Using Pan Bread Enriched with Chia Seeds to Reduce some Side Effects of Fatty Liver Induced with Fructose in Male Rats

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ABSTRACT:

Non- alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by excessive triglyceride accumulation in hepatocytes. NAFLD has increased in conjunction with obesity in Egypt. This research investigated the chemical analysis and sensory evaluation of pan bread and pan bread enriched with Chia seeds and the effects of the consumption of pan bread enriched with Chia seeds on fatty liver rats.Forty-eight male Albino rats were divided into six groups (n= 8 rats). The two negative control groups one of them was fed on basal diet and other fed on basal diet with pan bread and 32 rats were fed on basal diet and additional 25 % of fructose in water to induce fatty liver, and reclassified into positive control group which fed on basal diet and pan bread and 3 treated rat groups that treated with 300g Pan bread enriched with various three level (4%, 6% and 8% chia seeds powder / kg diet. The treated period was designed for 28 days after rat's administrated 25% fructose dissolved in drinking water for 8 weeks for inducing fatty liver.The Results showed that, Chia seeds contain high amounts of protein, dietary fiber and unsaturated fatty acids. It is also rich in phenolic compounds which considered as antioxidants.The results revealed that, the control (+ve) rat group showed significant decrease in body weight,food intak and food efficiency ratio but significant increase in serum (AST, ALT, ALP, GGT), bilirubin, glucose, T. lipids, TC, TG, LDL-c, urea, creatinine, and tumor necrosis factor (TNF,α) at $P \leq 0.05$ in compared with - ve control group and all treated groups.while all treated rat groups showed significant inecrease in body weight,food intak and food efficiency but significant decrease in serum (AST, ALT, ALP, GGT), bilirubin, glucose, T. lipids, TC, TG, LDL-c, urea, creatinine, and tumor necrosis factor (TNF,α) at $P \leq 0.05$ in compared with +ve control group. In conclusion, the administration of pan bread enriched with Chia seeds can lower the side effects of fructose to induced fatty liver in rats and improve the healthy status with improved of fatty liver function.

Key words: Non -alcoholic fatty liver, Fructose, Rats, Chia seeds and Biochemical analysis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the liver diseases which are decried firstly by storage of surplus of fat the hepatocytes in respect of a disorder of the homeostatic mechanisms that organized installation against consumption of fat in the liver **(Diehl and Day, 2017)**. Non-alcoholic fatty liver disease (NAFLD) is a public health problem lacking an approved pharmacological treatment. Omega-3 fatty acids have shown to reverse NAFLD **(Romero-Gómez *et al.*, 2017)**.

Moreover, hypercaloric nutrition, that has rich amount of trans/saturated fat and cholesterol, and fructose-sweetened beverages demonstrate to raise visceral adiposity and stimulate hepatic lipid amassment and into non-alcoholic steatohepatitis **(Fan and Cao, 2013).** Parallel to the obesity epidemic, non-alcoholic fatty liver disease (NAFLD) prevalence has markedly increased during the last years. Recent epidemiological studies have found that one of three adults has NAFLD, which has been associated with a cluster of metabolic abnormalities **(Ma *et al.*, 2017)**.Therapies that focus on the use of functional foods, rich in a variety of phytochemicals, mono/polyunsaturated fatty acids, antioxidants, minerals, and dietry fiber, have shown antioxidant, anti-inflammatory, and lipid-lowering effects, which could be useful in patients with NAFLD **(Silva Figueiredo *et al.*, 2018)**.

Lim *et al.* (2010) and Vos and Lavine (2013) reported that fructose is a ketonic monosaccharide existent in numerous plants, e.g. sugar cane, sugar beets, and corn. Moreover, fructose recognized as fruit sugar. Also,fructose is used as a sugar additive, which is the main component of some manufactured foods i.e. soft drinks, pastries, sweets and it has been confirmed as a reason related to the raised of nonalcoholic fatty liver disease, it is clear that, patients suffering from NAFLD and used a high ratios of fructose caused inflammation and damage of tissue liver **(Abdelmalek *et al.*, 2010).**

Bread is considered as a major processed food. The bread is poor in content of nutrients, due to the use of refined wheat flour in baking. For that reason, supplementation of wheat flour with cheap food sources such as various cereal, mixture of legumes, seeds and grains are modern methods to produce a high quality bakery products **(Kourkouta *et al.*, 2017)**. In addition, bread is an important staple food made of wheat flour, salt and yeast, and consumed worldwide. Nowadays, consumers prefer to eat healthier foods in order to prevent non-communicable diseases. For this reason industry and researchers are involved in optimizing bread making technology to improve the variety, quality, taste and availability of food products such as bread **(Hathorn *et al.*, 2008)**.

The chia seeds (*Salvia hispanica*, L.) is genesis which the most varied in the family Lamiaceae. It included about 900 types widely divided of various areas in the world, e.g. Southern Africa, (Central, North and South) America, and South-East Asia **(Takano, 2017)**.Chia seeds showed high concentrations of lipids, proteins, total dietary fiber, minerals like iron, manganese, boron, lead, aluminum, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, copper and antioxidant capacity than chia grown in MT and vitamin E.Moreover,the chia seed is an important source of protein, soluble dietary fiber, antioxidants, and polyunsaturated fatty acids (PUFA), particularly linolenic acid (omega3). These fibers may help to lower LDL cholesterol and slow down digestion, which can prevent blood sugar spikes after eating a meal and promote a feeling of fullness **(Mohd Ali *et al.*, 2012)**. Also,Chia seed is characterized by high contents of polyunsaturated fatty acids, mainly α-linolenic acid (ALA), which accounts for approximately 60% all fatty acids. Linoleic, oleic and palmitic acids are found in lower amounts. Furthermore, Chia seeds have greater contents of omega-3 acids than flaxseed. We also need to stress the advantageous ratio of omega-6 to omega-3 acids, which is approximately 0.3:0.35 **(Villanueva-Bermejo *et al.*, 2019)**. So,the use of chia must be stimulated since this food presents a high nutritional value and bioactive compounds that are related to benefits to the human health **(USDA, 2015)**. Thus,Chia seeds have been considered a promising food since it may be used in combating chronic diseases such as diabetes mellitus, hypertension, obesity, dyslipidemias, and metabolic syndrome,and Chia seed (*Salvia hispanica*) is the richest vegetal source of omega 3-fatty acids, antioxidants, and fiber. Although some animal models have suggested that chia could be used as an alternative to reduce intrahepatic fat content, its effect on NAFLD patients has not been studied yet (Chia seeds have been considered a promising food since it may be used in combating chronic diseases such as diabetes mellitus, hypertension, obesity, dyslipidemias, and metabolic syndrome.Chia seed (*Salvia hispanica*) is the richest vegetal source of omega 3-fatty acids, antioxidants, and fiber. Although some animal models have suggested that chia could be used as an alternative to reduce intrahepatic fat content, its effect on NAFLD patients has not been studied yet **(Toscano *et al.*, 2014; Montes Chañi *et al.*, 2018).**

Medina-Urrutia *et al.* (2020) reported that 25 g/day of milled chia ameliorates NAFLD. Chia is an accessible vegetal source of omega-3 fatty acids, antioxidants, and fiber, which could have the potential to prevent metabolic abnormalities in NAFLD patients. Considering that there is no pharmacological treatment approved for NAFLD, the findings of the present study suggest that a chia-supplemented diet could be an innovative alternative to control this disease.

This study aimed to investigate the effect of combining chia seeds with pan bread on ameliorating fatty liver injury in rats treated with fructose.

MATERIALS AND METHODS

Materials

Source of chia seeds

Chia seeds (*Salvia hispanica, L*) were obtained from one commercial retail at Imtenan Healthy Shop, Alexandria City, Egypt.

Ingredients of pan bread

For the producing of pan bread, other materials were used, i.e. wheat flour (%72) (food salt, sugar, yeast) and shortening, which they were purchased from the local market, Alexandria City. Egypt.

Chemical and kits:

Pure white crystalline cholesterol powder and saline solutions casein, cellulose, choline chloride powder, and DL methionine powder, were obtained from Morgan Co. Cairo, Egypt. Chemical kits used in this study (TC, TG, HDL-c, ALT, AST, ALP, urea, uric acid, and creatinine) were obtained from Al-Gomhoria Company for Drugs, Chemical and Medical Instruments, Cairo, Egypt. While, GSH, CAT, SOD kits was obtained from SIGMA Chemical Co., Cairo, Egypt.

