### Potential Therapeutic Effects of Chia and Sunflower on Rats Fed High Fat Diet

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### مجلة البحوث في مجالات التربية النوعية

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## Potential Therapeutic Effects of Chia and Sunflower on Rats Fed High Fat Diet.

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#### **ABSTRACT**

The present study was conducted to investigate the potential therapeutic effects of chia, sunflower seeds, and their mixture(CS, SFS and Mix) as rich sources of ALA in different concentrations (2.5 and 5%) on rats fed high fat diet. Forty-eight female albino rats weighting (150±10 g) were divided in eight equal groups(n=6), the first group: Fed on basal diet. All remaining rats (n=42) fed on high fat diet (HFD) and classified into the follow Group 2: positive control group. Groups 3 and 4: Fed on HFD +2.5 and 5 % CS, respectively. Groups 5 and 6: Fed on HFD + SFS, respectively. Finally, groups 7 and 8: Fed on HFD +2.5 and 5%Mix, respectively. The experiment lasted for 28 days. The best results for liver enzymes were recorded in group four (5%CS) with percent of change 40.00,48.75 and 22.96 for GPT, GOT and ALP, respectively. While, the best values of LDLc, serum glucose level and kidney function were recorded in group eight (5% Mix) where there was a significant reduction  $p \le 0.05$  with percent of change 60.00 and 51.7 % for LDLc and serum glucose level & 61.76 and 52.00 % for urea and creatinine. In conclusion, this study recommended the incorporation of CS and SFS as functional foods in diet daily routine to provide more effective health benefits.

**Key words:** powder;  $\alpha$ -linolenic acid; rats; functional foods; omega 3.

#### **INTRODUCTION:**

Consumption of high fat diet (HFD) is the main risk factor for various diseases, especially obesity and overweight prevalence and other related diseases (Woods et al., 2003 and Noeman et al., 2011). HFD is associated with a cardiovascular disease incidence through increasing low density lipoprotein cholesterol (LDLc) and reducing high density lipoprotein (HDLc) levels (Guay et al Additionally, it can induce insulin resistance, dysregulation of glucose and lipid metabolism as well as hepatic steatosis in liver thus HFD has an undesirable effect on liver diabetes and coronary heart diseases (Vieira et al., 2009and Suk and Shin, 2015). In the last decade,  $\alpha$ -linolenic acid (ALA) as a polyunsaturated n-3 (omega-3) fatty acid, has attracted the attention of scientific researchers to demonstrate its role in prevention and treatment of some diseases and they concluded that ALA could reduce the inflammation markers and this explains the mechanism of its beneficial effect on many diseases (**Poudyal** et al., 2013). Also, supplementation with ALA may prevent endothelial dysfunction in the process of diabetes (Zhang et al., 2013). Oilseeds and nuts classified as the richest sources of ALA (Richmond et al., 2013). Based on that chia and sunflower seeds were selected to be examined on rats fed high fat diet.

Chia seed (CS) (Salvia hispanica L.) is widely used as a functional food. It has a great potential to produce healthy improved nutritional value (Marcinek and products with Krejpcio, 2017 and Kwon et al., 2019). This potential may be correlated to its high content of fiber, protein, essential amino acids and polyunsaturated fatty acids (Capitani et al., 2012; Ding et al., 2018; Grancieri et al., 2019 and Melo et al., (2019). lipids ,15-40% protein ,26-41% It contains about 40 % carbohydrates and 18-30% fiber, the main fatty acid of CS is  $\alpha$ - linolenic acid ( $\omega$ - 3) with up to 68 % (Ayerza and Coates, 2004 and Nduko et al., 2018), so it could be used as a healthy supplement with nontoxic effect on human health. In addition, the presence of isoflavone and antioxidant components in CS such chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol, are also responsible for its effectiveness on various diseases (Martínez-Cruz and Paredes-López, 2014 and Yurt and Gezer, 2018). It must be mentioned that CS is gluten, cholesterol and trans fats free (Ullah et al., 2016).

Sunflower seed (SFS) with the scientific name of (Helianthus annuus L.) belongs to the Asteraceae family. It is used in many food applications for providing many benefits (Guo et al., 2017). SFS contains various nutrients that play helpful role in prevention and treatment of many diseases such as hypertension, diabetes, cardiovascular diseases and cancer (Saxena et al., 2007). The high content of phenolic compounds, flavonoids, polyunsaturated fatty acids, minerals, and vitamins of SFS participated in giving antioxidant and anti-inflammatory properties (Fowler, 2006 and Brătfălean et al., 2008). This seed contains about 17.00,26.10, 29.10 and 3.10 % for protein, fat, fiber, and ash, respectively (Olomu, 1995). SFS is the best natural dietary source of vitamin E and known with its rich content of polyunsaturated fat (Akande, 2011).

Therefore, this study was conducted to investigate the potential therapeutic effects of CS, SFS and their mixtures in different concentrations (2.5 and 5%) on rats fed high fat diet.

#### MATERIALS AND METHODS

#### **Materials**

Chia and sunflower seeds were obtained from Agricultural Seed, Spices and Medicinal Plants Co. (Harras), Cairo, Egypt. Casein, cellulose, choline chloride powder, and DL- methionine powder, were obtained from Morgan Co. Cairo, Egypt. Vitamins and salts mixture components were purchased from Techno-Gene, Chemical Co., El Doki, Egypt.

#### Rats

Forty-eight adult white female albino rats weighing (150  $\pm$  10 g), were obtained from research Institute of Ophthalmology, Medical Analysis Department Giza, Egypt.

#### **Methods**

#### **Samples preparation**

Chia and sunflower seeds were milled by electric grinder (Moulinex, France) to obtain powder form and kept stored at 4°C in clean bags until used.

#### **Diet**

The basic diet prepared according to (AIN, 1993). While vitamins and salts mixture components were formulated according to (Campbell, 1963 and Hegsted, 1941), respectively. The high fat diet was prepared according to (Woods *et al.*, 2003).

