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Study of the Healthy Effects of Different Fat Ratios Mixtures of Omega-3 to Omega-6 in Male Mice with Alloxan-Induced Diabetes

ABSTRACT

This study was conducted at the Iraqi Center for Cancer Research / Al-Mustansiriya University / Baghdad Governorate, for the period from November 2020 to January 2021, to know healthy effects of different fat ratios mixtures of Omega-3 to Omega-6 in male mice with type 2 diabetes induced by Aloxan at a concentration of 100 mg / kg of weight. 32 mice aged (2-3) months and their weight (25-30) g were included. Four mice were isolated from them as a healthy control group and the remaining mice were injected with alloxan. Infected mice were divided into seven groups, one of which was untreated group (infected control), while the rest were treated with mixtures of different proportions of omega 6 and 3 fats. After the 30-day experiment, the biological study showed that the introduction of experimental diabetes mellitus with alloxan led to a higher Significant ($p>0.01$) and ($P>0.05$) in the concentration of glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) compared with the proper control group. While it led to a significant decrease ($p>0.01$) and ($p>0.05$) in high-density lipoprotein-cholesterol (HDL-C) concentrations in the affected control group, but when using mixtures of different proportions of omega-6 fats: Omega-3 led to a decrease in the concentration of glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) compared with the infected and untreated control group and an increase in concentrations of high-density lipoproteins (HDL-C) cholesterol.

INTRODUCTION

Omega-3 and Omega-6 polyunsaturated fatty acids (PUFA) are important components of cell membranes, and necessary for the health and normal physiological functioning of the human body (Kapoor et al., 2021). Not all fatty acids can be produced endogenously due to the absence of some desaturases enzymes, so they must be taken either from food or from nutritional supplements, according to the body's need for them (De Souza et al., 2020). The prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide. Diabetes increases the risk of cardiovascular disease and mortality, through various abnormalities in glucose, lipid and lipoprotein metabolism, increased platelet synthesis, endothelial dysfunction, and increased risk of arrhythmias (Emerging Risk Factors Collaboration, 2011). Diet can play a role in the prevention of T2DM Polyunsaturated fatty acids (PUFAs) are of particular interest in the nutritional treatment of diabetes, given their potential role in many pathophysiological processes related to cardiovascular disease (CVD). Both Omega-3 and Omega-6 fatty acids are beneficial for improving lipids in healthy individuals and among people with type 2 diabetas, supplementation of Omega-3 fatty acids reduces triglycerides and VLDL cholesterol, however it may also increase LDL cholesterol (Jeppesen et al,. 2013). Terpstra et al. (2000) indicated that polyunsaturated fatty acids can play a major role in lowering cholesterol by stimulating the secretion of cholesterol from the intestines, and stimulating its oxidation to bile

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acids. Cholesterol is excreted from the body. Another indication is that alpha-linolenic acid may prevent the conversion of LDL to very low density lipoproteins (Valsta et al. 1995). With regard to type 2 diabetes (T2DM), there are many studies and research that praised the positive role played by Omega-3 acids in diabetic patients. Hu et al. (2018) indicated that a diet containing Omega-3 plays a critical role in the beginning of the progression of T2DM. Consumption of omega-3 polyunsaturated fatty acids is the most common anti-T2DM (Ulven and Christiansen, 2015). And Kromhout et al. (2010) showed in an analysis restricted to diabetic patients that the fatal CHD rate was lower among diabetic patients who took EPA-DHA, compared to placebo.

MATERIALS AND METHODS

1- Preparing the mixtures

Fish oil of German origin was purchased as an omega-3 source from a pharmacy in Baghdad, and pure Iraqi sunflower oil was purchased as an omega-6 source from local markets in Baghdad. Palej et al

. (2020) note that for every 100 grams of sunflower oil it contains 50 grams of omega-6. After confirming that it is safe and not expired. It was used in the experiment. These oils were mixed well in different proportions with the prepared feed depending on what was stated in (NRC, 1995) and kneaded by adding a little filtered water to it, then formed in specialized molds and placed in an oven at a temperature of (60°C) for no more than (15) minutes. Until dried and made palatable to animals (Al-Bayati et al., 2017), then placed in nylon bags with the name and treatment number written on it.

2- Animals used

White laboratory mice (Albino mice) were used, aged (2-3) months, and weighed (25-30) g. 32 males were used in this study. These animals were purchased from the animal house of the Iraqi Center for Cancer Research / Baghdad, and they were examined from Before using it in the experiment, a veterinarian specialized in the center ensures that it is safe, healthy, and free of diseases.