Experimental animals

48 adult male rats Albino of Sprague Dawely strain weighing 160 ± 10g were purchased from High Institute of Graduate Studies and Research, Alexandria University, Egypt.

Basal diet

Basal diet was prepared according to **Reeves *et al.* (1993)**. A mixture of vitamins and salts were prepared according to **Hegsted *et al.* (1941)** and **AOAC (1975)**.

Methods

Preparation of unenriched and enriched pan bread with chia seeds:

The method of **Lazaridou *et al.* (2007)** with some modifications was used to prepare pan bread. All ingredients were tabulated in table (1) as follows: Yeast (3%) was dissolved in 175 ml warm water (35°C) and then added to the dry ingredients (3% food salt, 2% sugar and 350 g wheat flour 72 % extraction and chia seeds powder). Chia seeds powder was added to pan bread by 4, 6,8,10 and12%. The control sample was prepared without mixing the chia seeds powder. The shortening (3%) was then added and the mixture was kneaded in the mixing bowl for 4 min at a low speed then for 2 min at high speed. The dough was fermented for 30 min at 30°C and 80-85% relative humidity in a fermentation cabinet. The dough was divided into 150g pieces, placed in the pan and proofed under the same conditions for 45 min. Bread dough were baked at 240 °C for 20-25 min following steaming for 10s. Baked pan bread was cooled down at room temperature for 60 min.

Table (1): Ingredients of pan bread

Ingredients (g)	Control sample	Pan bread with chia seeds powder				
		4%	6%	8%	10%	12%
Wheat flour 72 % extraction(g)	350	346	344	342	340	338
Chia seeds powder(g)	0	4	6	8	10	12
Sugar(g)	2	2	2	2	2	2
Food salt(g)	3	3	3	3	3	3
Yeast (%)	3	3	3	3	3	3
Shortening (%)	3	3	3	3	3	3
Warm water (ml)	175	175	175	175	175	175
Total	361	361	361	361	361	361

Determination of chemical composition for Chia seeds and its product

Gross chemical composition of Chia seeds and pan bread enriched with different levels of Chia seeds protein, fat, ash, and moisture were determined while total carbohydrates were calculated by difference as following: Carbohydrates % = 100 - (moisture % + protein % + fat % +ash) according to the methods of the **AOAC (2007)**. **Caloric values**: were calculated from the sum of the percentages of crude protein and total carbohydrates (N-free extract) multiplied by a factor of 4 (Kcal.g^{-}1) plus the crude fat content multiplied by a factor of 9 (Kcal.g^{-1}) according to **Zambrano *et al.* (2004)**.

Dietary fiber assessment (DF):

Dietary fibers were estimated method described by **Prosky *et al.* (1984).**

Determination of fatty acid using GLC:

Fatty acid determined using a Toxichron GLC, model B-5 800-1 equipped with FID detector (PE Auto System XL) using 180 x 0.3 cm. column of DEGS on Chromosorb W and nitrogen gas at a flow rate of 40 ml/min. Analyses were conducted isothermally at 185°C **(Taga *et al.*, 1984).**

Identification of phenolic compounds using HPLC:

Phenolic compounds determined using HPLC technique with some modifications; this was carried out according to **Kim *et al.* (2006)**.

Organoleptic evaluations of un-enriched and enriched pan bread with chia seeds powder:

The organoleptic characteristics were evaluated according to **Hooda and Jood (2005)**, using hedonic score consisting from 10 points. The statistical analysis showed the best sensory evaluation from different levels of chia seeds recorded for 4,6 and 8%, respectively, and its levels applied in the research plan.

Biological design:

Experimental design and animal groups:

Forty-eight male Albino rats were divided into six groups (n= 8 rats) were kept under observation for one week for adaptation and fed on basal diet. (n=16 rats) served as two negative control groups one of them was fed on basal diet and other fed on basal diet with pan bread and(n= 32 rats) were administration with 25% fructose dissolved in the drinking water to induce non-alcoholic fatty liver disease according to **Lozano *et al.* (2016)**. After eight weeks of fooding rats on fructose, and before starting the experimental diet, the injury fatty liver was confirmed by analyzing the serum level of lipid profile e.g. total cholesterol, triglycerides “TG” and liver enzymes e.g. ALT and AST. The findings of fatty liver groups recorded 193.6±13.52 mg/dl, 160.7±11.72 mg/dl & 78.22±3.61 U/L and 80.01 ±5.41 U/L, respectively. Then, reclassified into control positive group which fed on basal diet with pan bread and three treated rat groups that treated with 300g pan bread enriched with various three level (4%, 6% and 8% chia seeds powder / kg diet. Food and water was provided ad-libtum. Food intake was recorded daily and body weight of rats was measured once weekly.. At the end of the experimental period was designed for 28 days after rat’s administrated 25% fructose dissolved in drinking water for eight weeks for inducing fatty liver. The rats were anaesthetized by diethyl ether and sacrificed. Blood samples of each rat were withdrawn in test tubes of blood were left for coagulation then centrifuged at 3000 rpm for 15 minutes to obtain serum for estimation of some biochemical analysis.

Body weight gains (BWG), feed intake (FI), and feed efficiency ratio (FER):

During the experimental period (28 days) the net feed intake was daily recorded, while body weight was weekly recorded. The net feed intake and gained body weight were used for the calculation of feed efficiency ratios (FER) according to **Chapman *et al.* (1959)** as follow:

$$\text{BWG\%} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{F.E.R.} = \frac{\text{Gain in body weight (g)}}{\text{Feed intake(g/day)} \times \text{Experiment period(day)}}$$

Biochemical analysis:

Serum lipids profile:

Serum total lipid and cholesterol were determined according to the colorimetric method described by **Frings *et al.* (1972)** and **Allain *et al.* (1974)**, respectively. Serum triglycerides were determined by enzymatic method using kits according to **Fossati and Prencipe (1982)**. HDL-c was determined according to the method described by **Warnick *et al.* (1983)**.

VLDL-c was calculated in mg/dl according to **Crook (2006)** was using the following formula: VLDL-c (mg/dl) = Triglycerides / 5

LDL-c was calculated in mg/dl according to **Friedewald *et al.* (1972)**. as follows:

LDL-c (mg/dl) = Total cholesterol – HDL-c + VLDL-c

Calculation of atherogenic index (AI):

Calculation of atherogenic index = (VLDL-c+ LDL-c) / HDL-c

This atherogenic index was calculated as the method described by **Nwagha *et al.* (2010).**

Liver functions:

Determination of serum alanine aminotransferase (ALT), serum asparatate aminotransferase (AST), bilirubin and serum alkaline phosphatase

(ALP) were carried out according to the method of **Reitman and Frankel (1957) and Principato *et al.* (1985)**, respectively. Gamma-glutamal-transferase (GGT) was determined by kits from Barcelona, Spain,(Costa Brava 30), Biosystems S.A. **(Lorentz, 1997).**

Kidney functions:

Determination of serum urea and creatinine were done according to **Patton and Crouch (1977)**and **Jaffe (1980)**, respectively.

Measurement of glycemic index:

The levels of insulin and glucose in serum were determined when the rats were in a fasting condition for 8 hours. Glucose **(Trinder, 1969)**, insulin was determined using Monobind kit and Enzyme Linked Immuno Sorbant Assay (ELISA) method **(Turkington *et al.*, 1982)**. Homeostasis model assessment of insulin resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICK) were used to evaluate the resistance and sensitivity for insulin **(Vogeser *et al.*, 2007)** Final concentrations of blood glucose were multiplied by the concentration of insulin and then divided by 22.5 to get HOMA-IR for insulin resistance **(Sarafidis *et al.*, 2007)** using the formula: Insulin resistance = (insulin × glucose)/22.5.

Determination of antioxidant enzymes activities:

Liver catalase (CAT) was determined by **Luck (1974)**. Superoxide dismutase (SOD) activity was determined by using a measurement method developed by **Misra and Fridovich (1972)**.Glutathione peroxidase (GSH) activity was assayed with method of **Beutler *et al.* (1963).** Lipid peroxidation concentrations were expressed as thiobarbituric acid reactive substances (TBARS) per milligram of mitochondrial protein and were estimated using the method of **Tappel and Zalkin (1959).**

Tumor necrosis factor (TNF-α) were determined according to **Thorell and Lanner (1973).**

Statistical analysis:

Values were presented as means ± SD analyzed statistically by using one way ANOVA test, then Post Hoc test (LSD) was followed($P \leq 0.05$) was also used for indicating significance **(Kotz *et al.*, 1998)**.

