OPEN ACCESS ORIGINAL ARTICLE

EFFECT OF POLYUNSATURATED FATTY ACIDS PUFA ON LIPID PROFILE AND GLUCOSE LEVEL AMONG ALLOXAN – INDUCED TYPE – II DIABETES MALE ALBINO RATS

Sadia Khan[1], Mumtaz Ali Qureshi[2], Sumayya Qazi[3], Bakhtawar Qureshi[4], Kazbano Ramsha[5]

ABSTRACT

OBJECTIVE The purpose of this study was to determine the effect of polyunsaturated fatty acids on lipid profile and glucose level among alloxan induced diabetes male albino rats.**METHODOLOGY:** A total 50 male albino rats of Wister strain, weighing from 200 to 250 grams were taken for this experimental study. All the rats were procured from the animal husbandry of Sindh Agricultural University, Tandojam. They were maintained on balanced laboratory – based diet. The rats were also kept in 12 hour light- and 12 hour dark cycle. Water was freely given. All the rats were divided in five respective groups, named as control group-A, Diabetic control group-B and experimental group-C, D and E. Each group contained 10 rats and were kept in separate cages.**RESULT:** Mean fasting blood glucose in different group before induction showed insignificant difference among the group p=0.996. Mean triglyceride showed significant findings with p<0.001 in different groups showed insignificant findings with p=0.128 whereas mean level of LDL showed insignificant results among the group with p+0.961. **CONCLUSION:** The present study showed beneficial results of omega 3 fatty acids and omega 6 fatty acids when administered in combination (ratio of 1:1) on dyslipidaemia caused by insulin resistance in type II diabetes mellitus.

KEY WORDS: Omega -3, 6, lipid profile, Alloxan Albino rats.

1. Lecturer, Department of Biochemistry, Isra University,
2. Professor,Department of Biochemistry,Isra University,
3. Associate Professor,Department of Biochemistry,Isra University,
4. Isra University,drbakhtawarqureshi@gmail.com
5. Lecturer ,Department of Biochemistry ,Isra University,

Correspondence Author: Sumayya Qazi, Associate Professor, Department of Biochemistry, drsumayyaqazi@gmail.com

How To Cite This Article: Khan S [1], Qureshi MA [2], Qazi S[3], Qureshi B[4], Ramsha K [5] **EFFECT OF POLYUNSATURATED FATTY ACIDS PUFA ON LIPID PROFILE AND GLUCOSE LEVEL AMONG ALLOXAN – INDUCED TYPE – II DIABETES MALE ALBINO RATS.** JPUMHS;2023:13:04,61-66. http://doi.org/10.46536/jpumhs/2023/13.04.472

Received Aug 05 .2023, Accepted On 15 December 2023, Published On 31 December 2023.

INTRODUCTION

Around 41 million deaths globally are attributed due to non-communicable diseases (NCDs) that is equivalent to 74% of all deaths globally[1]. Among four major non-communicable disease including diabetes mellitus (DM), cardiovascular disease, chronic respiratory disease, and cancer[2], DM is now alarmingly increasing among general population over the last few decades, which is quite alarming. Eventually, diabetes-specific sequelae negatively influence the quality of life (QOL), increasing the need for healthcare services and financial costs[3]. Among all types of Diabetes, type-II diabetes mellitus (Type-II DM) is highly prevalent (90% of the population), affecting 537 million adults worldwide and is predicted to become 643 million by 2030 and 783 million by 2045[4]. The prevalence of Type-II DM in Pakistan has increased from 6.6 million in 2012 (ranked top 10th worldwide) to 70% in 2021 (ranked 3rd worldwide); predicted to be 66.2 million by

