15. The effect of chia seed ingestion on the immune system was examined and reported higher immunoglobulin E (IgE) concentrations in the rats receiving either chia seed oil (50 g/kg diet) or ground chia seeds (150 g/kg diet) for a month **Bartosz *et al*., (2019)**.

The main proposed mechanism that leads to the effect of chia seeds is due to its high fiber content. Chia seeds contain about 23–41% dietary fiber, making it a high fiber containing food. Fibers are known to induce satiety, causing lower food intake and greater weight changes **Wang *et al.*, (2023).** Furthermore, it also includes a significant number of bioactive constituents with strong antioxidant properties, including flavonoids like quercetin, kaempferol, epicatechin, rutin, and apigenin, -tocopherol, phenolic acids like gallic, caffeic, chlorogenic, cinnamic, and p-coumaric, and phytosterols**.** These compounds potentially play a crucial role in protecting organisms from the attack of reactive oxygen species (ROS) or nitrogen showing antioxidant, immune, and antimicrobial activities **Ahmadifar *et al.*, (2021).** The chia seed constitutes an important plant source of n-3 PUFA (Poly Unsaturated Fatty Acids) to be explored in different research models for human health and disease prevention, to its potential effect for the prevention of cardiovascular

diseases, fracture risk, obesity, and other obesity-associated disorders, such as diabetes **Grancieri *et al.,* (2019).** So, the main purpose of this research is to investigate the effect of chia seeds on eliminating the hazards of repeatedly heated oil of male rats.

Materials and Methods

Materials

Sunflower oil, sucrose, starch and chia seeds was purchased from Egyptian local market. Casein, dextrin, cellulose, L cysteine, choline chloride, D-L methionine, vitamins mixture and minerals mixture were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Thirty-five male albino rats Sprague-Dawley strain each weighed about (150±10g) were purchased from Helwan Farm, Cairo, Egypt. Biochemical kits were purchased from Gamma Trade, Giza, Egypt.

Methods

Preparation of reheated oil sample for

Sunflower oil was heated 15 times for 3 minutes at 180 °C and then cooled to room temperature. Fifty-gram potato pieces were fried in every frying interval as followed by **Çağlar *et al.,* (2012).**

Basal Diet

Basal diet was prepared according to **Reeves *et al.,* (1993).**

Experimental Design

Rats were kept in an animal house with controlled temperature at 25^{o}C, constant humidity of about 40–70% and 12 hr /12 hr light / dark cycle and fed with a standard diet and water *ad libitum* for one week as an adaptation period. After that, the rats were randomly distributed into five different groups (7 of each). Group 1 was fed basal diet containing sunflower oil for 8 weeks and served as -ve control. Group 2 (+ve control) as the same of group 1 and fed 4% reheated sunflower oil. Groups 3-5 as the same of group two and fed 2, 4 and 6% chia seeds powder, respectively. At the end of the experimental period, rats were fasted over the night and sacrificed.

Blood collection and serum separation

At the end of the experimental period (4 weeks), rats were fasted overnight before scarifying and blood samples were collected from each rat and were centrifuged at 3000 rpm for 15 min to obtain the serum for biochemical analysis.

Biological evaluation

Feed intake (FI) was recorded daily, body weight gain estimated weekly and feed efficiency ratio was calculated at the end of the experiment according to the method described by **Chapman *et al.,* (1959).**

Chemical analysis

Chia seeds chemical constituents, it's fatty acid content, peroxide value, total phenols and phenolic fraction and total flavonoids were determined according to **AOAC, (2005), ISO (2017),** Brand-**Williams *et al.,* 1995,** respectively**.**

Biochemical assays

Alanine and aspartate trans aminases were determined according to **Canepari *et al*., (1994)**. Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), very low-density lipoprotein (VLDL-c) and low-density lipoprotein (LDL-c) were determined according to the methods described by **Richmond, (1973)**, **Wahlefeld, (1974) and Albers *et al*., (1983),** respectively. Whereas, VLDL was calculated using TG/5 equation and LDL-c was calculated using TC – (HDL-c + VLDL-c) equation according to **Friedewald *et al*., (1972)**. Atherogenic index (AIP) was calculated according to **Dobiášová and Frohlich, (2001)** by the formula, log (TG/HDL-C). Blood urea nitrogen (BUN) was determined according to **Lear, (1950)** and creatinine (Cr) was determined according to **Moore and Sharer, (2017).** Malondialdehyde were determined according to **Shin, (2009)** and **Góth, (1991)**, respectively.