RESULTS AND DISCUSSION

Chemical composition of chia seeds:

Table (2) is presented the chemical composition of chia seeds. The moisture, protein, fat, ash, fiber, N-free extract, dietary fiber and energy value contents of chia seeds were found to be 0.00, 27.30, 37.10, 5.15, 23. 21, 7.24,58.01(g/100g DW) and 472.06 Kcal/100g, respectively. These results are parely with results of **Franklin and Hongu (2016)** they reported that the chemical composition of chia seeds consist of 15-25% protein, 30-33% fat, 26-41% carbohydrates, 18-30% fiber, and 4-5% ash. As well, these findings are not in accordance with study by **Kibui *et al.* (2018)** found that the chemical composition of chia seeds included moisture, ash, protein, lipids, fiber, carbohydrates and caloric value were (5.15,4.50,20.90,29.06,21.14,19.24 and 422.21 Kcal/100g, respectively.

These results are in disagreement with that of **Jaddu and Yedida (2018)** they reported that chia seeds are high in dietary fiber (34.6%) lipids (28.35 %) and protein (23.17 %). and the majority of fatty acids present in chia oil are α-linolenic (ALA) (64% of total oil), linoleic (LA) (21% of total oil), oleic, stearic, and palmitic acids.

According to **Ayerza (2009); Ayerza and Coates (2009) and da Silva *et al.* (2017)** the great variability in the physical and chemical characteristics of plant can be attributed to many factors, including the region where the plant was grown, climatic differences, fertility, soil pH and annual rainfall.

It is observable from previous results, the chia seed fits for the definition of nutraceutical which it contains vital nutrients that necessary in human nutrition and also, it provided protection against chronic disease e.g. obesity, diabetes and hyperlipidemia...etc.

Table (2): Chemical composition of chia seeds

Component	Values(g/100g DW)
Moisture	0.0 ± 0.00
Protein	27.30 ± 0.11
Ash	5.15 ± 0.10
Fat	37.10 ± 0.06
Crude fiber	23.21 ± 0.03
***NFE**	7.24 ± 0.01
Caloric value(Kcal/100g)	472.06 ± 0.08
Total dietary fiber	58.01 ± 0.25

*** N-free Extract (%) calculated by difference. Values are expressed in mean ± SD.**

Identification of fatty acids composition of chia seeds:

Data given in Table (3) displayed the identification of fatty acids composition of chia seeds. The obtained results indicated that the highest saturated fatty acids composition of chia seeds recorded for palmitic acid (C16:0) and stearic acid (C18:0). The values were 7.19 and 1.87 %, respectively. While, the lowest saturated fatty acids composition of chia seeds recorded for caprlyic acid (C8:0) and arachidic acid (C20:0), which were 0.01 and 0.01%, respectively.

In connection with, the mono unsaturated fatty acids, the higher value recorded for oliec acid (C18:1), while the lowest one recorded for tetradecanoic acid (phytoseric) (C14:1). The values were 5.99 and 0.50%, respectively.

Moreover, the polyunsaturated fatty acids, the higher polyunsaturated fatty acids recorded for linolenic acid C18:3 while the lowest one recorded for linoleic acid C18:2. The values were 63.99 and 17.01%, respectively. Data show that the ratio for both a saturated to unsaturated fatty acids and ω6:ω3 were1:9.4 and 1:3.8%, respectively.

These results are in agreement with **Nitrayová *et al.* (2014)** reported that the fatty acids composition showed the presence of palmitic, stearic, oleic, linoleic, α- linolenic and arachidic fatty acids in all tested samples. The α-linolenic acid constitutes on average 63.79% of the total fatty acids of chia seed, and for linoleic acid it was 18.89%. All seeds had low n-6 PUFA / n-3 PUFA ratio. Chia seed is the richest of n-3 PUFA α-linolenic fatty acid in the vegetable world. Chia seed are the good choice of healthy food to maintain a balanced serum lipid profile.

Also, **Jones *et al.* (2006)** mentioned that chia seeds had low n-6 PUFA / n-3 PUFA ratio. This observation has important health implications. The best way to lower the risk of coronary heart disease is to keep dietary n-6 PUFA / n-3 PUFA ratios as low as possible.

Table (3): Identification of fatty acids composition of chia seeds:

Fatty acids	**Concentrations %**	**Fatty acids**	**Concentrations %**
Caprlyic acid C8:0	0.01	Tetradecanoic acid (phytoseric) C14:1 n5	0.50
Lauric acid C12:0	0.22	Oliec acid C18:1n9	5.99
Myristic acid C14:0	0.04	**Total mono unsaturated fatty acids**	**6.49**
Palmitic acid C16:0	7.19	Linoleic acid C18:2n6	17.01
Stearic acid C18:0	1.87	Linolenic acid C18:3n3	63.99
Arachidic acid C20:0	0.01	**Polyunsaturated fatty acids**	**81.0**
Total saturated fatty acids	**9.34**	Saturated to unsaturated FA ratio	1:9.4
		ω6:ω3 FA ratio	1:3.8

Identification of phenolic compounds chia seeds:

Data illustrated in table (4) show the identification of phenolic compounds of chia seeds. It is clear to mention that the highest phenolics compounds of chia seeds recorded for caffeic acid, gentisic and rosmarinic acid. The values were 250.96, 205.12 and 148.02 µg/g, respectively. While, the lowest values were recorded for quercetin, kaempferol and syringic acid, the values were 0.33,0.42 and 0.62µg/g DW, respectively. Nevertheless, protocatechuic acid did not determine at this conditions. These results are conform with **Reyes *et al.* (2008)** reported that chia seeds contains a natural antioxidants e.g. asphenolic glycoside-Q and K, chlorogenic acid, caffeic acid, quercetin and kaempferol which protects consumers against some adverse health conditions, such as protection against some cardiovascular diseases and some types of cancer; as well as vitamins and minerals. In addition to a good nutritional composition of chia seed, can also be used a functional food or a nutraceutical which are having potential health benefits.

Table (4): Identification of phenolic compounds in chia seeds

Phenolic compounds	Concentrations (µg/g)
Caffeic acid	250.96
Gentisic	205.12
Rosmarinic	148.02
Cteachin	130.79
Rutin	93.01
Ferulic acid	25.30
Gallic acid	22.03
Vanillic acid	15.95
Cinnamic acid	11.99
P-hydroxybenzoic	6.01
Apigenin	5.07
P-coumaric acid	5.09
Apigenin-7-glucoside	2.93
Chrysin	1.77
Sinapic acid	0.82
Syringic acid	0.62
Kaempferol	0.42
Quercetin	0.33
Protocatechuic acid	ND*

ND* Not determined

Sensory evaluation for un-enriched and enriched pan bread with different levels of chia seeds:

Data tabulated in table (5) show the sensory evaluation of enriched pan bread with different levels of chia seeds. It is clear to notice that control pan bread samples recorded significantly (*P*≤0.05) the highest values for all tested sensory evaluation i.e. (taste, color, crust, odor, texture and overall acceptability) compared to the control and other pan bread enriched with chia seeds. The mean values were 17.69 ±0.01, 8.78 ±0.01, 8.58 ±0.01, 18.33 ±0.05, 18.95 ±1.26and 19.81 ±1.03, respectively.

On the contrary, pan bread with 10% and 12% chia seeds recorded the lowest significantly (*P*≤0.05) difference values for all tested sensory evaluation compared to the control and other pan bread enriched with chia seeds.The mean values were 10.98 ± 0.07, 5.27 ± 0.06, 5.13 ± 0.08, 11.25± 0.10, 12.89 ± 0.07and 13.69 ± 0.13 respectively vs. the values of pan bread with 12% chia seeds recorded11.49,4.88,4.76,13.47,11.0, and 11.9 respectively. After the previous discussion of the results, these treatments (pan bread with 10% and 12%) chia seeds were excluded from applying in this study. It could be noticed from the results that, pan bread with 8% chia seeds recorded the lowest value with significant difference (*P*≤0.05) for all tested sensory evaluation, except for overall acceptability compared to the control and other pan bread enriched with 4% and 6% chia seeds. The mean values were 14.20 ±0.23, 6.88 ±0.05, 6.38 ±0.23, 15.28 ±1.03, 15.34 ±2.28and 18.99 ±0.02, respectively, while, pan bread enriched with 4 and 6% chia seeds recorded moderate sensory evaluation. From the obtained results, it is observable that enriched pan bread with 8% chia seeds showing slight less overall acceptability compared to control (un enriched)but you can use it at commercial scale to improve nutritional value and health status.