3- Diabetes in animals

28 mice were injected with alloxan at a concentration of 100 mg/kg body weight after 24 h of starvation Mohammed et al. (2010) by subcutaneous injection as it was dissolved with 1 g of alloxan in 10 cm of normal saline physiological solution Owoyele et al. (2005). All injected mice were tested for diabetes by glucose test device and confirmed. And they became ill, as the blood sugar of the affected mice ranged between (250-270) mg/dL.

4-Experimental Design

The male mice used in the study were divided into eight groups, and each group has its own proportion that differs from the other in pedigree. The eight groups were left to feed on their allotted ration, as well as filtered water for 30 days, as we suggested that the proportions be as follows:

Group (A): The control group, which included 4 healthy mice, were fed with normal fodder and normal water.

Group (B): The control group with alloxan-induced diabetes who was fed a regular diet and normal water.

Group (C): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3: omega-6 (1:10).

Group (D): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3: omega-6 (1:5).

Group (E): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3: omega-6 (1:3).

Group (F): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3: omega-6 (3:1).

Group (G): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3: omega-6 (5:1).

Group (H): This group included four diabetic mice, fed on a diet in which the ratio was Omega-3:omega-6 (10:1).

5-Blood samples

After the end of the experiment period, the animals were starved for 12 h, then blood samples were drawn by stabbing the anesthetized animal's heart with a 5 ml plastic syringe, in anticoagulant-free test tubes and left for a quarter of an hour in water bath at 370 °C, then the serum was obtained by centrifugation at 3000 rpm for 15 min, and kept at -20 °C in new, clean plastic tubes until biochemical tests were performed (Al-Samarrai, 2019).

6- Biochemical Analysis

The blood glucose concentration was measured using the AGAPPE glucose test kit from India (Tietz, 1995), Serum triglyceride concentration was determined using an AGAPPE kit (Trinder, 1969), total serum cholesterol was determined using an AGAPPE kit, in which cholesterol was converted to the pink Quinonimine dye (Allain, 1974), The concentration of high-density lipoprotein in the blood was determined according to the supplier's instructions (AGAPPE) (Tietz, 1995). LDL-C was determined in the serum of groups according to the following relationship (Burtis and Ashwood, 1999): LDL-C concentration (mg / dl) = total cholesterol - (HDL-C) - (VLDL-C). The concentration ratio (VLDL) was determined in serum based on the following relationship (Burtis and Ashwood, 1999): VLDL concentration (mg/dL) = (triglycerides/5).

7- Statistical analysis

The results were analyzed statistically using (SAS, 2001) program and according to one-way analysis of variance. Analysis of Varience (ANOVA) of the parameters were tested using the Duncun multiple ranges test with a significance level of P≤0.05 to determine the significant differences between groups (Duncan and Mahaffey, 1994).

RESULTS AND DISCUSSION

The concentration of glucose in the blood serum

The results of the current study in Table No. (1) showed that the introduction of Aloxan diabetes mellitus led to a significant increase at the probability level (P < 0.01) in the blood glucose concentration of untreated diabetic male mice over the course of the experiment compared to the control group. These results are in agreement with Rahimi-Madiseh et al. (2017). The reason is that beta cells disintegrate and stop secreting the hormone insulin as a result of alloxan injections into mice.

Table (1) Effect of different proportion mixtures of omega-3 and omega-6 fats on the level of glucose in the blood serum of healthy and alloxan –induced diabetes male mice

Groups	Transactions	Glucose mg/ dl
A	control (normal	92.4 ^{h}
B	Diabetic control	262.58 ^{a}
C	Omega-3: omega-6 (1:10)	200.28^{b}
D	Omega-3: omega-6 (1:5)	187.75 ^{c}
E	Omega-3: omega-6 (1:3)	171.13 ^{d}
F	Omega-3:omega-6 (3:1)	152.52 ^{e}
G	Omega-3:omega-6 (5:1)	128.96 ^{f}
H	Omega-3:omega-6 (10:1)	99.24 ^{g}

The number of animals is four in each group, Different letters mean that there is a big difference in (P≤0.05) and (P≤0.01)

