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EFFECTS OF OPTIMIZED PROPORTION OF OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACIDS ON BLOOD GLUCOSE LEVELS OF TYPE 2 DIABETES MELLITUS ALBINO WISTAR RATS

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ABSTRACT

OBJECTIVE: The purpose of this study was to compare the effects of consuming omega 3, omega 6, and a combination of both omega 3 and omega 6 on blood glucose levels in type 2 diabetic Wistar rats with control and diabetic control group rats. **METHODOLOGY.** In this investigation, n=50 male Albino Wistar rats weighing 200-250gms were employed. The animals were obtained from the Sindh Agricultural University's Animal House in Tandojam after ethical authorization to use them for testing was granted. A group of ten rats were maintained in clear-sided plastic cages and were named after the groups. **RESULTS:** A significant reduction was seen in the Diabetic group (Group B). Diabetes rats treated with Omega 3 and Omega 6 showed a drop in body weight, but it was smaller than the diabetes controls (Group B). However, the Omega-3 and Omega-6 combination therapy group (Group E) demonstrated the greatest results, indicating that the combination therapy greatly reduced body weight loss. **CONCLUSION:** The benefits of combining omega-3 and omega-6 PUFA were found to be more beneficial in avoiding HbA1C increase than -3 and -6 alone.

KEY WORDS: Diabetes Mellitus Type 2, Blood Glucose, Glycated Hemoglobin, Fatty Acids ω-3 & ω-6

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorder that has affected 462 million individual across the globe comprising of 6.28% of world population[1-2]. It has been estimated that more than one million death has been attributed to T2DM in the year 2017 alone making it the ninth leading cause of mortality worldwide[3]. Evidences provided by number of literatures revealed that more than 90% of the cases are of T2DM a condition that is marked by inadequate secretion of insulin by islets of β-cells, increased in tissue insulin resistance and decrease in insulin secretory responses[4].According to an American based study the trend of developing both type 1 and type 2 diabetes among young adult population of US is soaring rapidly from 9.0%/ 100,000 population in 2002 to 2003 to 13.8 percent in 2015 with an estimated change of 4.8% percent

annually[5]. Literatures are also providing evidences that global cost of diabetes is soaring rapidly and currently an estimated cost of around $800bn is incurring in the management of DM[6]. Globally, the increase in the trend of DM can mainly be attributed with increase in obesity and decrease in physical activity along with rapid trend of increasing consumption of fast food among population of approximately all ages[7]. It has been estimated that 55.2% of the population mainly form low middle income countries including Pakistan consumed fast food at least once a week[8]. Besides that the same study has also estimated that the percentage consumption of fast food per week was lowest among Americans (8.3%) and highest among South East Asian population (17.7%)[8-9]. Researchers conducted to identify the impact of nutrition and life style modification on preventing the incidence of T2DM revealed that consuming nutritional rich food and lowering the intake of ultra-processed food along with increased in physical activity up to a moderate intensity can significantly reduces the incidence and progression of T2DM[9]. According to American Diabetes Association Mediterranean style diet rich in polyunsaturated fatty acid (PUFA) to be recommended to type 2 DM patients. PUFA includes omega-3, omega-6 and omega-9 fats are considered to be a potential source of energy and boosting metabolism[10]. According to the recommendation of the United Kingdom (UK) government an adult individual must consume 6.5% of energy as PUFA and suggesting an eating of daily proportion of oily fish each week that fulfills the need of approximately 0.45g/dl of long chain of omega-3[10-11]. American Heart Association has recommended supplements for those adults not eating enough fish oil[12]. According to the global burden of disease study the optimal omega 6 uptake is 11% of energy whereas as global intake is only 5% similarly the optimal uptake of omega 3 is 0.25g/day whereas its uptake is only 0.10g/day[13-15]. Although international societies and government has taken lots of initiatives in improving the awareness of consuming PUFA in a daily routine yet number of studies have suggested conflicting effects of consuming PUFA on glucose metabolism and hence arise a need for further studies. It is therefore the present study has been aimed to determine the effects of consuming omega 3, omega 6 and combination of both omega 3 and omega 6 on blood glucose levels of type-2 Diabetic Wistar rats and to compare the findings with control and diabetic control group rats.

Methodology

Study Design

Experimental Analytical study

Study Setting

The study was carried out at clinical laboratory of Isra University Hospital and in the animal house of Sindh Agricultural University, Tandojam.

Intervention Protocol

A total of n=50 male Albino Wistar rats weighing 200-250gms were used in this study. The animals were purchased from the Animal house of Sindh Agricultural University, Tandojam after the ethical permission granted to use them for experimentation. A group of 10 rats were kept in a clear-sided plastic enclosures and named according to the groups respectively. All rats were kept at room temperature 30±1oC with a 12-hour light and dim cycle and were given research facility arranged adjusted eating routine as roll and water not indispensable before any dietary control. The animals for the experimental purpose were equally divided into five groups with 10 animals per group and were fed on their allotted ration, as well as filtered water for 30 days. Alloxan intraperitoneally 120mg/kg of body weight were introduced to induce diabetes. Blood sample for the measurement of glucose levels was taken from the base of the tail at 72 hours (3rd Day) to ensure onset of diabetes. The day on which blood glucose level reached up to 250mg/dl that day was counted as first day of experimentation.