2045[4]. Recent studies have shown that diabetic patients with controlled lipid profile and blood pressures are less likely to have cardiovascular events thereby reducing mortality among diabetic subjects due to these risk factors[5-7]. Several studies demonstrated that persistent hyperglycemia causes underlying harm and damage to veins and tissues, resulting in a variety of complications such as diabetic neuropathy, retinopathy, hypertension, hyperlipidemia, cardiovascular diseases, and atherosclerotic coronary disease[8]. When considering the management and progression of diabetes mellitus and the consequences it causes, it is important to keep in mind the importance of dietary nutrients to lower the rate of incidence of type II diabetes mellitus and also the discomforts related with diabetes, particularly type II. The effect of diet is critical for the wellbeing of individuals. Modifying lifestyle and dietary habits can be exceptionally successful methods in prophylaxis and treatment of type-II diabetes. Studies show that dietary fat quality influences insulin and also the causes associated with type-II diabetes. Administration of Polyunsaturated Fatty Acids promotes good health and decreases the complications associated with type-II diabetes. Dietary fats are considered essential supplements and enhancers for endurance of diabetes[9]. Some important food components are not produced by the body and must be obtained from exogenous sources. Unsaturated fats are considered essential biochemical components for all body organs, including the lungs, heart, skin, and brain[10].Omega unsaturated fats are referred to as unsaturated solid fats capable of preventing a variety of clinical conditions, including depression, heart disease, mental illness, asthma, and joint pain[11]. Omega-3 unsaturated fats (-linoleic acid) are essential unsaturated fats that play a significant role in brain function, prevention of cardiovascular diseases and enhancing glycemic control[12-13]. Additionally, it has been established that docosahexanoeic acid (DHA), eicoapentaenoic acid (EPA), and alpha linoleic acid (ALA) are the primary members of this long chain unsaturated fat family. These fundamental unsaturated fats, which are capable of lowering blood fat levels[14, 15], are important for the health of the body. Omega-3 fats should be taken twice as often as omega 6 fatty acid. The recommended ratio is 1:1 – 1:4 for intake of n-3 FAs and n-6 FAs respectively[16]. These fundamental unsaturated fats are essential for normal development and have the ability to reduce blood fatty acid levels. Although dietary administration of Omega-3 and Omega-6 fatty acids has been a subject of a lot of research, the relationship between the effects of combination therapy with omega-3 and omega- 6 polyunsaturated fatty acids and lipid profile of diabetic subjects is still not well established. Hence, the current study was aim to identify the combined effects of Omega-3 fatty acids and Omega-6 polyunsaturated fats on the lipid profile of type II diabetic rats.

METHODS

Study Design

Experimental analytical study design.

Study Setting

This experimental analytical study has been carried out in the clinical laboratory of Isra University, Hyderabad and animal house of Sindh Agricultural University, Tandojam - Sindh.

Sample Size

N = 50 Male Albino Wistar Strain

Sampling Technique

Simple random sampling technique was adopted for the allocation of rats in study groups.

Sample Selection

Alloxan – induced type – II diabetic male Albino rats of Wistar Strains with minimum glucose concentration of 200 – 250 mg/dl and weighing 200-250grams. All those rats who were physically disabled/ sick or failed to meet required glucose level were excluded from the study.

Grouping of Rats

A sample of 50 rats were included in the trial that were equally divided n=10 in each of the five experimental groups;

Group – A (Normal Control Group)

The animals in this group were fed with normal diet for 30 days.

Group – B (Diabetic Control Group)

The animals were treated with alloxan intraperitoneally injection 120mg/kg.bw to induce diabetes.

Group – C (Experimental Omega – 3 Treated Group)

Diabetic induced animals were treated with 0.3gms/kg body weight of Omega – 3 for 30 days

Group – D (Experimental Omega-6 Treated Groups)

Diabetic induced animals were treated with Omega – 6 in a dose of 0.3gms/kg body weight for 30 days

Group – E (Experimental Combination Treated Groups)

Diabetic induced animals were treated with combination of Omega – 3 and Omega – 6 in a dose of 0.3gms/kg body weight for 30 days.