#### **Experimental design**

This work was carried out at the biology laboratory, Faculty of Home Economic, Menoufia University, Egypt. All rats fed a standard diet for 7 days for adaptation. The rats were kept in cylindrical wire cages with wire bottoms and were provided with diet in special food cups to avoid scattering. Also, water was introduced to the rats by glass tube projection through the wire cage. After adaptation period, rats were divided to eight groups (6 animals each) as following: Group 1: Fed on basal diet and kept as a control negative group. Group (2): Fed on High fat diet (HFD) contained 20 g of tallow /100 g of diet and kept as a control (+Ve). Groups (3and 4): fed on HFD+ 2.5% and 5% of chia seeds (CS), respectively while groups (5and 6) fed on HFD+ 2.5 and 5% of sunflower seeds(SFS) respectively, the last groups (7and 8) fed on Mixture of chia and sunflower seeds (1:1) with 2.5% and 5%, respectively. The experiment lasted for 28 days.

#### **Biological Evaluation**

#### **Blood sampling**

At the end of experimental period all rats were anesthetized with diethyl ether after 12 hours fasting, blood samples were received from portal vein into clean dry centrifuge tubes in which blood samples left to clot at room temperature then centrifuged for 10 minutes at 3000 rpm to separate the serum, all serum samples were stored frozen till analysis ( Malhotra, 2003).

#### **Biochemical analysis**

Different tested parameters in serum were determined using specific methods as follow: Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL-c) were determined by using methods of (Allain et al., 1974; Lopez-Virella, 1977 and Fossati & Prencipe, 1982) respectively. The determination of low-density lipoprotein cholesterol (LDLc) and very low-density lipoprotein cholesterol (VLDLc) were carried out according to the methods of (Lee and Nieman, 1996). Serum glucose was estimated according to (Rojas et al., 1999). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase activities (ALP) were determined according to (Belfied and Goldberg, 1971; Yound, 1975 and Tietz, 1976), respectively. Urea and creatinine levels were determined in serum according to the method described by (Hout, 1985).

#### Histopathological examination

After rats were scarified under ether anesthetized, liver and heart were removed, washed in slain solution, dried by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to (**Drury and Wallington**, 1980).

#### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD). The data were statistically analyzed using a computerized costat program by one-way ANOVA. Differences between treatments at p  $\leq$  0.05 were considered statically significant .

#### RESULTS AND DISCUSSION

### Effect of chia and sunflower seeds and their mixture on total cholesterol and triglyceride and lipids profile of rats

As shown in Tables (1 and 2) feeding rats on HFD resulted in a significant ( $p \le 0.05$ ) elevation in each total cholesterol(TC), triglycerides(TG), and LDLc levels with 43.33,34.83 and 72.09 %, respectively while, a significant ( $p \le 0.05$ ) reduction was recorded in HDLc level when compared to the negative control group. Findings were supported by the results of (**Guay** et al., 2012). Within treated groups LDLc and total cholesterol decreased significantly. The best treatment was 5% mixture, it produced a significant reduction in TC, TG and LDLc with 35.89,24.51 and 60.00 %, respectively. As well as, increasing in

HDLc level with 50.00%. The obtained findings agreed with (Kulczynski et al., 2019) who revealed that components of CS have a beneficial effect on improving lipid profile . This effect may be due to its high content of ALA that associated with beneficial changes in plasma lipids (Meng et al., 2013 and Santos-Lopez et al., 2017). Additionally, CS could improve altered lipid metabolism present in the skeletal muscle of rats (Ferreira et al., 2020). On the other hand, SFS groups also showed a significant improvement in serum lipids profile of treated rats. The cholesterol lowering effect of SFS were also evaluated by ( Richmond et al., 2013) who elicited that addition of SFS to the diet provided clinically beneficial effects on lipidand lipoprotein-mediated cardiovascular diseases (CVD) risk. This improvement might be due to its high content of vitamin E which has a positive impact on health especially reducing the risk of cardiovascular diseases as supported by results of (Burton, 1994 and Sozen et al., 2019). Another study contributed the desired effect on lipids profiles to poly unsaturated fatty acids (PUFA) high content of SFS that has a beneficial effect on reducing LDLc concentrations (Mensink and Katan, 1992 and Jiang et al., 2006).

Table (1): Effect of chia, sunflower seeds and their mixture on total cholesterol and triglyceride of rats.

Parameters	Total cholesterol		Triglycerides			
	(mg/dl)		(mg/dl)			
Groups	Mean±SD	% change	Mean±SD	% change		
G1 (Control -)	110.50±0.10 <sup>h</sup>	43.33-	101.00±0.20 <sup>f</sup>	34.83-		
G2 (Control +)	195.00±0.50 a		155.00±0.11 a			
G3 (2.5% CS)	147.50±0.40°	24.35-	133.00±0.20 °	14.19 -		
G4 (5% CS)	136.50±0.12 e	-30.25	124.00±0.30 <sup>d</sup>	-20 .00		
G5 (2.5% SFS)	153.00±0.15 <sup>b</sup>	21.53-	140.00±0.40 <sup>b</sup>	9.67 -		
G6 (5% SFS)	142.00±0.11 <sup>d</sup>	-27.17	131.00±0.20°	15.48-		
G7 (2.5% Mix)	131.00±0.12 <sup>f</sup>	-32.82	126.00±0.45 <sup>d</sup>	-18.70		
G8 (5% Mix)	125.00±0.15 g	35.89-	117.00±0.10 <sup>e</sup>	24.51-		
LSD (P ≤ 0.05)	4.622	2	3.351			

Each value represents mean  $\pm$  standard deviation. Means under the same column bearing different superscript letters are different significantly at P $\leq$  0.05.

Table (2): Effect of chia, sunflower seeds and their mixture on lipid profile of rats.