In brief, the superior three treatments (4&6 and 8% chia)which obtained high scores by panelist selected to apply in this study.

These results are in agreement with **Sayed-Ahmad *et al.* (2018)** demonstrated that the textural properties (higher moisture content and lower hardness),color, and sensory profiles of wheat / chia bread recorded 3.7 points in global acceptability score (1 to 5).

Also, these results are in assentment with **Youssif and Ghoneim (2017)** indicated that the sensory evaluation data demonstrated that, the multigrain mix (MGM) successfully replaced wheat flour to produce pan bread up to 15% without any unfavorable change.

Romankiewicz *et al.* (2017) observed that in comparison to the control product bread with chia seeds (CS) was characterized by a rich fatty acids composition and higher level of phenolic compounds. Most importantly, the results showed that the substitution of wheat flour with chia seeds up to 6% did not negatively affect the final product acceptance.

Also, **Folasade and Akinoso (2014)** mentioned that, crust color is a very substantial parameter in bread making that reflect the appropriateness of raw material used for the production and provides information about the quality of the bakery product. Crumb color was also significantly reduced by raising the level of substitution with the multigrain mix; it became darker than the control sample.

Table (5): Sensory evaluation of un-enriched and enriched pan bread with different levels of chia seeds:

	Taste	Color Crust		Odor	Texture	Overall acceptability
		Panelist External(10)	Panelist Internal(10)			
Control (un-enriched	17.69 ±0.01[a]	8.78 ±0.01 [a]	8.58 ±0.01 [a]	18.33 ±0.05 [a]	18.95 ±1.26 [a]	19.81 ±1.03 [a]
Enriched pan bread 4%chia seed	16.31±0.12[b]	8.54 ±0.23 [b]	8.11±0.02 [b]	17.77 ±0.03 [b]	17.63 ±1.71 [b]	15.97 ±0.09 [c]
Enriched pan bread 6%chia seed	15.78 ±0.12[c]	7.39 ±0.34 [c]	7.02 ±0.05 [c]	16.25 ±1.14 [c]	16.72 ±1.94 [b]	16.35 ±1.14 [c]
Eenriched pan bread 8%chia seed	14.20 ±0.23 [d]	6.88 ±0.05 [d]	6.38 ±0.23 [d]	15.28 ±1.03 [d]	15.34 ±2.28 [c]	18.99 ±0.02 [b]
Enriched pan bread 10%chia seed %	10.98 ± 0.07[f]	5.27 ± 0.06 [e]	5.13 ± 0.08 [e]	11.25± 0.10 [f]	12.89 ± 0.07 [d]	13.69 ± 0.13 [d]
Enriched pan bread 12%chia seed %	11.49±0.05[e]	4.88 ± 0.09 [f]	4.76 ± 0.09 [f]	13.47 ± 0.07 [e]	11.0 ± 0.12 [e]	11.99 ± 0.10 [e]
LSD	0.0758	0.1103	0.0672	0.3944	0.9398	0.396

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different (P≤0.05), and vice versa

Chemical composition of un- enriched and enriched pan bread with different levels for chia seeds:

The chemical composition of pan bread enriched with different levels of chia seeds are summarized in table (6). It is obvious that, mean values of moisture content for pan bread without and with addition chia seeds were 32.03 ±0.06, 31.33 ±0.02, 31.89 ±0.03 and 31.04 ±0.40(g/100g wt/wt), respectively.

Data displayed that the highest significant (P≤0.05) ratio of protein and ash content for pan bread enriched with 8% of chia seeds compared to pan bread enriched with 4 and 6% of chia seeds. Mean values 14.51 ±1.0 and 3.09

±0.13(g/100g DW), for 8% chia respectively. Also, these data indicated that had non-significant ratio of protein and ash content for pan bread enriched with 4% and 6%of chia seeds. By the antithesis, the lowest significant difference ($P \leq 0.05$) in protein and ash content for pan bread enriched without chia seed. The mean values recorded 9.33 ±0.01 and 1.82 ±0.03(g/100g DW), respectively.

Concerning the crude fat content, it is noticeable the reducing of level fat content for pan bread with chia seeds compared to the control. The lowest significant fat ($P \leq 0.05$) was for pan bread enriched with 8% of chia seeds being 1.52% D/w. whilst the highest significantly ($P \leq 0.05$) for pan bread enriched without of chia seeds being 3.81 ±1.09 (g/100gD/W). Further, fat values reveal more values which were a significant ($P \leq 0.05$) for pan bread enriched with 4% and 6%of chia seeds being 2.94 ±0.29and 2.01 ±0.27(g/100gD/W), respectively.

Furthermore, the crude fiber content raised gradually in the samples after adding chia seeds compared to pan bread without addition chia seeds. It is noteworthy, the highest significantly ($P \leq 0.05$) for pan bread enriched with 8% of chia seeds compared to the control and pan bread enriched with 4%and6% of chia seeds. Mean values of crude fiber classified from the highest to the lowest being 4.0 ±0.31, 3.54 ±0.25, 2.65 ±0.34and 2.09 ±0.17(g/100gD/W), respectively.

Regarding NFE, chia seeds mixed to pan bread explicated significant differences ($P \leq 0.05$) in NFE. The data show that means for NFE ranged from 50.92 ± 0.05, 49.87 ± 0.09, 49.14 ± 0.13, and 45.84 ± 0.0 (g/100g DW), respectively in the control and pan bread enriched with various levels of chia seeds.

As to energy values (Kcal/100g), the data exhibited that the highest significant ($P \leq 0.05$) in the control being 275.2±0.06Kcal/100g. Conversely, the lowest significant ($P \leq 0.05$) in pan bread enriched with 8% of chia seeds followed as 255.08± 0.01Kcal/100g. Moreover, means for energy values ranged from 270.78± 0.02and 260, 21±0.07 respectively in the pan bread enriched with 4% and 6% of chia seeds.

From the obtained results, it could be concluded that addition of 8% chia seeds to pan bread is recommended due to increase the nutritional values of pan bread. The obtained results were partly in accordance to that of **Guiotto *et al.* (2020)**, conducted that the gross chemical compositions of the bread samples containing different levels and types of chia flour showed non-significant differences were found in the moisture contents of the breads with the various substitution levels and types of chia flour in the systems. Notwithstanding, the proximate composition results showed that the incorporation of chia flour significantly ($P \leq 0.05$)increased the protein, dietary fiber and ash contents of the bread samples, while the carbohydrate content and energy value significantly ($P \leq 0.05$) decreased in comparison with the control sample. The contents of these components decreased as the substitution levels of the various chia flours augment in the bread samples.

Coelho and Salas-Mellado (2015) explained that the small incorporation of various types of chia flour in the formulations significantly increased the levels of proteins, dietary fiber and ash in the final products, compared with the wheat control. The formulations containing 5% wt/wt. of chia flour presented the higher technological potential. However, the higher substitution (10% wt/wt) of wheat flour with the various types of chia flour affected the quality parameters.

Table (6): Chemical composition of un- enriched and enriched pan bread with different levels of chia seeds

Nutrition value	Moisture	Protein	Ash	Fat	Crud fiber	NFE	Energy values (Kcal/100g)
			(g/100g DW)				
Control(72%wheat flour)	32.03 ±0.06[a]	9.33 ±0.01[c]	1.82 ±0.03[c]	3.81 ±1.09[a]	2.09 ±0.17[d]	50.92 ± 0.05[a]	275.2±0.06[a]
Enriched pan bread with 4%chia seed	31.33 ±0.02[c]	11.21 ±1.03[b]	2.0 ±0.14[b]	2.94 ±0.29[b]	2.65 ±0.34[c]	49.87 ± 0.09[b]	270.77±0.02[a]
Enriched pan bread with 6%chia seed	31.89 ±0.03[b]	11.39 ±0.27[b]	2.03 ±0.08[b]	2.01 ±0.27[c]	3.54 ±0.25[b]	49.14 ± 0.13[c]	260.16±0.07[c]
Enriched pan bread with 8%chia seed	31.04 ±0.40[d]	14.51 ±1.0[a]	3.09 ±0.13[a]	1.52 ± 0.01[d]	4.0 ±0.31[a]	45.84 ± 0.0[d]	255.08±0.01[d]
LSD	0.1289	0.4609	0.0666	0.3634	0.1729	0.0526	0.0288

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P \leq 0.05$)), and vice versa.