Animal Housing and Drug Dosage

The ethical committee of Isra University approved the use of laboratory animals. A total of 50 male rats having body weight of between 200 – 250gms were taken from the animal husbandry of Sindh Agriculture University, Tandojam and divided in 5 groups, each group having 10 rats. The rats were housed in a solitary clear sided plastic enclosures and were named appropriately. The animals were handled according to the guidelines issued by US and published by NIH. All rats were kept at room temperature 30±1^{o}C with a 12 hour light and dark cycle and were given fat rich diet. Additionally blood glucose levels were checked with glucometer. The experimental study was carried out for 30 days. A single dose of alloxan was administered according to their bodyweight (120 mg/kg) to induce diabetes. Rats were fasted 12 hours before and after injection of allaxon as unfed animals are more susceptible for allaxon induced diabetes. Diabetes was confirmed by laboratory analysis of blood from the tail area 72 hours after alloxan infusion. Rats with glucose level of more than 250 mg/dl was selected for further examination. Omega 3 and Omega 6 was administered orally once daily mixed with their fat rich chow. After the experiment, the weights of the control and experimental animals were measured. Each rat was deeply anesthetized by an overdose of chloroform and they were sacrificed by cervical dislocation. Blood was drawn by the method of cardiac puncture which was used for biochemical analysis.

Laboratory Analysis

The blood samples were brought to the clinical laboratory of Isra University Hyderabad, and the blood glucose levels were checked on automatic analyzer. Lipid profile levels were analyzed on Hitachi automatic analyzer. Values were compared between different groups to observe level of significance.

Lipid Profile

As per the Tietx et all Lipid profile was performed by convention utilizing Roche/Hitachi Diagnostics. Every one of the trial of lipid profile was completed on Roche/Hitachi, Cobas 311 programmed analyzer.

Serum Glutathione peroxidase (GPX)

Serum Glutathione peroxidase (GPX) levels were determined using AVIVA Diagnostic Assay Rat GPX Kit

Data Analysis

The data was analyzed by using SPSS (Statistical Packages for Social Sciences) version 22.0. The results were presented on tables and graphs. The statistical analysis of the differences of various quantitative variables between the experimental and control groups was evaluated by one way analysis of variance (ANOVA).

RESULTS

The study was conducted on 50 rats that were divided into 5 groups. FBS and RBS was measured before and after induction of diabetes which showed greatest FBS level in omega 6 group with mean value of 257.30 ±14.99 followed by diabetic control group; 233.24 ±18.62, omega-3 and 6 group 227.9±14.15, omega 3 group; 227.3 ±15.79 and normal control group 109.30 ±3.07 respectively. RBS levels significantly increase in all groups except for normal control with the highest mean difference of 93.46± 7.65 ($p<0.0001$) record in omega 3 & 6 group followed by omega 6, omega 3 and diabetic control group respectively. Details are mentioned in table 1.

Lipid profile was performed utilizing Roche/Hitachi Diagnostics. Results revealed a level of cholesterol in Diabetic control, Experimental Omega-6 groups, and Experimental Omega-3 group with a mean value of 151.34±18.98, 121.62±11.43, and 116.60±13.91 respectively. For triglyceride, all values recorded in the groups were found to be in the normal range limit of TG. For GHDL the highest value was recorded in group E provided with both omega 3 and 6 with a mean score of 33.56±6.07 followed by group C treated with omega 3 only with a mean score of 26.11±8.29. The lowest value of HDL were recorded in group B with a mean score of 18.90±7.03 respectively. The details are shown in table 2.

Table 1: Depicting FBS and RBS before and after induction of Diabetes											
Variable		**Normal Control**	**p value (<0.05)**	**Diabetic Control**	**p value (<0.05)**	**Omega 3**	**p value (<0.05)**	**Omega 6**	**p value (<0.05)**	**Omega 3 & 6**	**p value (<0.05)**
FBS	Before Induction	115.10 ±5.66	0.055	115.94±6.81	<0.001	111.37 ±4.96	<0.001	107.21± 4.81	<0.001	105.19± 5.28	<0.001
	After Induction	109.30 ±3.07		233.24 ±18.62		227.3 ±15.79		257.30 ±14.99		227.9±14.15	
RBS	Before Induction	147.54 ±4.88	0.09	158.34±5.69	<0.001	144.21± 4.94	<0.001	143.97± 4.83	<0.001	141.73± 5.85	<0.001
	After Induction	142.71 ±5.30		218.17 ±6.52		230.42 ±14.67		232.45± 11.73		235.19 ±13.50	