Parameters	HDL-c (mg/dl)		LDL-c (mg/dl)		VLDL-c (mg/dl)	
	Mean	%	Mean	%	Mean	%
Groups	±SD	change	±SD	change	±SD	change
G1 (Control -)	53.50±0.20 a	52.85-	36.30±0.10 <sup>h</sup>	-72.09	20.20±0.12 d	-34.83
G2 (Control +)	35.00±0.13 e		129.00±0.2 <sup>a</sup>		31.00±0.10 a	
G3 (2.5% CS)	45.50±0.22°	30.00	74.90±0.23 °	-41.93	26.60±0.15 b	-14.19
G4 (5% CS)	48.00±0.10 <sup>b</sup>	37.14	63.70±0.22 e	-50.62	24.80±0.30°	-20.00
G5 (2.5% SFS)	40.50±0.13 d	15.71	84.50±0.40 b	-34.49	28.00±0.40 b	-9.67
G6 (5% SFS)	45.50±0.40°	30.00	70.30±0.10 <sup>d</sup>	-45.50	26.20±0.11 <sup>d</sup>	-15.48
G7 (2.5% Mix)	47.00±0.15 b	34.28	58.80±0.12 d	-54.41	25.20±0.10°	-18.70
G8 (5% Mix)	50.00±0.14 b	42.85	51.60±0.30 g	-60.00	23.40±0.20 <sup>d</sup>	-24.51
LSD $(P \le 0.05)$	3.141		3.250		2.012	

Each value represents mean  $\pm$  standard deviation. Means under the same column bearing different superscript letters are different significantly at P $\leq$  0.05.

### Effect of chia, sunflower seeds and their mixture on glucose level

Data tabulated in table (3) illustrate the effect of CS, SFS and their mixture in different concentrations on glucose level. The highest value was recorded to the positive control group because of high fat diet that resulted in a significant increasing (p < 0.05)in serum glucose level when compared to negative control group . This finding agreed with (Lamont et al., 2016) who concluded that feeding high fat diet resulted in impairment of glucose tolerance. Within the treated groups, the best treatment was 5% Mix where there was a reduction in the elevated serum glucose level with 51.70% followed by 5% CS group with 47.86% when compared to the positive control group. The obtained results agreed with (Alamri, 2019) who concluded that chia seeds have a helpful role in improving blood glucose levels in diabetic rats via increasing glucose tolerance and insulin sensitivity in obese rats (Poudyal et al., 2012; Marineli et al, 2015 and Fonte-Faria et al. 2019) This hypoglycemic effect may be due to the presence of soluble fiber that could improve glycemic profiles (Tamargo et al., 2020). Also, chia seeds were used in bakery products as a functional food to reduce the glycemic index (Salgado-Cruz et al., 2017). In the same context, Sunflowers seeds contain many hypoglycemic nutrients that could participate in reducing the risk factor of type 2 diabetes (Luka et al. 2013).

Table (3): Effect of chia, sunflower seeds and their mixture on glucose level.

	Parameters	Glucose level (mg/dl)		
Groups		Mean±SD	% change	
G1 (Control -)		96.00± 0.30 g	58.97-	
G2 (Control +)		234.00± 0.10 <sup>a</sup>		
G3 (2.5% CS)		143.00± 0.20 °	38.88-	
G4 (5% CS)		122.00± 0.40 <sup>e</sup>	47.86-	
G5 (2.5% SFS)		151.00± 0.42 <sup>b</sup>	35.47-	
G6 (5% SFS)		136.00± 0.53 <sup>d</sup>	41.88-	
G7 (2.5% Mix)		121.00± 0.40 <sup>e</sup>	48.29-	
G8 (5% Mix)		$113.00 \pm 0.60^{\mathrm{f}}$	51.70-	
LSD (P ≤ 0.05)		4.061		

Each value represents mean  $\pm$  standard deviation. Means under the same column bearing different superscript letters are different significantly at P $\leq$  0.05.

### Effect of chia and sunflower seeds and their mixture on liver functions of rats

As shown in Table (4), feeding rats on high fat diet increased the levels of GPT, GOT and ALP with values 40.00 .59.58 and 24.44% respectively, when compared to negative control group. This finding agreed with (Mendes de Castro et al., 2013) who indicated that HFD induced a significant increasing in liver enzymes level. While, within the treated groups there was a significant reduction (P  $\leq$  0.05) in liver enzymes level when compared to negative control group. The best values were recorded to group 4 (5% CS) with percent of change 40.00,48.75 and 22.96 for GPT, GOT and ALP, respectively. In addition, these results were supported by (Fernández-Martínez et al., 2019) who concluded that consumption of CS may improve the health state of non-alcoholic fatty liver disease (NAFLD) and could prevent hepatic cirrhosis or hepatocellular carcinoma (HCC).

Table (4): Effect of chia and sunflower seeds and their mixture on liver functions of rats

Parameters	GPT (U/L)		GOT (U/L)		ALP (U/L)	
	Mean	%	Mean	%	Mean	%
Groups	±SD	change	±SD	change	±SD	change
G1 (Control -)	12±0.10 <sup>f</sup>	- 40	48.50±0.11 <sup>f</sup>	-59.58	102.0±0.16 e	-24.44
G2 (Control +)	20±0.13 <sup>a</sup>		120.0±0.13 a		135.0±0.10 a	
G3 (2.5% CS)	13±0.11 <sup>e</sup>	-35	89.0±0.14 °	-25.83	110.0±0.15 <sup>d</sup>	-18.51
G4 (5% CS)	12±0.12 <sup>f</sup>	-40	61.50±0.10 <sup>e</sup>	48.75-	104.0±0.12 e	-22.96
G5 (2.5% SFS)	$16\pm0.16^{c}$	-20	95.0±0.11 b	-20.83	118.0±0.13 °	-12.59
G6 (5% SFS)	15±0.14 <sup>d</sup>	-25	$77.0\pm0.16^{d}$	-35.83	121.0±0.10 °	-10.37
G7 (2.5% Mix)	$17\pm0.12^{b}$	-15	63.50±0.12 e	47.08-	119.0±0.12 °	-11.85
G8 (5% Mix)	16±0.11°	-20	56.0±0.13 <sup>f</sup>	-53.33	125.0±0.14 b	-7.40
LSD $(P \le 0.05)$	0.752		2.851		3.504	

Each value represents mean  $\pm$  standard deviation. Means under the same column bearing different superscript letters are different significantly at  $P \le 0.05$ .