Statistical analysis:

All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to **Emtage and Duthy, (2002)**. All differences were considered significant if P-values were ($P< 0.05$).

Results and Discussion

The thermally-induced oxidation of glycerol-bound polyunsaturated fatty acids (PUFAs) in foods and culinary oils during standard frying or cooking episodes is a process that involves the prior generation of isomeric conjugated hydroperoxydiene (CHPD) species. These CHPDs fragment to form alkoxyl radicals that, in turn, undergo 8-scission to generate a wide range of aldehydic products. In view of the extremely toxic nature of the aldehydic end-products generated, the employment of PUFA-containing culinary oils for domestic or commercial fryinghoking episodes poses health hazards that have recently attracted much public and clinical interest **Grootveld *et al.,* (2001)**.

Indeed, these cytotoxic agents have been implicated in the development and progression of atherosclerosis and its associated pathological sequelae such as ischemic heart disease and peripheral vascular disease, and have also been shown to exert gastropathic, pro-inflammatory **Benedetti *et al.,* (1990)**, and geno toxicological properties. These phenomena are undoubtedly attributable to the extremely high reactivity of aldehydes with critical biomolecules (e.g., thiols such as glutathione; DNA, forming covalently-modified base adducts; and the apolipoprotein B component of low-density lipoprotein, altering its biological characteristics.)

Leong *et al.,* (2015) stated that when frying oil is heated at high temperatures, hydroperoxides and aldehydes are formed. These toxic products are absorbed by the food, and eventually into the gastrointestinal tract and thereafter enter the systemic circulation after ingestion. Consumption of repeatedly heated frying oils is associated with increased risk of

hypertension. The practice of reusing frying oil leads to detrimental health risks such as hypertension, histological abnormalities and alterations in genetic material. Free radicals generated during the frying process could damage membrane lipids through lipid peroxidation, subsequently leading to oxidative stress.

The Peroxide value of unused and reused sunflower oil was showed in Table (1). Peroxide value of sunflower oil was 1.86 $mEqO_2$\kg and the reused sunflower oil was 15 $mEqO_2$/kg. The peroxide value was highly increased due to reheating process of the sunflower oil for 15 times at 180 °C for three minutes. The results obtained proved that reheating increase the oxidative process and so the peroxide value increases **(Rastogi *et al.,* 2006)**.

Vegetable oils are thought to be the better alternative option compared to animal fats due to the fact that vegetable oils contain high quantity of unsaturated fatty acids and lack of cholesterol. But, today's fast-moving world, ready-made deep-fried food is the most preferable food and frequently consumed by the people. This proves the consumption of highly oxidized fatty acids. The main component in these fried food products is edible vegetable oil. Repeated heating of the oil speeds up the oxidative breakdown of lipids, creating dangerous reactive oxygen species and reducing the cooking oil's natural antioxidant content. Consuming dishes cooked in warmed oil over an extended period of time may seriously weaken one's antioxidant defense system, resulting in diseases like hypertension, diabetes, and vascular inflammation. Consuming warmed oil has negative effects that damage simple oxidative to cellular antioxidant defense **(Leong *et al.* 2015)**.

The sunflower oil extracted from seeds has higher oxidative stability in relation to cold-pressed sunflower oil during storage. The sunflower oil contains linoleic, polyunsaturated, omega-6 and oleic fatty acid, whereas the linolenic acid content is always less than 0.3% **(Crapiste *et al.,* 1999)**. So, this above researches were in harmony with this research findings.

Likewise, **(Godswill *et al.,* 2018)** evaluated the peroxide value of sunflower oil and found that the PV value raised from 10.6359 mEq/kg to 19.3101 mEq/kg on frying Irish potato chips. Similar reports were found with **(Goswami *et al.,* 2015)** who reported the peroxide value of sunflower oil increased from 0.40 mEq/kg to 8.42 mEq/kg when fried for five times. Furthermore, the peroxide value of sunflower oil with repeated frying increased 75.0% correspondingly related to their initial values of fresh oil samples **(Paunović *et al.,* 2020).**

These results are comparable with the reports of **(Shastry *et al.,* 2011)** who analyzed the Peroxide value of reused oil, which showed a significantly increase from 6.6 meq/kg of fat Fresh sunflower oil to 17.3 meq/kg of reused sunflower oil. This increase in peroxide values indicated in relation to increased lipid peroxidation by-products content, mainly the peroxides that were formed in the oil during heating process. But the food prepared using reheated oil for the long-term ingestion could severely confronts one’s antioxidant defense network in turn lead to diseases like hypertension, diabetes and vascular inflammation **Clemente *et al.,* (2009).**