The results showed that groups C, D and E had a significant decrease (P < 0.01) in blood sugar compared to the infected control group, and we note the superiority of group E compared to groups C and D, which led to a decrease in blood sugar. This results are in agreement with Wu et al. (2017) who indicated that omega-6 improves blood sugar and insulin resistance. The reason for the decrease in blood glucose level may be due to the effect of omega-6 on insulin receptors, as Kröger et al. (2015) indicated that incorporation of linoleic acid into phospholipids changes the membrane fluidity and may modulate the insulin receptor. This results are also consistent with Imamura et al. (2016) who indicating that dietary PUFAs predominantly linoleic acid improved blood sugar,

insulin resistance and insulin secretion capacity, compared to carbohydrates and saturated fats. The results also showed a significant decrease at ($P < 0.01$) in treatments F, G and H, in blood glucose compared to the infected control group, and groups C, D, E. We note that the superiority of treatment H compared to treatment F, G gave the mice of 99.24 mg/dL. This results agree with Elhabiby et al. (2018) and Komal et al. (2020) that supplementation with Omega-3 PUFAs has healthy effects on lipid and glucose levels (Komal et al.,2020). They also agreed with Talukdar et al. (2010). Because omega-3 is a component of the cell membrane and acts as an anti-inflammatory and antioxidant, it is useful for preventing diabetes.

Effect on total cholesterol

The results of the study in Table No. (2) showed a significant increase ($p < 0.01$) of total cholesterol concentration in the affected control group compared to the healthy control group. This result is in agreement with the findings of Jassim et al. (2017); Mans and Aburjai (2019). The increase in total cholesterol in the blood may be due to the release of free fatty acids (FFA) from lipolysis in adipose tissue, where fats are used as an energy source instead of glucose when the body suffers from diabetes (Al-Safi and Abdel-Latif, 2018). The results showed a significant decrease in the concentration of cholesterol in the blood ($p < 0.01$) in groups C, D, and E where the use of omega-6 was higher than omega-3, and this may be due to the ability of omega-6 to inhibit the formation of triglycerides and cholesterol in the liver by decreasing the induction of enzyme secretion [Hepatic 3-hydroxyl-3-Methylglutaryl Coenzyme A (HMG-CoA)]. (The union of two molecules of acetyl CoA from mevalonate (Lehninger, 1982). This results also showed a significant decrease in the concentration of cholesterol in the blood ($p < 0.01$) in the groups F, G, and H in which the proportion of omega-3 was used higher than that of omega-6, where the arithmetic mean of the groups was (140.04, 112.78 and 130.17) mg/dL, respectively, compared with the infected control group (174.66) mg/dL. We note that treatment H recorded the lowest percentage of total cholesterol (112.78) mg/dL, while treatment C recorded the highest percentage (165.30) mg/dL compared to the infection control group.

Table (2) Effect of different proportion mixtures of omega-3 and omega-6 fats in (Cholesterol, T.G, HDL, LDL, and VLDL) the blood serum of healthy male mice and alloxan-induced diabetes

Groups	Total cholesterol (mg / dl)	T.G (mg / dl)	HDL (mg / dl)	LDL (mg / dl)	VLDL (mg / dl)
A	120.22 g	84.79 g	40.00 b	63.26 g	16.958 f
B	174.66 a	134.04 a	19.89 f	127.96 a	26.809 a
C	165.30 b	125.85 b	21.03 f	119.10 b	25.169 b
D	160.15 c	121.28 c	25.89 e	110.01 c	24.255 c
E	145.78 d	110.87 d	30.77 d	92.84 d	22.175 d
F	140.04 e	101.89 e	35.40 c	84.26 e	20.378 d
G	112.78 h	90.15 f	40.97 b	71.16 f	18.030 e
H	130.17 f	81.80 g	45.76 a	50.66 h	16.359 f

The number of animals is four in each group, Different letters mean that there is a big difference in ($P \leq 0.05$) and($P \leq 0.01$), (A) the control (normal), (B) Diabetic control, (C) Omega-3:omega-6 (1:10) , (D) Omega-3:omega-6 (1:5) , (E) Omega-3:omega-6 (1:3) , (F) Omega-3:omega-6 (3:1) , (G) Omega-3:omega-6 (5:1) , (H) Omega-3:omega-6 (10:1)

The results showed that the groups F, G, and H containing higher levels of omega-3 had a significant decrease in total cholesterol compared to the control group. The reason may be the role of omega-3 in inhibiting the enzymes that form triglycerides and cholesterol (HMG-CoA). These results are consistent with those of Goh et al. (1997) when examining the effect of omega-3 fatty acids on plasma lipids, cholesterol, lipoprotein and fatty acid content in NIDDM patients. The cholesterol-lowering mechanisms of omega-3 fatty acids in mice due to activation of AMP-activated protein kinase, as this protein plays a role in inhibiting cholesterol synthesis, activating fatty acid oxidation genes, and suppressing fatty acid synthesis in the liver (Tillander et al., 2014 Yang et al., 2016).