Table 2 : Showing cholesterol, triglyceride and HDL levels among rats									
Groups	**Cholesterol**			**Triglyceride**			**HDL**		
	Mean ± SD	**F-value**	**p-value (<0.05)**	**Mean ± SD**	**F-value**	**p-value (<0.05)**	**Mean ± SD**	**F-value**	**p-value (<0.05)**
Group A. (Control)	64.40±11.47	56.39	0.001	58.10±10.45	61.55	0.0001	19.10±4.95	9.25	0.0001
Group B. (Diabetic Control)	151.34±18.98			135.11±18.57			18.90±7.03		
Group C. (Experimental Omega – 3 Treated Group)	116.60±13.91			111.56±12.49			26.11±8.29		
Group D. (Experimental Omega-6 Treated Groups)	121.62±11.43			127.42±10.43			23.04±4.32		
Group E. (Experimental Combination Treated Groups)	98.70±9.56			85.13±10.03			33.56±6.07		

DISCUSSION

In this study, the impact of Omega-3 fatty acids and 6 fatty acids have been explored on the various factors of lipid profile like cholesterol, TGs, and HDL among type-II diabetic animal models. The results revealed that Various studies are conducted to find out the effects of Polyunsaturated Fatty Acids on blood glucose level and also on insulin in diabetic grown-ups. As per a few researches, utilization of both Omega 3 FAs and Omega 6 FAs might be useful not only on glycemic control but also on insulin resistance[17]. It is also detailed that the Omega 3 Fatty Acids essentially help type-II diabetics to deal with the glycemic status and dyslipidemia.

A portion dependent investigation in 2015 uncovered that the ingestion of Omega 3 unsaturated fats assisted with treating insulin obstruction and dyslipidemia, caused inside Wistar rats utilizing fructose. This study concluded that Omega 3 unsaturated fats changed the metabolic pathways and managed hepatic lipogenic quality which eventually causes an improvement towards insulin obstruction and dyslipidemia. Comparative outcomes have likewise been seen by the current examination in regards to insulin affectability and dyslipidemia.

Rossmeisi et al. in 2012 investigated on dietary corpulent mice with corn oil-based high fat eating routine and announced that replacing upto 44% of dietary lipids by Omega 3 as either fatty oils or as phospholipids may ensure betterment of dyslipidemia, glucose homeostasis and insulin obstruction as supported by present study[18].

Another study conducted in 2021 revealed the effects of different ratio mixtures of Omega-3

to Omega-6 in male rats with type 2 diabetes and concluded that Balanced proportions of Omega 6: Omega 3 plays a very important role in maintaining the health of body. This is also supported by results from present study that the groups of rats that were fed balanced proportions of Omega 3 FAs and Omega 6 FAs gave improvement as they decreased inflammatory processes and lowered free radicals, also improved blood glucose levels and lipid profile in their respective groups[19]. Sarbolouki et al in 2013 directed a controlled randomized clinical trial on 67 over weight patients with type-II diabetes mellitus for 3 months to decide impacts of EPA which is a class of n-3 PUFA. The examination subjects were isolated in two different control groups which got 2 grams of corn oil every day and the other group got 2 grams of EPA on regular schedule. The outcomes proved that after 3 months of EPA supplementation, there was huge decrease in fasting plasma glucose ($p < 0.001$), HbA1c ($p=0.01$) and HOMA-IR ($p=0.032$) when contrasted with other group. Hence discoveries of glucose homeostasis of present examination is predictable with previously mentioned study[20]. All these studies shows that both omega 3 and 6 fatty acids helps maintain lipid profile, thus it is thus recommended that further studies should be conducted to assess the optimized portions of triple Omega fatty acids in combination instead of individual fatty acids on molecular level.

CONCLUSION

It has been concluded from current study that dietary intake which is abundant in both Omega-3 fatty acids and Omega-6 fatty acids proved beneficial in improving the lipid profile in type II diabetic subjects. Individual therapy of Omega-3 fatty acids and Omega-6 fatty acids showed limited improvement on dyslipidemia caused by type II Diabetes as compared to combined therapy.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

FUNDING: The work was not financially supported by any organization. The entire expense was taken by the authors

ACKNOWLEDGEMENTS: We would like to thank the all contributors and staff and other persons for providing useful information.