### Effect of chia, sunflower seeds and their mixture on kidney functions of rats

Data in table (5) illustrate the effect of CS, SFS and their Mix on kidney functions of rats. As shown ,consumption of HFD induced renal changes in positive control group when compared to the negative control group , these changes were observed by increasing in serum creatinine level with 58.28 % this result agreed with (Gomez-Guerrero et al., 2005). In the same context, urea and uric acid in positive control group increased with % of 64.70 and 61.01 respectively comparing to negative control group. On the other hand, Data showed that treatment with CS or SFS and their Mix at different concentration caused a significant ( $p \le 0.05$ ) decrease in uric acid, creatinine and urea levels compared to positive control group, this effect might be due to the high content of omega 3,this finding is in agreement with (Fayez et al., 2014) who concluded that omega 3 possesses a protective effect against renal dysfunction in rats.

Table (5): Effect of chia and sunflower seeds and their mixture on kidney functions of rats

Parameters	Urea (mg/dl)		Uric acid (mg/dl)		Creatinine (mg/dl)	
	Mean	%	Mean	%	Mean	%
Groups	±SD	change	±SD	change	±SD	change
G1 (Control -)	2.40±0.10°	64.70	23.0±0.10 <sup>d</sup>	61.01	0.73±0.10 <sup>b</sup>	-58.28
G2 (Control +)	6.80±0.13 a		59.0±0.13 a		1.75±0.13 a	
G3 (2.5% CS)	3.90±0.11 b	-42.64	40.0±0.15 b	-32.20	1.01±0.15 b	-42.28
G4 (5% CS)	2.90±.011 °	-57.35	29.0±.011 °	-50.84	0.92±.016 b	-47.42
G5 (2.5% SFS)	4.90±.016 b	27.94-	42.0±0.11 b	-28.81	1.65±0.11 a	-5.71
G6 (5% SFS)	4.50±0.13 b	-33.82	39.0±0.10 <sup>b</sup>	-33.89	1.60±0.13 a	-8.57
G7 (2.5% Mix)	4.10±0.15 b	-39.70	36.0±0.13 b	-38.98	1.25±0.10 <sup>b</sup>	-28.57
G8 (5% Mix)	2.60±.011 °	-61.76	30.0±.016 °	-49.15	0.84±.011 b	-52.00
LSD $(P \le 0.05)$	1.151		3.40		0.532	

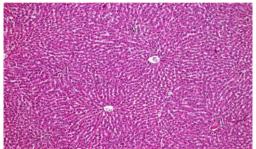
Each value represents mean  $\pm$  standard deviation. Means under the same column bearing different superscript letters are different significantly at P $\leq$  0.05.

#### Histopathological examination

Microscopic examination of different sections of liver of control rats showed normal histological structure of central vein, portal areas and hepatic parenchymal cells (Figure 1). Heart of control rats showed also normal histological structure of the cardiac muscle fibers (Figure 2). While microscopic picture of liver and heart of control positive rats showed marked histological alterations. Regarding liver of control positive rats, examination of which revealed severe congestion of the central vein and hepatic sinusoids, vacuolar degeneration and necrosis of the hepatic cells (Figure 3). The necrotic cells appeared either with pyknotic or fragmented nuclei or without any nuclear structure. The portal areas showed congestion of the portal vessels, moderate proliferation of the bile duct epithelium and mild fibrous proliferation (Figure 4). While heart of control positive rats showed severe swelling of the cardiac muscle fibers with vacuolar and granular degeneration and necrosis (Figure 5). Some muscle fibers appeared eosinophilic hyalinized. Some of the muscle fibers which showed vacuolar degeneration revealed signet ring appearance with intermuscular edema (Figure 6). These findings are in agreement with (Al-Hayder et al., 2020) who noticed that tallow administration by rats resulted in abnormal histopathological changes in rat liver Concerning examination of tissues of different treated groups, revealed that the best curative effect was observed on using 5 %

CS (group 4) and 2.5 %Mix (group 7) followed by 2.5% CS (group 3), 5% SFS (group 6) and 5 % Mix (group 8) while the least was for drug 3 (group 5). The liver tissue of group 3 showed good restoration of the hepatic parenchymal cells with only scattered degenerated and necrotic ones as well as congested central veins and dilated and congested sinusoids (Figure 7). Heart of group 3 showed mild necrobiotic changes of the cardiac muscle fibers (Figure 8). Livers of group 4 showed near to normal appearance of the hepatic cells with mild proliferation of bile duct epithelium, mild portal inflammatory cells infiltration and congested portal vessels (Figure 9). Hearts of those rats showed good degree of cardiac muscle fibers restoration with few degenerated fibers and mild intermuscular edema (Figure 10). Livers of group 5 showed widening and congestion of the hepatic sinusoids, scattered degenerated and necrotic hepatic cells (Figure 11). Hearts of group 5 showed mild degeneration and necrosis of the cardiac muscle fibers and intermuscular edema (Figure 12). Livers of group 6 showed mild degeneration, nuclear pyknosis and necrosis of the hepatic cells, congestion of some sinusoids (dashed arrow) and mild inflammatory cells infiltrating the portal areas (Figure 13). While hearts of those rats showed good degree of restoration of the cardiac muscle fibers with few with vacuolar degeneration and others necrotic as well as some intermuscular edema (Figure 14). Livers of group 7 showed near to normal appearance of the hepatic cells with scars necrotic cells and some activated Kupffer cells (Figure 15). Cardiac tissue of group 7 showing few necrotic muscle fibers and mild intermuscular edema (Figure 16). Livers of group 8 showed congestion of the central veins and mild necrobiotic changes of the hepatic cells with few cells with pyknotic nuclei (Figure 17). Hearts of group 8 showing few necrotic muscle fibers. scars fibers with vacuolar degeneration and mild intermuscular edema (Figure 18). These findings were in the same context of results found by (El-Yamany, 2020) who revealed that consumption of 5% chia seeds by diabetic rats showed a slight hydropic degeneration of hepatocytes. Also, the obtained results were supported by (Pintapagung et al., 2020) who found that oral administration of 4% chia seeds as extract has the potential to improve wound healing in diabetic mice. Another study by (Silva et al., 2019) concluded that concentrations of inflammatory markers in female rats could be decreased by chia seeds consumption.