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 fed with normal diet and treated with alloxan intraperitoneally 120mg/kg of body weight to induce diabetes.

Group C (Experimental Omega – 3 Treated Group): Diabetic induced animals were fed diet mixed with 0.3gms/kg of body weight of Omega – 3 fatty acid for 30 days.

Group D (Experimental Omega – 6 Treated Group): Diabetic induced animals were fed diet mixed with 0.3gms/kg of body weight of Omega – 6 fatty acid for 30 days.

Group E (Experimental Combination Treated Groups): Diabetic induced animals were fed diet mixed with equal amount of

Omega – 3 and Omega – **i**6 fatty acid of 0.3gms/kg of body weight for 30 days.

Outcome Measures

Fasting Blood Sugar and Random Blood Sugar levels: A fasting and random blood sugar level were estimated using an Accu-Check active glucose meter kit[16]. The blood was taken by pricking the tail of rats after cleaning it with alcohol gauze swabs. Ear marking method was used for the labeling of rats for keeping the records of blood sampling.

Glycated Hemoglobin HbA1C: Glycated Hemoglobin (HbA1C) levels were monitored at baseline and after 30 days of intervention by using Elisa commercial kit for estimating HbA1C (Crystal Chem, Chicago, IL; USA)[17]. The blood was taken by pricking the tail of rats after cleaning it with alcohol gauze swabs. Ear marking method was used for the labeling of rats for keeping the records of blood sampling.

Data Analysis : The analysis were performed on SPSS version 25 for descriptive analysis frequency and percentage tables and plot were drawn. Inferential statistics were performed at within group and between group comparisons. Paired t-test were applied for within group analyses and analysis of variance (One-way) for between group comparison. Level of significance were kept at 95% of Confidence Interval $p<0.05$.

RESULTS

A total number of n=50 Wistar rats were equally divided n=10 rats into each of the five groups. Demographics description had revealed no significant difference in the mean weight (between group analyses) of rats taken at baseline where as significant difference in the weight were observed after one month of intervention. Analyses had revealed that mean post experimental body weight and ± SD in the groups A, B, C, D and E was noted as 247.30 ± 6.27, 194.50 ± 9.34, 210.30 ± 5.27, 205.20 ± 6.56 and 230 ± 10.33 grams respectively (F-value 73.78, P=0.001). Significant decrease was noted in the Diabetic group (Group B). Omega 3 and Omega 6 treated diabetic rats (group C and D) also revealed a decrease in body weight but it was less compared to the diabetic controls (Group B). However, the Omega-3 and Omega-6 combination therapy group (Group E) showed the best results which reveals the combination therapy prevented the body weight loss significantly (table 1).

Table 1. Demographic description between group variation in weight after intervention

Groups	Mean	SD ±	F-Value	P-Value
Group A. (Control)	247.30	6.27	73.78	0.001
Group B. (Diabetic Control)	194.50	9.34		
Group C. (Experimental Omega – 3 Treated Group)	210.30	5.27		
Group D. (Experimental Omega-6 Treated Groups)	205.20	6.56		
Group E. (Experimental Combination Treated Groups)	230.80	10.33		

Mean level of FBS before induction in Normal control was 113.70±5.47, in diabetic control was 112.90±4.01, in Omega 3 treated was 109.20±4.96, in Omega 6 treated group was 98.43±4.81 and in Omega 3 & 6 was 108.60±5.69 and Mean level of FBS after induction in Normal control was 108.20±3.52, in Diabetic Control was 230.80±15.49, in Omega 3 treated group was 224.40±15.79, Omega 6 treated group was 253±12.43 and in Omega 3 & 6 treated group was 224.0±14.15. Paired t test showed the statistically significant at p value <0.05 and <0.001. The impact on RBS had revealed that the Mean level of RBS before induction in Normal Control was 144.50±4.67, in Diabetic Control was 151.20±5.02, in Omega 3 treated was 140.20±4.56, in Omega 6 treated was 151.4±6.21 and in Omega 3&6 treated was 143.70±4.27 .Mean level of RBS after induction RBS in Normal Control was 140.60±5.21, in Diabetic Control was 215.10±8.06, in Omega 3 treated was 229.0±16.43, in Omega 6 treated was 226.5±10.43 and in Omega 3&6 treated was 232.60±12.50 .paired t test showed the statistically significant difference before and after induction at p value <0.001. (Table 2)