Effect on the concentration of triglycerides

The current results in Table No. (2) showed that the second group (B) represented by the diabetes control group experienced a significant increase in the concentration of triglycerides (TG) in the blood serum compared to the control group. This result agreed with Francis et al. (2018) in diabetic mice. This increase may be due to the activity of the lipoprotein lipase enzyme due to the lack of insulin. We also note from the results that the H group (Omega-3:omega-6 (10:1)) It gave the lowest arithmetic mean and showed a significant ($p>0.01$) decrease in triglyceride (TG) concentration compared to the infection control group. This result agreed with Faselian et al., (2021) that omega-3FA supplementation significantly reduces TC and TG levels. Another reason cited by Leaver et al., (2007) show that omega-3s can reduce TG and LDL levels and increase fatty acid oxidation in the liver by activating peroxisome proliferator-activated receptor (PPAR) molecules. Or they are activated by omega-3s, whose main function is regulatory binding factors that act as regulators of lipid homeostasis. Omega-3 fatty acids also reduce all markers of chronic inflammation associated with atherosclerosis including diabetes mellitus, thus reducing high-sensitivity C-reactive protein, oxidized LDL, and lipoprotein-associated phospholipase A2. Thus, omega-3 fatty acids contribute to reducing high levels of triglycerides (Ferrieres, 2020).

Effect on cholesterol high-density lipoproteins (HDL-C)

The results in Table (2) show a significant decrease at the probability level ($p<0.01$) of HDL-C values in the blood serum of the affected control group compared to the control group. These results are in agreement with the result of Gao et al.(2020). The decrease in HDL-C in diabetes may be due to the increased activity of cholesterol transferase, which transfers cholesterol esters from HDL-C to VLDL-C, leaving HDL-C rich in TG and less affinity for Apo-A. This makes it free and easy to filter through the kidneys (Al-Jubouri, 2008) We note through the results of the superiority of the H group and it gave a significant increase in the level of HDL and showed a statistical difference from the other arithmetic averages, and the average was 45.76 mg / dL compared to the infected control group of 19.89 mg / dL. While the lowest mean among the treated groups in group C was 19.89 mg/dL. The increase in HDL cholesterol levels in groups C, D and F may be due to the positive effect of omega-6, and this finding is consistent with Shearer and Wallker (2018) who indicated that omega-6 fatty acids reduce the risk of heart disease. It lowers total cholesterol levels, reduces bad cholesterol, and raises levels of "good" HDL cholesterol. However, omega-6 is not as effective as omega-3. The results showed that the groups in which the proportions of omega-3 were higher than omega-6 were superior, and the result was in agreement with Al-Zubaidi (2012) and Othman (2015) who indicate that omega-3 acids increase the proportion of protein seborrhoeic; High-density HDL transports cholesterol from the body to the liver, and lowers the rate of low malignancies. LDL, which transports cholesterol from the liver to the liver. around the body. This may be due to the multiple roles of omega-3s as anti-inflammatory and antioxidant and thus reduce free radicals caused by oxidative stress, or through activation of lipoprotein lipase and TG removal of VLDL and chylomicron molecules (Bays et al., 2008; Brewery,20004)

Effect on low-density lipoprotein cholesterol (LDL-C)

The results in Table (2) showed a significant increase ($P < 0.01$) in the level of low-density lipoprotein cholesterol in the control group of infected animals compared to the healthy control group. This result is in agreement with Al-Jafar et al. (2020) on male mice exposed to oxidative

stress. The cause may be free radicals from alloxan use and pancreatic B-cell damage, which leads to an increased level of oxidative stress, leading to a defect in LDL-C receptors in the liver (Brewery, 20004). We note from the results that the groups C, D, and E whose omega-6 levels were higher than omega-3 showed less improvement in LDL levels compared to the F, G, and H groups that had the highest omega-3 levels. These results agreed with Mensink et al. (2003) and also agreed with Hodson et al. (2001) and Wu et al. (2017) who indicated that unsaturated fatty acids have a positive effect on blood lipid metabolic pathways, leading to a decrease in total cholesterol and an increase in good cholesterol. The C, D, and F groups that gave a slight improvement in LDL levels may be due to the fact that omega-6 promotes oxidative stress, as shown by Harris et al. (2009) that diets high in LA may increase oxidative susceptibility. Low-density lipoprotein LDL oxidation in vivo, and can promote oxidized LDL. The results also showed a significant decrease ($P < 0.01$) in groups F, G, and H in LDL levels compared to the infected control group and groups C,D and F We also note the superiority of group H compared to groups G, and f , which gave the mean Mathematical 50.66 mg//dl. These results are consistent with study of Lee et al. (2013) who show that combination therapy with 3 omega fatty acids can lead to a significant benefit in terms of LDL particle size and TG level in dyslipidemic patients with type 2 diabetes.