Effect of enriched pan bread with different levels of chia seeds on body weight gain (BWG), food intake (FI) and food efficiency ratio (FER) of (–ve) control and fatty liver rats:

The effect of different levels of pan bread enriched with chia seeds on body weight gain, (BWG), food intake (FI) and food efficiency ratio (FER) of fatty liver rats are shown in table (7). It is clear to notice that the higher body weight gain (BWG) recorded for the negative control groups which fed on (basal diet and basal diet including un- enriched pan bread), while the positive control group which fed on (basal diet including un- enriched pan bread) recorded the lower value with a significant difference $P \leq 0.05$. The mean values were 60.33± 0.02, 56.21±0.12and 22.10±0.21(g/28 day), respectively.

On the other hand, the higher body weight gain of treated groups (fatty liver groups) recorded for pan bread +8% chia seed, while the lower value recorded for pan bread +4% chia seed with a significant difference $P \leq 0.05$. The mean values were 48.25±0.42 and 40.62±0.23 (g/28 day), respectively vs. the positive control group recorded 22.10±0.21(g/28 day).

In case of food intake (FI), it could be concluded that the higher food intake recorded for the negative control groups which fed on (basal diet and basal diet including un- enriched pan bread), while the positive control group which fed on (basal diet including un- enriched pan bread) recorded the lower value with a significant difference $P \leq 0.05$. The mean values were 26.12 ±0.01, 25.75±1.20 and 17.45±1.22g/day, respectively. Moreover, the highest food intake of treated groups (fatty liver groups) recorded for pan bread +8% chia seed, while the lowest value recorded for pan bread +4% chia seed with a significant difference $P \leq 0.05$. The mean values were 24.15±1.20and 22.70±1.14g/day, respectively as compared with the positive control group which recorded 17.45±1.22g/day.

With regard to food efficiency ratio (FER), it could be noticed that the higher food efficiency ratio recorded for the negative control groups that fed on (basal diet and basal diet including un- enriched pan bread), while the positive control group recorded the lower value with a significant difference $P \leq 0.05$. The mean values were 0.0825 ± 0.001, 0.078 ± 0.004and 0.045 ± 0.002, respectively.

Furthermore, the higher food efficiency ratio of treated groups (treated groups) recorded for pan bread +8% chia seed, while the lower value recorded for pan bread +4% chia seed with significant differences $P \leq 0.05$. The mean values were 0.071 ±0.005and 0.064 ±0.003, respectively as compared with the positive control group which were (0.045 ± 0.002)

Medina-Urrutia *et al.* (2020) showed that 25 g/day of milled chia ameliorates NAFLD. Chia is an accessible vegetal source of omega-3 fatty acids, antioxidants, and fiber, which could have the potential to prevent metabolic abnormalities in NAFLD patients. Considering that there is no pharmacological treatment approved for NAFLD, the findings of the present study suggest that a chia-supplemented diet could be an innovative alternative to control this disease.

Also, **Jin *et al.* (2012)** proved the effect of whole and milled chia seeds on plasma α-linolenic acid (ALA) concentrations, finding that, independently of body weight, plasma ALA was increased by 24% when supplementing the habitual diet with 25 g/day of milled chia during 7 weeks.

Table (7): Effect of different levels of pan bread enriched with chia seeds on body weight gain (BWG), food intake (FI) and food efficiency ratio (FER) of (-ve) contral and rats induced fatty liver

	Groups \ Parameters	BWG (g/28 day)	FI (g/day)	FER (g/day)
Healthy groups	Control (-ve)	60.33± 0.02 [a]	26.12 ±0.01[a]	0.0825 ± 0.001[a]
	Control (-ve) pan bread	56.21±0.12[b]	25.75±1.20[a]	0.078 ± 0.004[b]
Fatty liver groups treated with	Control (+ve) pan bread	22.10±0.21[f]	17.45±1.22[d]	0.045 ± 0.002[e]
	Pan bread with4% chia seed	40.62±0.23[e]	22.70±1.14[c]	0.064 ±0.003[d]
	Pan bread with 6% chia seed	42.37±0.10[d]	23.27±1.23[bc]	0.065 ±0.003[d]
	Pan bread with% chia seed	48.25±0.42[c]	24.15±1.20[b]	0.071 ±0.005[c]
	LSD	0.1998	0.9825	0.0043

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P \leq 0.05$), and vice versa.

Effect of enriched pan bread with different levels of chia seeds on lipid profile and atherogenic index of(−ve) control and fatty liver rats:

Data presented in table (8) depicted the effect of pan bread enriched with different levels of chia seeds on lipid profile of fatty liver rats. It is evident to observation the value of total lipid (TL) for the positive control group was higher than the negative control groups which fed on (basal diet and basal diet including un- enriched pan bread) with a significant differences ($P \leq 0.05$). The mean values were 688.66±0.00, 486.66± 0.04 and 501.86±0.32mg/dl, respectively. On the other hand, the highest total lipid of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While the lowest value recorded for pan bread with 8% chia seeds with significant differences ($P \leq 0.05$). The mean values were 584.89±0.92 and 510.03±1.18mg/dl, respectively vs. the positive control group which fed on un- enriched pan bread being (688.66±0.00 mg/dl).

Regarding total cholesterol (TC), it is obvious that the value of TC of the positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 202.15±0.31, 147.72±0.52 and 138.29±0.0 mg/dl, respectively. Moreover, the highest TC of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences ($P \leq 0.05$). The mean values were 189.35±0.35 and 160.36±0.31mg/dl, respectively when compared with the positive control group which were 202.15±0.31mg/dl. These data are harmonious with **Alamri (2019)** confirmed that the proportion of TC were significantly reduced in treated groups fed on white and black chia seeds compared with the positive control groups.

Coelho *et al.* (2018) pointed that chia components are chia proteins and bioactive peptides that inhibited main factors of the cholesterol structure dissertation, i.e.3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase).

In case of triglycerides (TG), the value of TG of the positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 162.9±0.05, 129.67±0.01and 119.46^{f}±0.08mg/dl, respectively. Further, the highest TG of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. whilst, the lowest value recorded for pan bread with 8% chia seeds with significant differences ($P \leq 0.05$). The mean values were 154.29±0.04 and 136.93±0.05mg/dl, respectively compared with the positive control groups as followed 162.9±0.05mg/dl.

These finding are identical to **Oliva *et al.* (2013)** indicated that the ratio of TG significantly higher in the positive control groups, compared to treated groups provided with chia seeds for 21days.

In connection with high density lipoprotein (HDL-c), it is conspicuous to mention that the value of HDL-c of the negative control groups were higher than the positive control with significant differences ($P \leq 0.05$). The mean values were 63.15±0.04, 57.39±0.39 and 35.12±0.12 mg/dl, respectively. Conversely though, the highest HDL-c of treated groups (fatty liver groups) recorded for pan bread with 8% chia seeds. While, the lowest value recorded for pan bread with 4% chia seeds with significant differences ($P \leq 0.05$). The mean values were 50.87±0.07 and 40.96±0.02 mg/dl, respectively when compared to the positive control 35.12±0.12 mg/dl.These findings are congruent with **da Silva *et al.* (2016)** demonstrated that, concentrations of HDL-c in treated groups provided with chia seeds had significantly higher compared to the positive control group.With respect to low density lipoprotein (LDL-c), the value of LDL-c of the positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 134.44±0.04, 64.40±0.02 and 51.25±0.04mg/dl, respectively. Nevertheless, the highest LDL-c of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences ($P \leq 0.05$). The mean values were 117.53±0.01 and 82.10±0.05 mg/dl, respectively vs. the positive control group which being 134.44±0.04 mg/dl.