When the oil is exposed to elevated temperatures in the presence of air and moisture, the complex reactions such as oxidation, free radicals, hydrolysis, isomerization, and polymerization

take place in a series. These reactions and influence quality attributes of the final product such as flavour, texture, shelf life and nutrient composition **(Omer *et al.,* 2015)**. During frying, oils are subjected to thermal oxidation, polymerization, and hydrolysis and in turn, these reactions lead to a decrease in tocopherols and total phenols (TP). There is also an increase in the peroxide value (PV) and decomposed products formed are of high molecular weight contains polar compounds and polymeric triacyl glycerides **(Karakaya and Şimşek, 2011)**.

The repeated use of frying oils produces undesirable constituents that may cause health hazards**.** In the current study, the health hazards caused due to the use of reused frying oil reduction was studied by the application of chia seeds in male albino rats fed with reused oil in their diets. Chia seeds are attributed with high nutritive value mainly because of their high contents of dietary fibre and fat **(Bartosz *et al.,* 2019).** The chemical composition of chia seeds (100g) used in our study was evaluated and found that the presence of high content of fats (28.22 g) followed by fibre content of 24.88g. Also, the presence of 19g of carbohydrates, 17.3 g of protein, 4.7g of ash and 5.9 g of moisture was found in 100g of chia seeds (Table 2).

Similar results were obtained by **(Marineli *et al.,* 2015)** reported that the chia seeds 30–34 g dietary fibre that exceeds even dried fruits, cereals or nuts. Further, it approximately 60% fatty acids mainly high contents of polyunsaturated fatty acids, α-linolenic acid (ALA) and lower amounts of linoleic, oleic and palmitic acids are found in lower amounts. Also, the proteins present in the chia seeds are rich in endogenous amino acids, in particular alanine, serine and glycine, glutamic and aspartic **acids (Nitrayova *et al.,* 2014).**

Table **2** showed the chemical composition and fatty acid content of chia seeds. Chia seeds contain fats, fibre, carbohydrates, protein and a small quantity of ash and moisture with values of 28.22, 24.88, 19, 17.3, 4.7 and 5.9 g/100g of moisture, respectively. Chia seeds contains palmitic acid, palmitoleic acid, γ- linolenic acid (GLA) and linolenic acid with values 0.24, 4.81, 25.46 and 69.49%, respectively. It can be observed that chia seeds contain high concentration of linolenic acid followed by γ- linolenic acid.

γ- linolenic acid acts in several ways to exert its effects, including the modulation of eicosanoids (PGs, LTs) and cytokines, and by regulating genes that affect apoptosis and cell growth. GLA is functionally EFA because it can correct the symptoms of EFA deficiency so studies have confirmed its anti-inflammatory properties of GLA **Rezapour-Firouzi, (2017).**

An intact inner-mitochondrial membrane made of the phospholipid, cardiolipin, is required for producing ATP. The optimal functional configuration of cardiolipin is enriched with four linoleic acid side chains. Linoleic Acid enriched cardiolipin provides the scaffold for the electron transport chain proteins to efficiently conduct ATP synthesis. Because humans cannot endogenously synthesize linoleic acid, diet is the sole source of linoleic acid to synthesize 4 linoleoyl-cardiolipin. During oxidative phosphorylation involving the electron transport chain, cardiolipin is remodelled by exchanging oxidized linoleic acid for new non-oxidized linoleic acid molecules **Martha *et al.,* (2022).**

In the current study, the total phenolic and flavonoid content present in the chia seeds were also evaluated and represented in the Table 3. It indicated that, 182.5 g of total phenols and 51.5 g of total flavonoids were found in 100g of chia seeds. It is observed in the result that, the presence of total phenols was comparably higher than the flavonoid content.