AUTHORS' CONTRIBUTIONS: All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared.

REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases
2. https://www.emro.who.int/noncommunicable-diseases/diabetes/index.html#:~:text=Diabetes%20is%20one%20of%20the,the%20insulin%20it%20does%20produce.
3. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019 Jan;62:3-16.
4. IDF
5. Low Wang CC et al. Clinical update : Cardiovascular disease in diabetes mellitus – Mechanisms, management and clinical consideration. Circulation 2016;133(24):459-2502.
6. M et al. Heart disease and stroke statistics report 2502. Circulation 2016;133(4):447-454.
7. Preist SR et al. Trends in all cause cardiovascular disease mortality in young onset diabetes: type – II diabetes. Circulation 2009;119(13):1728-1735.
8. Akhtar S, Khan Z, Rqfiq M, Khan A, Prevalence of type – II diabetes in District Dir lower in Pakistan. Pak J Med Sci 2016;32(3):622-631.
9. Ansari RM, Hosseinzadeh H, Zwar NA. Primary health care system of Pakistan : Challenges to self management of Type – II diabetes. Lipids in Heart Disease 2016;13(1):183-189.
10. Salem N, Lin Y, Moriguchi T, Lim Sy, Salem N et al. Distribution of Omega 6 and

Omega 3 polyunsaturated fatty acids in the whole rat body and 25 compartments. Prostaglandins Leukot Essent fatty acids 2015;100:13-20.

11. Akhtar S, Khan Z, Rqfiq M, Khan A, Prevalence of type – II diabetes in District Dir lower in Pakistan. Pak J Med Sci 2016;32(3):622-631.
12. Muley P, Shah M, Muley A. Omega – 3 fatty acid supplementation in children to prevent asthma J. Allergy 2015;201:50-50.
13. Lalia AZ, Lanza IR. Insulin sensitizing of Omega – 3 fatty acids. Lost in translation. Nutrients 2016;8(6):40-49.
14. Djousse L, Biggs ML, Lemaite RN, King IB, Song X et al. Piasma Omega – 3 fatty acids and incident diabetes in older adults. Am J Clin Nutr. 2011;94(2):527-533.Mazaherioun M, Sacdisomeolia A, Javanbakht MH, Eshraghian Djalali M. Beneficial effect of N – 3 fatty acids on adiponectin levels and adipo R gene expression in patients with diabetes mellitus : a randomized placebo control double – blind clinical trial. Arch Med. Sci 2017;13(4):716-720.
15. Artemis P. An increase of Omega – 6 to omega – 3 fatty acids ratio increases the risk for obesity. Nutrients 2016;8(3):128-132.
16. Ali. M. Atallah * Faryal. F. Hussein. Study of the Healthy Effects of Different Fat Ratios Mixtures of Omega-3 to Omega-6 in Male Mice with Alloxan-Induced Diabetes. ISSN:1813-1646 (Print); 2664-0597 (Online) Tikrit Journal for Agricultural Sciences.2021.
17. Huang W, Bansode RR, Bal NC, Mehta M, Mehta KD. Protein kinase Cβ deficiency attenuates obesity syndrome of ob/ob mice by promoting white adipose tissue remodeling. Journal of lipid research. 2012 Mar 1;53(3):368-78.
18. Ali M. Atallah Faryal F. Hussein. Study of the Healthy Effects of Different Fat Ratios Mixtures of Omega-3 to Omega-6 in Male Mice with Alloxan-Induced Diabetes. ISSN:1813-1646 (Print); 2664-0597 (Online) Tikrit Journal for Agricultural Sciences.2021.
19. Sarbolouki S, Javanbaht MH., Derakhshanian H, Hosseinzadeh P, Zareei M, Hashemi SB, Dorosty AR, Eshraghian MR. and Djalali M, 2013. Eicosapentaenoic acid improves insulin sensitivity and blood sugar in overweight type 2 diabetes mellitus patients: a double-blind randomsed clinical trial. Singapore Med J, 54(7),pp.387-390.