Concerning low density lipoprotein (VLDL-c), the value of VLDL-c of positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 32.59±0.02, 25.93±0.01 and 23.88±0.10mg/dl, respectively. On the contrary, the highest VLDL-c of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences (P≤0.05). The mean values were 30.86±0.04 and 27.39±0.03 mg/dl, respectively as compared to the positive control group that were32.59±0.02 mg/dl.

These data are in similar study by **da Silva *et al.* (2016)** stated that rats provided with traditional and processed chia seed led to decrease in the levels of LDL-c and VLDL-c.

Regarding atherogenic index (AI), the value of AI of the positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 4.70±0.06, 1.57±0.03and 1.19±0.02%, respectively. On the other hand, the highest AI of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences ($P \leq 0.05$). The mean values were 3.60±0.01and 2.15±0.05 %when compared to the positive control group 4.70±0.06mg/dl.

It could be concluded from the displayed of the previous results that the positive effect for serum lipid parameters of rats after fooding on chia seeds especially ratio8% has the preferable for 28 days.

Medina-Urrutia *et al.* (2020) reported that dietary chia supplementation induced an increase in plasma α-linolenic acid (ALA) concentration (75%) and dietary fiber (55%) consumption. After chia supplementation, total cholesterol (2.5%), non-high density lipoprotein cholesterol (3.2%), and circulating FFA (8%) decreased. Furthermore, NAFLD regressed in 52% of the treated patients ($P < 0.05$ for all).

Montes Chañi *et al.* (2018) mentioned that chia seed (*Salvia hispanica*) is the richest vegetal source of omega 3-fatty acids, antioxidants, and fiber. Although some animal models have suggested that chia could be used as an alternative to reduce intrahepatic fat content.

Table (8): Effect of enriched pan bread with different levels of chia seeds on lipid profile and atherogenic index of(−ve) control and fatty liver rats

	Parameters / Groups	TL	TC	TG	HDL-c	LDL-c	VLDL-c	AI
		mg/dl						%
Healthy groups	Control (-ve)	486.66± 0.04f	138.29± 0.0 f	119.46± 0.08 f	63.15± 0.04 a	51.25± 0.04 f	23.88± 0.10 f	1.19± 0.02 f
	Control (-ve)pan bread	501.86± 0.32e	147.72± 0.52e	129.67± 0.01e	57.39± 0.01b	64.40± 0.02e	25.93± 0.01e	1.57± 0.03e
Fatty liver groups treated with	Control (+ve) pan bread	688.66± 0.00a	202.15± 0.31a	162.9± 0.05a	35.12± 0.05f	134.44± 0.04a	32.59± 0.02a	4.70± 0.06a
	Pan bread with 4% chia seed)	584.89± 0.92b	189.35± 0.35b	154.29± 0.04b	40.96± 0.02e	117.53± 0.01b	30.86± 0.04b	3.60± 0.01b
	Pan bread with 6% chia seed	562.34± 0.79c	177.64± 0.36c	145.88± 0.03c	42.58± 0.03d	105.88± 0.03c	29.18± 0.01c	3.17± 0.02c
	Pan bread with 8% chia seed)	510.03± 1.18d	160.36± 0.31d	136.93± 0.05d	50.87± 0.07c	82.10± 0.05d	27.39± 0.03d	2.15± 0.05d
	LSD	**0.6289**	**0.3102**	**0.0443**	**0.036**	**0.2992**	**0.0367**	**0.0091**

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P \leq 0.05$), and vice versa.

TL= total lipid. TC= total cholesterol. TG= triglycerides. HDL-c= high density lipoprotein. LDL= low density lipoprotein. VLDL-c= very low density lipoprotein. AI= Atherogenic index.

Effect of enriched pan bread with different levels of chia seeds on liver functions and bilirubin of(−ve) control and fatty liver rats:

Data given in Table (9) displayed the effect of pan bread enriched with different levels of chia seeds on liver functions (ALT, AST, ALP, GGT, and bilirubin) of fatty liver rats. It is obvious that the value of AST liver enzyme of the positive control was higher than the negative control groups with significant differences ($P \leq 0.05$). The mean values were 79.17±0.64, 49.72±0.31 and 40.31±0.01 U/L, respectively. On the other hand, the highest AST of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences (P≤0.05) (P≤0.05). The mean values were 59.84±1.29 and 50.33±0.00 U/L, respectively vs. the positive control group followed as 79.17±0.64U/L.

As for ALT liver enzyme, it is noticeably that the value of ALT liver enzyme activiting of the positive control was higher than the negative control groups with significant differences (*P*≤0.05). The mean values were 81.64±0.50, 47.87±0.01and 41.22±0.06 U/L, respectively. On the contrary, the highest ALT of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. However, the lowest value recorded for pan bread with 8% chia seeds with significant differences (P≤0.05).The mean values were 65.25±0.91 and 51.27±1.28 U/L, respectively as compared to the positive control group recorded 81.64±0.50U/L.

These data are harmonious with **Alamri (2019)** confirmed that the levels of ALT and AST were significantly higher in the negative and the positive control group compared with the rats fed on chia seed groups.

In regard to ALP liver enzyme, data indicated that the value of ALP liver enzyme of the positive control was higher than the negative control groups with significant differences (*P*≤0.05). The mean values were 222.33±0.60, 130.10±0.35 and 122.62±0.08U/L, respectively. Further, lower activity recorded for pan bread 4% chia seeds. Whilst, the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05). The mean values were 185.28±0.82 and 149.34±9.30 U/L, respectively when compared with the positive control group recorded 222.33±0.60 U/L.

In connection with GGT, it could be notice that the value of GGT of the positive control was higher than the negative control groups with significant differences (*P*≤0.05). The mean values were 98.55±4.45, 60.11±1.96 and 52.81±0.07 U/L, respectively. On the contrary, the highest GGT of treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. While, the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05). The mean values were 85.31±3.67 and 72.10 ±0.26 U/L, respectively vs. compared to the positive control group 98.55±4.45 U/L.

The value of bilirubin of the positive control was higher than the negative control groups with significant differences (*P*≤0.05). The mean values were 1.91±0.24, 0.40 ±0.07and0.21±0.08 g/dl, respectively. On the contrariwise, the highest of bilirubin treated groups (fatty liver groups) recorded for pan bread with 4% chia seeds. Whereas, the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05).The mean values were 0.75±0.08 and 0.59±0.07 g/dl, respectively as compared to the positive control which mean value was 1.91±0.24g/dl.

These results are in agreement with **Marineli Rda *et al.* (2015)** reported that the consumption of white chia seeds was found to be effective for reducing liver damage by reducing AST and ALT levels. An improvement in lipid profiles and liver function has been observed following the consumption of both black and white chia seeds, which can be attributed to the high level of omega-3 fatty acids in chia seeds.

Fernandez-Martinez *et al.* (2019) reported that the hypolipidemic and hepatoprotective effects of chia may be correlated to its high content of α-linolenic acid (omega-3), fiber, protein, and phenolic compounds.

Kumagai *et al.* (2013), they reported that bilirubin is the breakdown product of haeme, found in haemoglobin, the prime constituent of red blood cells (RBCs). Hepatic group showed an increase in direct and total bilirubin level compared to the normal range, indicating a decreased bilirubin clearance from blood.

Table (9): Effect of enriched pan bread with different levels of chia seeds on liver functions and bilirubin of (−ve) control and fatty liver rats

	Parameters / Groups	AST	ALT	ALP	GGT	Bilirubin
		U/L				g/dl
Healthy groups	Control (-ve)	40.31±0.01[e]	41.22±0.06[f]	122.62±0.08[f]	52.81±0.07[f]	0.21±0.08[e]
	Control (-ve) pan bread	49.72±0.31[d]	47.87±0.01[e]	130.10±0.35[e]	60.11±1.96[e]	0.40±0.07[d]
Fatty liver groups treated with	Control (+ve) pan bread	79.17±0.64[a]	81.64±0.50[a]	222.33±0.60[a]	98.55±4.45[a]	1.91±0.24[a]
	Pan bread with 4% chia seed	59.84±1.29b	65.25±0.91[b]	185.28±0.82[b]	85.31±3.67[b]	0.75±0.08[b]
	Pan bread with 6% chia seed	52.38±1.07[c]	59.19±0.17[c]	165.31±2.10[c]	77.67±4.06[c]	0.62±0.01[c]
	Pan bread with 8% chia seed	50.33±0.00[d]	51.27±1.28[d]	149.34±9.30[d]	72.10±0.26[d]	0.59±0.07[c]
	LSD	**0.6684**	**0.6089**	**3.512**	**2.6827**	**0.104**

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P \leq 0.05$) and vice versa.