The total phenolic content present in the chia seeds were also evaluated in the current study. The results indicated that 182.5 g of total phenols and 51.5 g of total flavonoids were found in 100g of chia seeds (Table 4). Phenolic components of chia seeds were illustrated in Table (4). The gallic acid, vanillic acid, chlorogenic acid, β-coumaric, hesperidin, myricetin, quercetin, rosemarinic, apigenin and kaempferol content in chia seeds were 0.55, 2.94, 1.68, 1.36, 3.57, 6.75, 142.11, 3.61, 2.30 and 4.73%, respectively. From these data, it could be observed that, the percent of quercetin was high in chia seeds, followed by myricetin and rosemarinic, respectively. The total phenols found in chia seeds may be particularly gallic, caffeic, chlorogenic, cinnamic and ferulic acids, quercetin, kaempferol, epicatechin, rutin, apigenin and **β**-coumaric acid. Also, the total flavonoids present in chia seeds may contain Isoflavones like daidzein, glycitein, genistein and small amounts of genistein **(Bartosz *et al*., 2019).**

Pertaining to the biological effect of feeding chia seeds, Table (5) illustrated the effect of chia seeds on body feed intake (FI), weight gain, (BWG) and feed efficiency ratio (FER) of experimental male albino rats. Feed intake values of +ve control group increased compared with -ve control rats with mean values 20.16 vs. 19.63g/d, respectively. Whereas, rats fed on 2, 4 and 6% chia seeds mean values decreased compared with +ve control rats with mean values 20.01, 19.55 and 18.24 vs. 20.16 g/d, respectively.

With regard to the body weight gain, rats of +ve control nonsignificant increase compared to -ve control rats 54.21 vs. 52.25 %, respectively. In the other hand, rats fed 4 and 6 % chia seeds were nonsignificant decreased compared to +ve control rats with mean values 49.99 and 50.78 vs. 54.21 %, respectively. Whereas, rats of group 2 which fed 2% of chia seeds nonsignificant increase compared with +ve group with mean values 57.16 vs. 54.21 %, respectively.

Furthermore, the calculation of feed efficiency ratio (FER) indicated that the +ve control group nonsignificant increase compared with -ve control rats with mean values 0.096 vs. 0.095, respectively. Whereas, rats fed on 2 and 6% chia seeds showed nonsignificant increase compared with +ve control group with mean values 0.102, 0.099 vs. 0.096, respectively. Rats fed 4 % chia seeds have the lowest value among all tested groups with nonsignificant decrease compared to +ve group 0.091 vs. 0.095, respectively.

David *et al.*, (2009), reported that the high dietary fiber and *α*-linolenic (ALA) contents of chia seed would induce a small but significant decrease in body weight and fat and improve disease risk factors. The high fiber content of chia seeds may improve satiety, decrease energy intake, and promote weight loss **Howarth *et al.*, (2007).**

Table (6) showed the effect of chia seeds powder on alanine transaminase and aspartate trans aminase of rats fed on diet containing reheated sunflower oil. The ALT mean values of +ve control group was significant increase compared to -ve control rats with mean values 36.60 vs. 19.80µl/L, respectively. Whereas all tested groups which fed 2, 4, 6% chia seeds powder were significant decrease compared to +ve control group. The highest improvement was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values 23.60, 27.20 and 29.0080µl/, respectively.

Wayne, (2011), stated that the consumption of chia seeds was found to be effective for reducing liver damage by reducing AST and ALT levels. Chia seeds contain substance that has been found on it to improve liver enzymes is a-linolenic acid. This substance is present at high levels in chia seeds, ranging from 14 to 20 g/100 g**.**

Table (7) showed serum total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low-density lipoprotein of male rats. All these parameters except HDL of +ve control group were significant increased compared to -ve control rats with mean values 197.20, 140.27, 146.75, 28.05 vs. 120.58, 55.08, 51.36 and 11.02 mg /dl, respectively. High density lipoprotein of +ve control significant decreased compared with -ve control rats with mean values 22.40 vs. 58.20 mg/dl, respectively. All lipid profile parameters except HDL were significant decreased compared to +ve control rats. HDL was significant increased compared to +ve control rats. The highest improvement in TC levels was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values142.25, 156.20 and 170.06mg/dl, respectively. The highest improvement in TG levels was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values 80.09, 102.50 and 117.20mg/dl, respectively. The highest improvement in LDL levels was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values 76.63, 94.50 and 115.62 mg/dl, respectively. Additionally, the highest improvement in VLDL levels was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values 16.02, 20.50 and 23.44mg/dl, respectively. Whereas, the lowest improvement in HDL levels was in rats fed 6% chia seeds followed by 4 and 2% chia seeds with mean values 49.60, 41.20 and 31.00mg/dl, respectively.