**In conclusion**, chia and sunflower seeds as high sources of ALA, fiber, vitamin E and phenolic compounds provided a desirable effect on each serum lipids profile, serum glucose level and liver functions and could improve the histological alterations happened in rats as a result of feeding HFD. Therefore, we recommended the incorporation of CS and SFS as functional foods in concentration (2.5 and 5%) to provide more effective health benefits.



**Fig. 1:** Liver of control rat showing normal histological structure of central vein (CV), portal areas (arrow) and hepatic parenchymal cells (HCs). (H&E, X100).

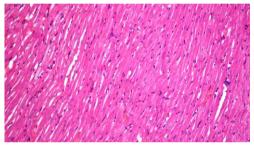


Fig. 2: Heart of control rat showing normal histological structure of the cardiac muscle fibers (arrow). (H&E, X200).

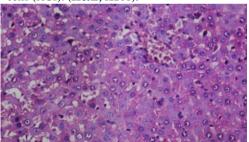


Fig. 3: Liver of control positive rat showing severe congestion of the central vein and hepatic sinusoids (arrow), vacuolar degeneration (dashed arrow) and necrosis (short arrow) of the hepatic cells. (H&E, X400).

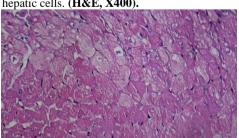
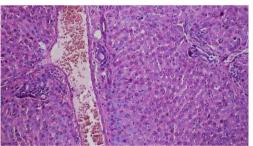


Fig. 5: Heart of control positive rat showing severe swelling of the cardiac muscle fibers



**Fig. 4:** Liver of control positive rat showing congestion of the portal vessels (arrow), moderate proliferation of the bile duct epithelium (dashed arrow) and mild fibrous proliferation (short arrow). (**H&E, X200**).

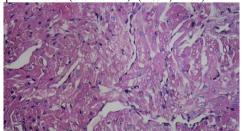


Fig. 6: Heart of control positive rat showing vacuolar degeneration with signet ring

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with vacuolar (arrow) and granular (dashed arrow) degeneration and necrosis (short arrow). (H&E, X400).

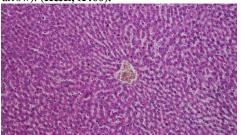
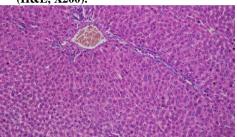
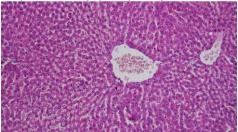


Fig. 7: Liver of control positive rat and treated with drug 1 (group 3) showing good restoration of the hepatic parenchymal cells with only scattered degenerated and necrotic (arrow) ones as well as congested central vein (CV) and dilated and congested sinusoids (dashed arrow). (H&E, X200).



**Fig. 9:** Liver of control positive rat and treated with drug 2 (group 4) showing near to normal appearance of the hepatic cells with mild proliferation of bile duct epithelium (arrow), mild portal inflammatory cells infiltration (short arrow) and congested portal vessels (dashed arrow). (**H&E, X200**).



**Fig. 11:** Liver of control positive rat and treated with drug 3 (group 5) showing widening and congestion of the hepatic sinusoids (arrow), scattered degenerated and necrotic hepatic cells (dashed arrow). **(H&E, X200).** 

appearance (arrow) and necrosis (dashed arrow) of the muscle fibers and intermuscular edema (short arrow). (H&E, X400).

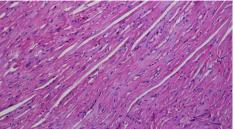
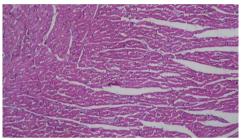
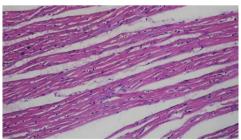


Fig. 8: Heart of control positive rat and treated with drug 1 (group 3) showing mild necrobiotic changes (arrow) of the cardiac muscle fibers. (H&E, X200).



**Fig. 10:** Heart of control positive rat and treated with drug 2 (group 4) showing good degree of cardiac muscle fibers restoration with few degenerated fibers and mild intermuscular edema (Ed). **(H&E, X200).** 



**Fig. 12:** Heart of control positive rat and treated with drug 3 (group 5) showing mild degeneration and necrosis (arrow) of the cardiac muscle fibers and intermuscular edema (Ed). (**H&E**, **X200**).

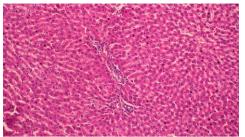
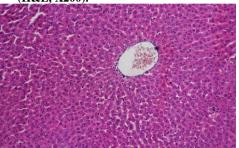
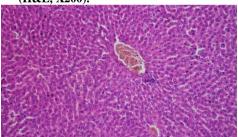


Fig. 13: Liver of control positive rat and treated with drug 4 (group 6) showing mild degeneration, nuclear pyknosis (short arrow) and necrosis (arrow) of the hepatic cells, congestion of some sinusoids (dashed arrow) and mild inflammatory cells infiltrating (thin arrow) the portal areas. (H&E, X200).



**Fig. 15:** Liver of control positive rat and treated with drug 5 (group 7) showing near to normal appearance of the hepatic cells with scars necrotic cells (arrow) and some activated Kupffer cells (dashed arrow). **(H&E, X200).** 



**Fig. 17:** Liver of control positive rat and treated with drug 6 (group 8) showing congestion of the central veins (arrow) and mild necrobiotic changes of the hepatic cells with few cells with pyknotic nuclei (dashed arrow). (**H&E**, **X200**).