Effect on very low density lipoprotein cholesterol (VLDL)

The results of Table (2) show a significant increase ($P \leq 0.01$) in very low-density lipoprotein cholesterol in the serum of diabetic experimental mice throughout the experiment period (infected control) compared to the healthy control group. These results are in agreement with those Mohammed (2016) and Jandal and Naji (2021) in diabetic rats. High very low-density lipoprotein cholesterol may be caused by adipose tissue damage due to impaired oxidative protective systems and exacerbation of free radical damage from oxidative stress as a result of injury. Diabetes, leading to an increase in free fatty acids in VLDL-C synthesis by the liver (Zhang, 2001). We note through the results of a significant decrease ($p < 0.01$) in the levels of very low-density lipoprotein cholesterol in the blood serum of male mice with alloxan-induced diabetes in the groups treated with unsaturated fatty acids represented by omega 3 and omega 6 compared to the infection control group. These results agree with Shearer et al,(2012) that omega-3 reduces the rate of LDL synthesis in the liver. The reason may be that omega-3 impairs VLDL secretion by inhibiting triglyceride synthesis. Bays et al. (2008) indicated that omega-3 fatty acids decrease the synthesis and secretion of very low-density lipoprotein (VLDL) particles, and increase TG clearance of VLDL particles and chylomicrons by regulating enzymes, such as lipoprotein lipase.

CONCLUSION

Balance of food Omega 6: Omega 3 is very important in maintaining the health and vitality of the body. We found through the results that the groups that contain the levels of Omega 3 in a positive relationship with Omega 6 gave a positive improvement as they reduced inflammatory processes and free radicals, lowered glucose levels, and improved blood glucose levels. Blood lipids in the affected groups.

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دراسة التأثيرات الصحية لخلطات نسب الدهون المختلفة من أوميغا 3 إلى أوميغا 6 في ذكور الفئران المصابة بداء السكري المستحث بالألوكسان

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الكلمات المفتاحية:
أوميغا-3 ، اوميغا-6 ، الكلوكوز ، داء السكري ، دهون الدم

الخلاصة

أجُريت الدراسة في المركز العراقي لأبحاث السرطان / الجامعة المستنصرية / محافظة بغداد ، للفترة من تشرين الثاني 2020 إلى كانون الثاني 2021 ، لِمعرفة الآثار الصحية لخلطات مختلفة النسب من أوميغا- 6: دهون أوميغا- 3 في ذكور الفئران المصابة بداء السكري النوع الثاني المستحدث بالألوكسان بتركيز 100 ملجم / كجم من الوزن. إذ تم تضمين 32 فأر تتراوح أعمارهم بين (2-3) أشهر و أوزانهم (25-30) جم. تم عزل أربعة فئران منها مجموعة سيطرة سليمة وحُقنت الفئران المتبقية بالألوكسان. قُسمت الفئران المصابة إلى سَبع مجموعات ، إحداها كانت مجموعة غير معالجة (سيطرة مصابة) ، بينما عولجت البقية بخلطات مختلفة النسب من دهون أوميغا 6 و 3. وبعد إنتهاء التجربة التي أستمرت 30 يوماً، أوضحت الدراسة البايولوجية إنَّ استحداث داء السكري التجريبي بالألوكسان أدى إلى إرتفاع معنوي عند ($p>0.01$) و ($P>0.05$) في تركيز الكلوكوز(glucose) والكوليسترول الكلي (TC) والكليسريدات الثلاثية(TG) والبروتين الدهني واطئ الكثافة للكوليسترول (LDL-C) والبروتين الدهني واطئ الكثافة جدا للكوليسترول(VLDL-C) مقارنة مع مجموعة السيطرة السليمة . بينما أدت إلى إنخفاض معنوي ($p>0.01$) و ($P>0.05$) في تراكيز البروتينات الدهنية عالية الكثافة للكوليسترول (HDL-C) في مجموعة السيطرة المصابة ، أمَّا عند إستخدام خلطات مختلفة النسب من دهون أوميغا-6 :أوميغا-3 أدت إلى إنخفاض في تركيز الكلوكوز(glucose) والكوليسترول الكلي(TC) والكليسريدات الثلاثية (TG) والبروتين الدهني واطئ الكثافة للكوليسترول(LDL-C) والبروتين الدهني واطئ الكثافة جدا للكوليسترول (VLDL-C) مقارنة مع مجموعة السيطرة المصابة وغير المعالجة . وإرتفاع في تراكيز البروتينات الدهنية عالية الكثافة للكوليسترول (HDL-C).