An improvement in lipid profiles has been observed following the consumption chia seeds, which can be attributed to the high level of omega-3 fatty acids in chia seeds **Sheisa *et al.,* (2019)**. Additionally, some studies have indicated that dietary omega-3 fatty acids reduce the plasma levels of triglycerides. Additionally, substance that has been found to improve lipids is a-linolenic acid. This substance is present at high levels in chia seeds, ranging from 55 to 65.8%. Caffeic acid and chlorogenic acid are the main antioxidants in chia seeds, and can inhibit lipid peroxidation. These substances have been shown to be significantly stronger and more effective than other antioxidants such as vitamin E and vitamin C **Valdivia-López and Tecante, (2015)**. Another antioxidant which is present in chia seeds is quercetin, which can prevent the oxidation of lipids. Additionally, the antioxidant properties of quercetin are considered to be stronger than those of some flavonol compounds **Kweon *et al.,* (2001)**.

Table (8) showed blood urea nitrogen (BUN) and serum creatinine (CR) of rats fed deferent levels of chia seeds powder. A significant increase in serum BUN and CR was observed in +ve control group compared to healthy rats of group one with mean values 28.00 and 0.82 vs. 13.80 and 0.32 µl/L, respectively. All tested groups showed significant reduction of both biochemical parameters. Regarding to BUN and CR, the highest improvement was in group fed 6% chia seeds followed by 4 and 2% with mean values 17.40, 19.60, 20.00 for BUN and 0.39, 0.47, 0.69 µl/L, respectively.

These results are in agreement with **Mostafa, (2021) and Helal *et al.,* (2023)** stated that revealed treatment with CS at various concentrations resulted in a substantial decrease in uric acid, creatinine, and urea levels as compared to the positive control group. Also, **Fayez *et al.,* (2014)** mentioned that omega-3 fatty acids have a preventive effect against renal impairment in rats.

Table (9) showed mean values of malondialdehyde (MDA) and catalase (CAT) of rats fed different levels of chia seeds. Pertaining to MDA, +ve control group significant increase compared to -ve control rats with mean values 4.06 vs. 0.39 nmol/mL, respectively. Groups fed 2,4 and 6% chia seeds powder significant decreased compared to +ve control rats with mean values 2.93, 2.07, 1.08 vs. 4.06 nmol/mL, respectively. Regarding to CAT, rats fed 2,4 and 6% chia seeds powder significant increase compared to +ve control rats with mean values 0.98, 1.10, 1.52 vs. 0.33 nmol/mL, respectively. The most improvement in MDA and CAT was in group 5 which fed 6% chia seeds powder.

These results are in harmony with **Marineli *et al.*, (2015)** they found that chia intake increased the concentration of SOD and catalase, without altering the oxidative stress (MDA and NO) and maintained the total antioxidant activity of plasma and liver, increasing the activity of antioxidants enzymes which have ability to defend the body against the oxidative stress. The same effect was observed by **Rincón-Cervera *et al.,* (2016)** concluded that chia intake was able to increase antioxidant markers concentrations in Wistar rats. Plasma catalase concentration was higher in the animals that consumed chia in **Mahfouz, (2020)** research owing to a positive effect of the compounds present in chia seeds on the activity of the antioxidant enzyme capable of decomposing hydrogen peroxide. Also, **Ferreira et al. (2018)** they reported that the administration of a chia-based diet, caused restores the activity of some enzymes, including catalase and superoxide dismutase, that decrease the hepatic production of some oxidation and inflammation markers.

Table (1) Peroxide values of reused and unused Sunflower oil

Peroxide value (PV)	$mEq/O_2\backslash kg$
Unused Sunflower oil	**1.86**
Reused Sunflower oil	**15.00**

Table (2) Chemical composition and fatty acid content of Chia seeds

Chia seed contents	g\100g	Fatty acid	%
Ash	4.70	C16:0(palmitic acid)	0.24
Fats	28.22	C16:1(palmitoleic acid)	4.81
Proteins	17.30	C18:3 (γ-linolenic acid)	25.46
Fibres	24.88	C18:3(linolenic acid)	69.49
Total carbohydrates	19.00	-	-
Moisture	5.90	-	-

Table (3) Total Phenolic and Flavonoid content of raw chia seeds

Contents of Chia seeds	mg \100 g
Total phenols	182.46
Total flavonoids	51.52

Table (4): phenolic components of chia seeds

Constituent	mg/100g	Constituent	mg/100g
Gallic acid	0.55	Myricetin	6.75
Vanillic acid	2.94	Quercetin	142.11
Chlorogenic acid	1.68	Rosemarinic	3.61
P coumaric	1.36	Apigenin	2.30
Hesperidin	3.57	Kaempferol	4.73