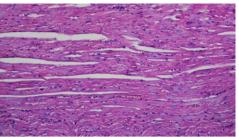
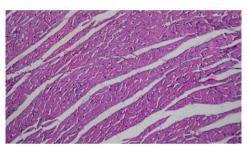


Fig. 14: Heart of control positive rat and treated with drug 4 (group 6) showing good degree of restoration of the cardiac muscle fibers with few with vacuolar degeneration (arrow) and others necrotic (dashed arrow) as well as some intermuscular edema (Ed). (H&E, X200).



**Fig. 16:** Heart of control positive rat and treated with drug 5 (group 7) showing few necrotic muscle fibers and mild intermuscular edema. **(H&E, X200)** 

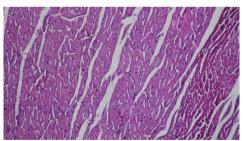


Fig. 18: Heart of control positive rat and treated with drug 6 (group 8) showing few necrotic muscle fibers (arrow), scars fibers with vacuolar degeneration (dashed arrow) and mild intermuscular edema. (H&E, X200).

#### REFERENCES

- **AIN** (1993): American institute of nutrition purified diet for laboratory Rodent, Final Report. J. Nutrition, 123: 1939-1951.
- **Akande, K.E.** (2011): Proximate and Amino acid Analyses of Full-fat Sunflower (*Helianthus annuus* L.) Seed Meal. Singapore Journal of Scientific Research, 1: 179-183.
- **Alamri, E. (2019):** The influence of two types of chia seed on some physiological parameters in diabetic rats. International Journal of Pharmaceutical Research & Allied Sciences, 8(3):131-136.
- Al-Hayder, N. M.; Al-Mayyahi, S. R. and Abdul-Razak, S. A. (2020): Effects of sunflower oils and beef tallow on serum parameters and liver histopathology in experimental rats. Obesity Medicine, 18: e100232.
- Allain, C.C; Richmond, N. and Rosechloy, P. (1974): Cholestrol enzymatic colorimetric test. Chem. Clin., 19 (20): 1350 1361.
- **Ayerza,R. and Coates ,W.,(2004):**Composition of chia (Salvia Hispanica) grown in six tropical and subtropical ecosystems of South America. Trop. Sci., 44:131-135.
- Belfied, A. and Goldberg, D.M. (1971): Alkaline phosphatase colorimetric method. Journal of Enzyme, (12):561-569.
- Brătfălean, D.; Cristea, V.M.; Agachi, P.Ş.and Irimie, D.F. (2008): Improvement of sunflower oil extraction by modelling and simulation. Rev. Roum. Chim. ,53:881–888.
- **Burton, G.W. (1994):** Vitamin E: molecular and biological function. Proc Nutr Soc., 53(02):251–262.
- **Campbell, J.A.** (1963): Methodology of Protein Evaluation "RAG Nutr., Document R.10, Led.37. June meeting, New York.
- Capitani, M.I.; Sportano, V. Nolasco, S.M. and Tomas, M.S. (2012): Physicochemical and functional characterization of

- by -products from chia *Salvia hispanica* L.seeds of Argentia.LWT-Food Sci Tecnol.,45:94-102.
- Ding, Y. Lin, H.; Lin, Y.; Yang, D.; Yu, Y.; Chen, J.; Wang, S. and Chen, Y.(2018): Nutritional composition in the chia seed and its processing properties on restructured ham-like products. Journal of Food and Drug Analysis, 26:24-34.
- **Drury, R.A. and Wallington, E.A. (1980):** Carlton's Histological Technique. 5<sup>th</sup> ed. Oxford University.
- **El-Yamany, A.M. (2020):** The effects of moringa leaves and chia seeds on some blood parameters and histopathology of liver and kidney to boost immunity of diabetic rats. Journal of Home Econ., 36 (1): 149-169.
- Fayez, M.A.; Awad, S.A.; El-Naa, M.M.; Kenawy, A,S. and El-Sayed, E.M. (2014): Beneficial effects of thymoquinone and omega-3 on intestinal ischemia/reperfusion-induced renal dysfunction in rats. Bulletin of Faculty of Pharmacy, 52(2): 171-177.
- Ferreira, M.D.R.; Olivaa, M.E.; Aiassaa, M. and D'Alessandroa, E. (2020): Salvia hispanica L. (chia) seed improves skeletal muscle lipo toxicity and insulin sensitivity in rats fed a sucrose-rich diet by modulating intramuscular lipid metabolism. Journal of Functional Foods, 66:103775.
- Fernández-Martínez, E.; Lira-Islas ,I.G.; Cariño-Cortés ,R.; Luis E Soria-Jasso ,E.L.; Pérez-Hernández ,E. Pérez-Hernández,N.(2019): Dietary chia seeds (*Salvia hispanica*) improve acute dyslipidemia and steatohepatitis in rats. J Food Bio chem., 43(9): e 12986.
- Fonte-Faria, T.; Citelli, M.; Atella, G. C.; Raposo, H. F.; Zago, L., de Souza, T. and Barja-Fidalgo, C. (2019): Chia oil supplementation changes body composition and activates insulin signaling cascade in skeletal muscle tissue of obese animals. Nutrition, 58, 167–174.