Effect of enriched pan bread with different levels of chia seeds on kidney functions, and glycemic index of (−ve) control and fatty liver rats:

Data tabulated in table (10) show the effect of pan bread enriched with different levels of chia seeds on kidney functions and **glycemic index** e.g. (glucose, HOMA- IR and insulin) of fatty liver rats.

The findings show that the uric acid of the positive control group was higher than negative control groups with significant differences (*P*≤0.05). The mean values were 79.99±0.47,45.77± 0.14 and 40.33± 0.10 mg/dl, respectively.

Moreover, the uric acid of treated groups (fatty liver groups) recorded the highest value for pan bread with 4% chia seeds, while the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05). The mean values were 75.63±0.41 and 49.27± 0.42 mg/dl, respectively when compared to the positive control group being 79.99 ±0.47 mg/dl.

Concerning of creatinine level, data indicated that the creatinine of the positive control group was higher than the negative control groups with significant differences (*P*≤0.05). The mean values were 1.11±0.07, 0.61±0.11 and 0.53±0.01 mg/dl, respectively.

Nevertheless, creatinine level of treated groups (fatty liver groups) recorded the highest value for pan bread with 4% chia seeds, but the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05). The mean values were 0.82±0.13 and 0.70± 0.12 mg/dl, respectively vs. the positive control group which value was 1.11 ±0.07 mg/dl.

These results are in the same line with **Makris (2018)** who reported that urea is a water-soluble side product of protein catabolism, mainly produced by the liver and excreted by the kidneys. The nitrogen content of BUN is more commonly evaluated by clinicians. Creatinine superiorly reflects the glomerular filtration rate, and hence, renal functionality. Even so, BUN and creatinine are the first-order tests to evaluate renal function.

Also, **Levey and Inker (2016)** mentioned that chia-rich diet can alter renal function which was evaluated by measuring BUN, creatinine, and BCR. The controversy may be originated from the differences between species, age, weight, and even the amount of water ingested daily. Noteworthy, the glomerular filtration rate was not measured in this study. Although urea and creatinine are valuable indicators, the gold standard for assessing renal function is the glomerular filtration rate.

From the same table (10), data showed that the glucose level of the positive control group was higher than that of the negative control groups with significant differences (*P*≤0.05). The mean values were 285.34± 0.38, 125.38± 0.29 and 120.11± 0.01mg/dl, respectively. Notwithstanding, the glucose level of treated groups (fatty liver groups) recorded the highest value for pan bread with 4% chia seeds, while the lowest value recorded for pan bread with 8% chia seeds with significant differences (*P*≤0.05). The mean values were 176.78±

1.19 and 156.82±0.51 mg/dl, respectively as compared to the positive control group being 285.34± 0.38mg/dl.

The obtained data were in the line with **da Silva *et al.* (2016)** conducted that treated groups presented that the chia seed decreased in the concentration of the blood glucose compared to the positive control. Also, *In vivo* study by **Ho *et al.* (2013)** who demonstrated that bread fortified with chia seeds improved the concentration for blood glucose in humans.

In case of insulin value, data appeared that the concentration of insulin in the negative control groups was higher than that of the positive control group with significant differences ($P \leq 0.05$). The mean values were 21.70± 0.0,27.53± 1.11 and 12.34±1.28µU/ml, respectively. Moreover, the concentration of insulin for treated groups (fatty liver groups) recorded the highest value for pan bread with 8% chia seeds, while the lowest value recorded for pan bread with 4% chia seeds with significant differences ($P \leq 0.05$). The mean values were 21.01± 0.03 and 17.82± 0.54µU/ml, respectively *vs.* the positive control group where the mean value was 12.34± 1.28µU/ml. These data are variance with **Creus *et al.* (2016)** noted that the levels of revealed insulin non-significant in values for treated groups after the end experiment.

Within regard to of insulin-resistance level, the finding reveal that the level of insulin-resistance in the negative control groups was lower than that of the positive control group with significant differences ($P \leq 0.05$). The mean values were 115.84±0.01,153.43±0.0 and 158.0±0.02, respectively. Moreover, the concentration of insulin resistance for treated groups (fatty liver groups) recorded the highest value for pan bread with 8% chia seeds, while the lowest value recorded for pan bread with 4% chia seeds with significant differences ($P \leq 0.05$). The mean values were 140.02±5.16and 146.43±0.67, respectively when compared to the positive control group followed as 158.0±0.02.

It was evident from the previous discussion of the results obtained that an improvement in glycemic index of fatty liver rats which practiced the consumption of enriched pan bread with chia seeds was due to this content of chia seeds which is rich dietary in fiber **Parker *et al.* (2018)**.

Elleuch (2008) pointed that the viscosity of the intestinal lumen can be raised by the utilization of soluble dietary fiber, that can reduce the assimilation of glucose due to the lowering in connect between glucose and the enterocyte.

Oliva *et al.* (2013) reported that insulin-resistance and NAFLD animal model studies have shown that the administration of a chia-based diet was useful, aside from decreasing de novo lipogenesis and improving betaoxidation. These findings suggest an improvement in adipose tissue insulin sensitivity and reduction in de novo lipogenesis as a result of chia intake.

Table (10): Effect of enriched pan bread with different levels of chia seeds on kidney functions, and glycemic index of(−ve) control and fatty liver rats

Groups	Parameters	Uric acid	Creatinine mg/dl	Glucose	Insulin (µU/ml)	HOMA- IR*
Healthy groups	Control (-ve)	40.3±0.10[f]	0.53±0.01[e]	120.11±0.01[f]	21.70±0.0[b]	115.84±0.01[f]
	Control (-ve)	45.77±0.14[e]	0.61±0.11[d]	125.38±0.29[e]	27.53±1.11[a]	153.43 ± 0.0[b]
Fatty liver groups treated with	Control (+ve)	79.99±0.47[a]	1.11±0.07[a]	285.34±0.38[a]	12.46±1.28[f]	158.0± 0.02[a]
	Pan bread with 4%chia see	75.63±0.41[b]	0.82±0.13[b]	176.78±1.19[b]	17.82±0.54[e]	140.02 ± 5.16[d]
	Pan bread with 6%chia seed	52.58±0.88[c]	0.78±0.01[bc]	162.29±0.96[c]	18.54±0.39[d]	133.72 ± 3.63[e]
	Pan bread with 8%chia seed	49.27±0.42[d]	0.70±0.12[c]	156.82±0.51[d]	21.01±0.03[c]	146.43 ± 0.67[c]
	LSD	**0.4273**	**0.0798**	**0.6147**	**0.6583**	**2.3201**

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P \leq 0.05$) and vice versa. **IR** *= insulin-resistance.

Effect of pan bread enriched with different levels of chia seeds on TBARS Anti-oxidative enzyme and TNF-α of(−ve) control and fatty liver rats:

Data given in table (11) show the effect of pan bread enriched with different levels of chia seeds on TBARS, anti- oxidative enzyme and TNF-α of fatty liver rats.

The results reveal that the TBARS of the positive control group was higher than that of the negative control groups with significant differences ($P \leq 0.05$). The mean values were 0.98±0.02, 0.58±0.0 and 0.50±0.10 nmol/ml, respectively.

On the other hand, the TBARS of treated groups (fatty liver groups) recorded the highest value for pan bread with 4% chia seeds, while the lowest value recorded for pan bread with 8% chia seeds with significant differences($P \leq 0.05$). The mean values were 0.83±0.13 and 0.60±0.01nmol/ml, respectively when compared with the positive control group being0.98±0.02 nmol/ml.

In case of glutathione (GSH), it is clear to notice that the negative control groups was higher than that of positive control group with significant differences ($P \leq 0.05$). The mean values were 0.60±0.02,0.55±0.01 and 0.23±0.03U/ml, respectively.

Moreover, the treated groups (fatty liver groups) recorded the highest value of GSH for pan bread with 8% chia seeds, while the lowest value recorded for pan bread with 4% chia seeds with significant differences ($P \leq 0.05$). The mean values were 0.50± 0.01and 0.40±0.09nmol/ml, respectively vs. the positive control group being 0. 23±0.03U/ml.