Table (5) Biological effect of chia seeds on feed intake (FI), Body weight gain (BWG) and feed efficiency ratio (FER) of male rats

Groups	FI (g/d/rat)	BWG (%)	FER
G1- Control (-ve)	19.63	52.25±04.42^{a}	0.095±0.001^{a}
G2- Control (+ve)	20.16	54.21±08.45^{a}	0.096±0.001^{a}
G3- Chia seeds 2%	20.01	57.16±06.33^{a}	0.102±0.002^{a}
G4- Chia seeds 4%	19.55	49.99±07.11^{a}	0.091±0.005^{a}
G5- Chia seeds 6%	18.24	50.78±05.27^{a}	0.099±0.002^{a}

The mean values are expressed as Mean ± SE. The mean values which have the same subscript letters in the same column were insignificant at P ≤ 0.05.

Table (6): Effect of chia seeds on alanine transaminase (ALT) and aspartate transaminase (AST) of male rats treated with reused oil

Groups	ALT	AST
	µl/L	
-ve Control	19.80±1.24^{d}	99.20±8.18^{d}
+ve Control	36.60±1.07^{a}	133.60±8.47^{a}
G3- Chia seeds 2%	29.00±1.30^{b}	118.80±4.31^{b}
G4- Chia seeds 4%	27.20±1.24^{b}	115.20±9.06^{b}
G5- Chia seeds 6%	23.60±0.50^{c}	104.20±5.32^{c}

The mean values are expressed as Mean ± SE. The mean values which have the same subscript letters in the same column were insignificant at P ≤ 0.05.

Table (7): Effect of chia seeds on blood urea nitrogen (BUN) and creatinine (Cr) of male rats treated with reused oil

Groups	TC	TG	HDL	LDL	VLDL
	mg/dl				
-ve Control	120.58 ±09.96^{e}	55.08 ±07.12^{e}	58.20 ±02.90^{a}	51.36 ±01.52^{e}	11.02 ±00.69^{d}
+ve Control	197.20 ±11.33^{a}	140.27 ±11.43^{a}	22.40 ±02.38^{e}	146.75 ±02.68^{a}	28.05 ±00.23^{a}
G3- Chia seeds 2%	170.06 ±12.58^{b}	117.20 ±09.26^{b}	31.00 ±02.53^{d}	115.62 ±01.89^{b}	23.44 ±00.18^{b}
G4- Chia seeds 4%	156.20 ±11.86^{c}	102.50 ±06.52^{c}	41.20 ±02.37^{c}	94.50 ±03.11^{c}	20.50 ±01.74^{b}
G5- Chia seeds 6%	142.25 ±17.91^{d}	80.09 ±07.08^{d}	49.60 ±03.76^{b}	76.63 ±02.40^{d}	16.02 ±02.23^{c}

The mean values are expressed as Mean ± SE. The mean values which have the same subscript letters in the same column were insignificant at P ≤ 0.05.

Table (8): Effect of chia seeds on blood urea nitrogen (BUN) and creatinine (Cr) of male rats treated with reused oil

Groups	BUN	Cr
	µl/L	
-ve Control	13.80±1.06^{d}	0.32±0.02^{e}
+ve Control	28.00±1.14^{a}	0.82±0.02^{a}
G3- Chia seeds 2%	20.00±0.70^{b}	0.69±0.02^{b}
G4- Chia seeds 4%	19.60±1.02^{b}	0.47±0.01^{c}
G5- Chia seeds 6%	17.40±1.32^{c}	0.39±0.02^{d}

The mean values are expressed as Mean ± SE. The mean values which have the same subscript letters in the same column were insignificant at P ≤ 0.05

Table (9): Effect of chia seeds on malondialdehyde (MDA) and catalase (CAT) of male rats treated with reused oil

Groups	MDA (nmol/ml)	CAT(µ/L)
-ve Control	**0.93±0.003^{d}**	**1.74±0.002^{a}**
+ve Control	**4.06±0.002^{a}**	**0.33±0.002^{d}**
G3- Chia seeds 2%	**2.93±0.010^{b}**	**0.98±0.001^{c}**
G4- Chia seeds 4%	**2.07±0.002^{b}**	**1.10±0.004^{c}**
G5- Chia seeds 6%	**1.08±0.005^{c}**	**1.52±0.001^{b}**

The mean values are expressed as Mean ± SE. The mean values which have the same subscript letters in the same column were insignificant at P ≤ 0.05.

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