- **Fossati, P.; and Prencipe, L.** (1982): Serum triglycerides determined colorimetrically with enzyme that produces hydrogen peroxide. J. of Clin. Chem., 28: 2077-2088.
- **Fowler, M.W. (2006):** Plants, medicines and man. J Sci Food Agric.,86(12):1797–1804.
- Grancieri, M.; Duarte-Martino, H.S. and Gonzalez de Mejia, E. (2019): Chia seed (Salvia hispanica L.) as a source of proteins and bioactive peptides with health benefits: a review. Comprehensive Reviews in Food Science and Food Safety, 18(2):480-499.
- Gomez-Guerrero, C.; Hernandez-Vargas, P.; Lopez-Franco,O. and Egido, G. (2005): Mesangial cells and glomerular inflammation: From the pathogenesis to novel therapeutic approaches. Curr Drug Targets Inflammation Allergy.,4:341-51.
- Guay, V.; Lamarche, B.; Charest, A.; Tremblay, A.J. and Couture, P.(2012): Effect of short-term low- and high-fat diets on low-density lipoprotein particle size in normolipidemic subjects. Metabolism, 61(1): 76-83.
- **Guo**, **S.**; **Yan Ge**, **Y. and Jom, K.N.(2017):** A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus L.*). Chem Cent J., 11: 95.
- **Hegsted, D.; Mills, R. and Perkin, E. (1941):** Salt mixture. J. Biol. Chem., 18:138-142
- **Hout, O. (1985):** In: Interpretation of clinical laboratory tests-Siest,G.; Henny, J.; Schiele F, eds. london: Youn, D.S. Biomedical Puplication.
- **Jiang, R.; Jacobs, D.R. and Mayer-Davis, E. (2006):** Nut and seed consumption and inflammatory markers in the multiethnic study of atherosclerosis. American Journal of Epidemiology, 163(3):222–231.
- Kulczynski, B.; Kobus-Cisowska, J.; Taczanowski, M.; Kmiecik, D. and Gtamaza-Michalowska, A.(2019): The

- chemical and nutritional value of chia seeds-current stage of Knowledge. Nutrients, 11(6): 1242.
- Kwon, H. C.; Bae, H.; Seo, H. G. and Han, S. G. (2019): Short communication: Chia seed extract enhances physiochemical and antioxidant properties of yogurt. J. Dairy Sci., 102:4870–4876.
- **Lamont, B.J.; Waters, M.F. and Andrikopoulos, S. (2016):**A low -carbohydrate high fat diet increases weight gain and does not improve glucose tolerance ,insulin secretion or β-cell mass in NZO mice .Nutrition & Diabetes ,6:1-7.
- Lee. R. and Nieman, D. (1996): Nutritional Assessment. 2 nd, Mosby, Missouri, USA.
- **Lopez-Virella, M.F. (1977):** High density lipoprotein cholesterol by selective precipitin. Clin Chem., 23(5): 882-4.
- Luka, C.D.; Tijjani, H.; Joel, E.B.; Ezejiofor, U.L. and Onwukike, P.(2013): Hypoglycaemic properties of aqueous extracts of *Anacardium occidentale*, *Moringa oleifera*, *Vernonia amygdalina* and *Helianthus annuus*: a comparative study on some biochemical parameters in diabetic rats. Inter J Pharm Sci Invent., 2(71):16–22.
- Marineli, R.D.; Moura, C.S. and Moraes, É.A. (2015): Chia (Salvia hispanica L.) enhances HSP, PGC-1α expressions and improves glucose tolerance in diet-induced obese rats. Nutrition., 31(5):740-748.
- Malhotra, V.K. (2003):"Practical Biochemistry for Students" Fourth edition, Jaypee Brothers Medical Publishers (P) LTD, New Delhi.
- Mensink, R.P.and Katan, M.B. (1992): Effect of dietary fatty acids on serum lipids and lipoproteins: a meta- analysis of 27 trials. Arteriosclerosis and Thrombosis. ;12(8):911–919.
- Marcinek,K. and Krejpcio,Z. (2017):Chia seeds (*Salvia hispanica*):Health promoting properties and therapeutic applications- a review .Roez Panstw Zakl Hig,67(2):123-129.

- **Martínez-Cruz,O. and Paredes-López,O.(2014):** Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica L.*) by ultra-high performance liquid chromatography. Journal of Chromatography A, 1346 (13):43-48.
- Melo, D.; Machado, T.B.; Beatriz, M. and Oliveira, P.P. (2019): Chia seeds: an ancient grain trending in modern human diets. Food & Function., 10(6):3068-3089.
- Meng, S.; Cao, J.; Feng, Q. Peng, J. and Hu, Y. (2013): Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evid Based Complement Alternat Med., 801457, doi: 10.1155/2013/801457.
- Mendes de Castro, U.G.; Santos, R.A.; Silva, M.E.; Lima, W.G.; Campagnole-Santos. M.J. and Alzamora, A.C. (2013): Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids in Health and Disease, 12 (136): doi.org/10.1186/1476-511X-12-136.
- Nduko, J.M; Maina, R.W.; Muchina, R.K. and Kibitok, S.K. (2018): Application of Chia (*Saliva Hispanica*) seeds as a functional component in the fortication of pineapple jam .Food Sci.Nutr.,6(8):2344-2349.
- Noeman, S.A.; Hamooda, H.E. and Baalash, A.A. (2011): Biochemical study of oxidative stress markers in the liver, kidney and heart of high Fat diet induced obesity in rats. Diabetology & Metabolic Syndrome, 3(17). doi.org/ 10. 1186/1758-5996-3-17.
- **Olomu, J.M., (1995):** Monogastric Animal Nutrition-Principles and Practice. Jachem Publications, Benin City, Nigeria.
- Pintapagung, T.; Asawapattanakul, T.; Buasrithong, N.; Phunnanon, M.and Thaingkhan, W. (2020): Effect of oral administration of chia (*Salvia hispanica* L.) seed extract on wound healing property in diabetic mice. Veterinary Integrative Sciences, 18(3): 153-171.