As for superoxide dismutase (SOD), it is clear to notice that the negative control groups was higher than that of positive control group with significant differences ($P \leq 0.05$). The mean values were 2.97±0.01, 2.02±0.11, and 1.54±0.0U/ml, respectively.

Further, the treated groups (fatty liver groups) recorded the highest value of SOD for pan bread with 8% chia seeds, while the lowest value recorded for pan bread with 4% chia seeds with significant differences ($P \leq 0.05$). The mean values were 2.62± 0.04and 2.11± 0.07U/ml, respectively as compared to the positive control group being 1.54±0.0U/ml.

Concerning catalase (CAT) enzyme, data showed that the negative control groups was higher than that of the positive control group with significant differences ($P \leq 0.05$). The mean values were 55.83±0.03, 52.85±0.05 and 24.45±0.06U/ml, respectively.

Whilst, the treated groups (fatty liver groups) recorded the highest value of CAT for pan bread with 8% chia seeds, while the lowest value recorded for pan bread with 4% chia seeds with significant differences. The mean values were 47.89±0.04 and 40.67±0.10U/ml, respectively when compared with the positive control group that recorded 24.45±0.06U/ml.

As to, TNF-α, it is clear to notice that the positive control group was higher than that of the negative control groups with significant differences ($P \leq 0.05$). The mean values were 9.91±0.04, 3.59±0.09 and 4.00±0.05 pg/ml, respectively.

Notwithstanding, the treated groups (fatty liver groups) recorded the highest value of TNF-α for pan bread with 4% chia seeds, while the lowest value recorded for pan bread with 8% chia seeds with significant differences. The mean values were 5.39±1.03 and 4.08±0.01pg/ml, respectively *vs*. the positive control group being 9.91±0.04 pg/ml.

These results are in harmony with **Marineli Rda *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 SOD concentrations in *Wistar* rats. Plasma catalase concentration was higher in the animals that consumed chia in our study,owing to a positive effect of the compounds present in this food on the activity of the antioxidant enzyme capable of decomposing hydrogen peroxide.

Also, **Ferreira *et al.* (2018)** they reported that insulin-resistance and NAFLD animal model studies have shown 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 (11): Effect of enriched pan bread with different levels of chia seeds on TBARS, anti-oxidative enzyme and TNF-α of(-ve) control and fatty liver rat

Groups	Parameters	TBARS (nmol/ml)	GSH (U/ml)	SOD (U/ml)	CAT (u/min/ml)	TNF-α* (pg/ml)
Healthy groups	Control (-ve)	0.50 ± 0.10^{e}	0.60 ± 0.02^{a}	2.97 ± 0.01^{a}	55.83 ± 0.03^{a}	$3.59^{e}\pm0.09^{e}$
	Control (-ve) pan bread	0.58 ± 0.00^{d}	0.55 ± 0.01^{b}	2.02 ± 0.11^{e}	52.85 ± 0.05^{b}	$4.00^{d}\pm0.05^{d}$
Fatty liver groups treated with	Control (+ve) pan bread	0.98 ± 0.02^{a}	0.23 ± 0.03^{e}	1.54 ± 0.00^{f}	24.45±0.06f	$9.91^{a}\pm0.04^{a}$
	Pan bread with 4% chia seed	0.83 ± 0.13^{b}	0.40 ± 0.09^{d}	2.11 ± 0.07^{d}	40.67 ± 0.10^{e}	$5.39^{b}\pm1.03^{b}$
	Pan bread with 6% chia seed	0.67 ± 0.11^{c}	0.43 ± 0.02^{d}	2.47 ± 0.00^{c}	44.64 ± 0.06^{d}	$4.81^{c}\pm0.08^{c}$
	Pan bread with 8% chia seed	0.60 ± 0.01^{d}	0.50 ± 0.01^{c}	2.62 ± 0.04^{b}	47.89 ± 0.04^{c}	$4.08^{d}\pm0.01^{d}$
	LSD	**0.0708**	**0.037**	**0.0502**	**0.0537**	**0.3819**

Values are expressed as mean ± SD.Mean value with different letters in the same column are significantly different ($P\leq0.05$),and vice versa.
TNF-α*: Tumor necrosis factor α.

CONCLUSION

As conclusion, chia seeds are the richest vegetal sources of various compounds such as dietary fiber, phenolic compounds and nutrients. Additionally, the results obtained from the current study that, treated groups (fatty liver) fed on pan bread enriched with chia seeds especially 8% has the superior influence for improving levels of glucose, kidney functions, liver enzymes and lipid profile. Consequently, the current study recommends promoting and encouraging the use of chia seeds products as they are functional foods protecting us from different degenerative diseases.

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استخدام خبز القالب المعزز ببذور الشيا للحد من بعض الآثار الجانبية للكبد الدهني الناتج عن تناول الفركتوز في ذكورالفئران.

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مرض الكبد الدهني غير الكحولي (NAFLD) هو اضطراب فى التمثيل الغذائى يتميز بالتراكم المفرط للدهون الثلاثية في خلايا الكبد. لوحظ ازدياد نسبة مرض الكبد الدهني غير الكحولي بالتزامن مع السمنة في مصر. كان الهدف من هذه الدراسة هو تقدير التركيب الكيميائي والتقييم الحسي وتاثير استهلاك خبز القالب الكنترول والخبز القالب المعزز ببذور الشيا علي الفئران المصابه بالكبد الدهني وكما تم استخدام ثمانيه و أربعون من ذكور الفئران البيضاء قسمت إلي ست مجموعات (كل مجموعة = ٨ فئران) ،حيث تم استخدام (١٦) فئران كمجموعتين ضابطتيين سالبتين الاولي تتغذت على النظام الغذائي الأساسي والثانية تغذت علي النظام الغذائي الأساسي مع الخبز الغير معزز. بينما باقى المجاميع ٣٢ من ذكور الفئران تم تغذيتها على النظام الغذائي ألاساسي مع ٢٥% فركتوز مذاب فى ماء الشرب لمدة ثمانية أسابيع متتالية للاصابة بالكبد الدهني ، تم إعادة تقسيم الفئران كمجموعة ضابطه موجبة والتي تتغذت على النظام الغذائي الأساسي مع الخبز الغير معزز ثم باقى ثلاث مجموعات من الفئران تمت معالجتها بمستويات مختلفة من خبز القالب المستبدل بدقيق بذور الشيا بنسبة ٤ ٪ ، ٦ ٪ ، ٨ ٪. واستمرت فترة التجربة لمدة ٢٨ يومًا بعد الاصابة بالكبد الدهنى بتناول الفركتوز لمدة ثمانية اسابيع. أظهرت النتائج أن بذور الشيا غنية بالمركبات الفينولية التي تعتبر من مضادات الأكسدة. كما أشارت النتائج إلى أن المجموعة الضابطة الموجبة اظهرت نقص معنوي في الزيادة في الوزن والمتناول من الطعام ونسبة كفاءة الغذاء ومستوى الدهون مرتفعة الكثافة (HDL-c). وزيادة معنوية عالية في سيرم الدم لانزيمات الكبد والكلى والبيليروبين ومستوى الجلوكوز والدهون الكلية والكوليستيرول الكلى والجلسريدات الثلاثية وعامل تكوين الاورام والدهون منخفضة الكثافة.وذلك بالمقارنة بالمجموعة السالبة والمجموعات المعالجة كما اظهرت نتائج جميع المجموعات المعالجة زيادة معنوية في وزن الجسم والطعام المتناول ونسبة كفاءة الغذاء و مستوى الدهون مرتفعة الكثافة (HDL-c). ونقص معنوي في سيرم الدم لانزيمات الكبد والكلى والبيليروبين ومستوى الجلوكوز والدهون الكلية والكوليستيرول الكلى والجلسريدات الثلاثية وعامل تكوين الاورام والدهون منخفضة الكثافة.وذلك بالمقارنة بالمجموعة الموجبة ونستخلص من نتائج الدراسة ان تناول خبز القوالب المدعم ببذور الشيا يعمل علي تقليل الآثار الجانبية للكبد الدهني ويساعد علي تحسين وظائف الكبد الدهني في الحالة الصحية في الفئران المصابة بالكبد الدهني من الفركتوز.

الكلمات الأفتتاحية: الكبد الدهني غير الكحولي ـ الفئران ـ الفركتوز – بذور الشيا ـ التحاليل الكيميائية الحيوية.