Table 2 Within group Analyses using a paired t test for Fasting and Random Blood sugar levels					
Fasting Blood Sugar levels					
Variables	**Pre mean ± Sd**	**Post mean ± Sd**	**t-stat**	**t-critical**	**Level of Significance**
Group A	113.7±5.47	108.2±3.52	1.25	2.56	<0.055
Group B	112.9±4.96	230.8±15.49	-7.99		<0.001
Group C	109.2±4.8	224.0±15.79	-5.35		<0.001
Group D	109.2±4.81	253.4±12.45	-6.56		<0.001
Group E	108.6±5.69	224.4±14.59	-8.56		<0.001
Random Blood Sugar levels					
Group A	144.5±4.67	140.6±5.12	2.02	2.56	0.09
Group B	151.2±5.02	215.1±8.06	-6.5		<0.001
Group C	140.2±4.56	229±16.43	-4.65		<0.001
Group D	151.4±6.2	226.5±0.43	-5.62		<0.001
Group E	143.7±427	232.6±15.5	-6.66		<0.001

Further glycated hemoglobin levels were determined and between group analyses were performed after completion of intervention. Analyses had revealed that Mean level of HbA1c(%) in Normal Control was 3.03±0.76, in Diabetic Control was 8.13±2.47in Omega 3 treated was 4.92±1.49, in Omega 6 treated was 5.86±1.52 and in Omega 3&6 treated was 3.94±0.19. The serum HbA1c levels were found to be significantly elevated in the diabetic control group. Omega 3 and Omega 6 treated diabetic rats (group C and D) also revealed arise in serum HbA1c levels but it was less compared to the diabetic controls (Group B). However, the Omega-3 and Omega-6 combination therapy group (Group E) showed the significant results which reveals the combination therapy has Prevented the rise in HbA1c levels significantly.(table 3)

DISCUSSION

The findings of our study had revealed that both omega 3 and omega 6 were turned out to be effective in preventing rise in blood sugar levels after alloxan induction at both random and fasting blood sugar test. Moreover levels of glycated hemoglobin were also found to be significantly lowered in comparison to experimental control group. Analyses of findings further revealed that the combine effects of omega 3 and omega 6 had provided much better effects on Hba1c levels in comparison to individual effects of omeaga-3 and omega-6, hence suggesting the use of combine form of polyunsaturated fatty acids (PUFA) in diet to control diabetes and maintain normal blood sugar levels among T2DM patients. A study that was performed to determine the cardio protective effects of metformin and ω-3 PUFA on T2DM rat model had revealed that both drugs were turned out to be effective in suppressing metabolic changes associated with T2DM with emphasis on the use of combine therapy for more effective results[18]. Similarly in another study that was conducted to determine the effects of n-3 PUFA supplementation on gene expression of NF-E2-related factor-2 (NRF2) that considers as an important factor in inducing the expression of several protein with antioxidant effects revealed that significant improvement in the

Table 3 One way Analyses of Variance for between group comparison				
Groups	**Mean**	**SD ±**	**F-Value**	**P-Value**
Group A. (Control)	3.03	0.76	17.11	0.001
Group B. (Diabetic Control)	8.13	2.47		
Group C. (Experimental Omega – 3 Treated Group)	4.92	1.49		
Group D. (Experimental Omega-6 Treated Groups)	5.86	1.52		
Group E. (Experimental Combination Treated Groups)	3.94	0.19		

expression of NRF2 and overall improvement in antioxidant capacity, hence considered beneficial in the amelioration of oxidative stress and prevention of T2DM complications[19]. A meta-analysis that was performed to determine the effects of fish oil supplementation on glucose control and lipid levels among patients with T2DM. The meta-analysis had involved a 12 Randomized controlled trial and concluded that fish oil supplementation had shown no significant impact on fasting plasma glucose levels effect size=0.13, however significant effects was notice on lipid profile level where levels of HDL was increased and triglyceride levels were reduced. The meta-analysis concluded that fish oil supplementation had shown no significant effects on blood glucose levels of T2DM patients[20]. A study conducted to determine the effects curcumin and long chain of omega-3 PUFA supplementation on glycemic control among individuals at high risk of developing T2DM. The findings provided evidences that combine use of omega-3 and curcumin appears to be an effective strategy in lowering the risk of T2DM[21]. The findings of our study were found to be inconsistent with the findings of previously conducted researchers where most of the evidences were supporting the findings of our study yet conflicting results were also available suggesting no such impact of PUFA on blood sugar levels hence suggesting the need for further studies. In addition to there are has some strength of our study such as it has highlighted one of the most prevalent condition that is T2DM and the effects of PUFA in prevention of T2DM and also that present study can be made a bench mark for future studies on determining the effects pf PUFA on T2DM patients. Besides that during the conduct of this study few limitations that were come across while performing the study were: the study was limited in its approach in determining the effects of PUFA on FBS, RBS and Hba1C and no efforts were made to determine the effects on Lipid Profile levels. Similarly no efforts were made to identify the effect of ω-3 and ω-6 on other metabolic and inflammatory markers like Interlukin-6 (IL-6) and Tumor necrosis factor (TNF-α).

CONCLUSION

The study has concluded that long chains of omega-3 and omega-6 PUFA were turned out to be effective in preventing the rise in blood sugar levels and glycated hemoglobin concentration. The effects of combine use of omega-3 and omega-6 PUFA however found to be more effective in preventing the rise HbA1C than ω-3 and ω-6 alone. Hence combine use of PUFA is recommended for more effective results.

ETHICS APPROVAL: The ERC gave ethical review approval

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

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