- **Poudyal, H.; Panchal, S.K.; Waanders, J.; Ward, L. and Brown, L. (2012):** Lipid redistribution by α-linolenic acidrich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. The Journal of Nutritional Biochemistry,23(2): 153–162.
- Poudyal, H.; Panchal, S.k.; Ward, L.C. and Brown, L. (2013): Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. The Journal of Nutritional Biochemistry, 24(6): 1041-1052.
- Richmond, K.; Williams, S.; Mann, J. Brown, R. and Chisholm, A. (2013): Markers of cardiovascular risk in postmenopausal women with type 2 diabetes are improved by the daily consumption of almonds or sunflower kernels: a feeding study. ISRN Nutr., 19: e626414.
- Rojas, E.; Herrera, A.L.; Poirier, L.A. and Ostrosky Wegman, P. (1999): Are metals dietary carcinogens? Mutat. Res., 44: 157-181.
- Salgado-Cruz, M.P.; Ramirez-Miranda, M.; Diaz-Ramirez, M. and Alamilla-Beltran, L.G. (2017): Microstructural characterization and glycemic index evaluation of pita bread enriched with chia mucilage. Food Hydrocolloids, 69:141-149.
- Santos-Lopez, J.A.; Garcimartin, A; Lopez-Olive, M.E. and Bautista, M. (2017): Chia oil-enriched restructured pork effects on oxidative and inflammatory status of aged rats fed high cholesterol/high fat diets .Journal of Medicinal food, 20(5):526-534.
- Saxena, R.; Venkaiah, K.; Anitha, P.; Venu, L. and Raghunath, M. (2007): Antioxidant activity of commonly consumed plant foods of India: contribution of their phenolic content. Int J Food Sci Nutr., 58(4):250-60.
- Silva, B.P.; Toledo, R.C.; Mishima, M.D.V.; Moreira, M.E.; Vasconcelos, C.M.; Pereira, C.E.R.; Favarato, L.S.C.; Costa, N.M.B. and Martino, H.S.D. (2019): Effects of chia (Salvia hispanica L.) on oxidative stress and inflammation in

- ovariectomized adult female wistar rats. Food Funct., 7(10): 4036-4045.
- **Sozen, E.; Demirel, T. and Ozer, N.K. (2019):** Vitamin E: Regulatory role in the cardiovascular system. IUBMB Life., 71(4):507-515.
- **Suk, M. and Shin, Y. (2015):** Effect of high-intensity exercise and high-fat diet on lipid metabolism in the liver of rats. J Exerc Nutrition Biochem., 19(4): 289–295.
- Tamargo, A.; Martin, D.; Navarro del Hierro, J.; Moreno-Arribas, M.V. and Muñoz, L.A. (2020): Intake of soluble fiber from chia seed reduces bio accessibility of lipids, cholesterol and glucose in the dynamic gastrointestinal model simgi®. Food Research International, 137: e109364.
- **Tietz, N. W. (1976):** Fundamentals of Clinical Chemistry. Philadelphia. B.W. Standers, P.243.
- Ullah, R.; Nadeem, M.; Khalique, A.; Imran, M.; Mehmood, S.; Javid, A. and Hussain, J. (2016): Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): A review. J. Food Sci. Technol., 53:1750–1758.
- Vieira, V.J.; Valentin, R.J.; Wilund, K.R. and Woods, J.A. (2009): Effects of diet and exercise on metabolic disturbances in high-fat diet-fed mice. Cytokine, 46(3): 339-345.
- Woods, S.C.; Seeley, R.J.; Rushing, P.A. D'Alessio, D. and Tso, P. (2003): A Controlled High-Fat Diet Induces an Obese Syndrome in Rats. The Journal of Nutrition, 133(4): 1081–7.
- **Yound, D.S.** (1975). Determination of GOT. Clinical Chemistry, 22 (5): 21-27.
- **Yurt, M. and Gezer, C. (2018):** Functional characteristics of chia seed (*Salvia hispanica*) and its effects on health. Food .43(3):446-460.
- Zhang, W.; Fu, F.; Tie, R. Lieng, X.; Tian, F.; Xing, W.; Ji, L.; Xing, J.; Sun, X. and Zhang, H. (2013): Alpha-linolenic acid intake prevents endothelial dysfunction in high-fat diet-fed streptozotocin rats and underlying mechanisms. Vasa.,42: 421-428.

# التأثيرات العلاجية المحتملة لبذور الشيا وعباد الشمس على الفئران التي يتم تغذيتها على غذاء عالى في الدهون

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#### نبذه:

صممت هذه التجربة لدراسة التأثيرات العلاجية المحتملة لبذور الشيا وعباد الشمس ومخلوطهما بتركيزات مختلفة على الفئران التي يتم تغذيتها على غذاء عالى في الدهون حيث أجريت الدراسة باستخدام ثمانية واربعون فأرا من اناث فئران الألبينو والتي تزن (150±150) جرام تم تقسيمهم الى ثمان مجموعات متساوية (ن =6) ، المجموعة الأولى: تغذت على الغذاء الأساسي كمجوعة ضابطة سالبة، المجموعة الثانية: تغذت على غذاء عالى في الدهون كمجوعة ضابطة موجبة، المجموعة الثالثة والرابعة تم تغذيتهم على غذاء عالى في الدهون مع اضافة (2.5 ، 5%) من بذور الشيا على التوالي . المجموعة الخامسة والسادسة تم تغذيتهم على غذاء عالى في الدهون مع اضافة (2.5 ، 5%) من بذور عباد الشمس على التوالي . المجموعة السابعة والثامنة تم تغذيتهم غذاء عالى في الدهون مع اضافة (2.5 ،%5) مخلوط على التوالي و أستمرت التجربة لمدة 28 يوم . وسجلت افضل نتائج لوظائف الكبد للمجموعة الرابعة حيث أدت الى تحسن معنوى بنسبة 48,75،، 40,00 و 22,96% في كلا من درجة نشاط انزيمات 48,75، 40,00 على التوالي. وسجلت افضل نتائج الكولسترول المنخفض الكثافة والجلوكوز للمجموعة الثامنة ايضا حيث ادت الى انخفاض معنوى بنسبة 60,00، 51,70% في الكولسترول المنخفض الكثافة والجلوكوز على التوالي الذا اوصت هذه الدراسة بأهمية دمج واضافة بذور الشيا وعباد الشمس كأغذية وظيفية غنية في حمض الفا الينولينك لنمط الغذاء اليومي للأستفادة من فوائدها الوقائية وربما العلاجية للأمراض.

الكلمات الافتتاحية: مسحوق ; حمض الفا لينولينك; الفئران ; الاغنية الوظيفية ; أوميجا 3 .