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Study Effect of Evening Primrose Oil Supplement on Type 2 Diabetes Mellitus - Associated Metabolic Parameters

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Abstract

Natural products are reported to be useful for controlling or preventing T2DM as an anti-inflammatory, AMPK activators, insulin secretion stimulators, alpha-glucosidase / disaccharidase or amylase inhibitors. Evening primrose oil (*Oenothera biennis)* is a substantial source of omega-6 essential fatty acids, mostly gamma-linolenic acid (GLA). The activity of Δ-6-desaturase enzyme responsible to convert linolenic acid (LA) to forms GLA is compromised in patients with type 2 diabetes. Accordingly, this study aimed to evaluate the effect of evening primrose oil in amelioration metabolic parameters of type 2 diabetes mellitus. Twenty six overweight or obese patients newly diagnosed with type 2 diabetes were enrolled. Thirteen patients received metformin 500 mg tablets twice daily alone (as a conventional therapy) for 3 month therapy, and 13 patients received metformin 500 mg plus evening primrose oil 2 gm capsule twice daily. Serum fasting glucose, total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein cholesterol, very low density lipoprotein cholesterol, fasting serum insulin (FSI) was measured. Insulin resistance and β-cell function was determined by using homeostatic model assessment (HOMA). There was statistically significant elevation in baseline level of fasting serum glucose, glycated hemoglobin (HbA1c), total cholesterol, low density lipoprotein-cholesterol (LDL-C), fasting serum insulin, insulin resistance (HOMA- IR), and in both patient groups 1 and 2 compared to control subjects, ($P< 0.001$). High reduction in these parameters post treatment was found in both patient groups compared to pre treatment level, significantly with fasting serum insulin in group 2 patients ($P< 0.001$). Inversely, lower levels were seen in HDL-C, HOMA-B, significantly elevated post treatment in both groups. No difference was found in triglyceride and VLDL-C in both patient groups compared to control subjects, but significantly reduced post treatment. In conclusion, early intervention with natural oil rich in gamma linolenic acid with traditional hypoglycemic drugs can improve therapeutic outcome and represent a promising strategy to slow the progression of diabetes complications.

1 Introduction

The prevalence of type 2 diabetes parallel to obesity is increasing worldwide[1]. Approximately, 23% of patients with morbid obesity have type 2 diabetes, and the spread of screening-detected diabetes is 8%[2]. T2DM have raising rates in overweight and obesity in adults as well as in youth[3].Many of oral antidiabetic agents have been developed through the last 40 years[4,5]. Natural compounds reported to be useful in controlling or preventing T2DM namely as anti-inflammation, AMPK activators, insulin secretion stimulators, alpha-glucosidase / disaccharidase or amylase inhibitors, and acting with an unknown mechanism[6]. Evening primrose- *Oenothera*

biennis is a wild flower that belongs to the genus of *Oenothera*[7]. Historically, evening primrose (*Oenothera* spp.) has been recognized as a valid health care product[8]. Evening primrose was a fundamental food to improve ailments such as bruising, stomachaches, and shortness of breath[9]. Evening primrose oil is a substantialsource of omega-6 essential fatty acids, mostly gamma-linolenic acid (GLA) and linoleic acid (LA), both major components of myelin and the neuronalcell membrane[10]. Researchers have found that the high concentrations of GLA found in evening primrose oil can be used to treat several pathological conditions in humans caused by GLA deficiencies, and minimize the severity of many diseases[9].

Gamma-linolenic acid has anti-inflammatory, antithrombotic, and lipid reducing effect. It also enhances smooth muscle relaxation and vasodilatation. In addition, EFAs including GLA are substantial constituents of membrane phospholipids,including the mitochondrial membrane, where they promote the integrity and fluidity of the membrane[11]. LA forms GLA by Δ-6-desaturase enzyme, and the activity of this enzyme is compromised in patients with T2DM[10]. Lifestyle factors like stress, smoking, over consumption of alcohol, saturated and trans-fatty acids and nutritional deficiencies of VitaminB6, zinc, and magnesium suppress Δ-6- desaturase[12]. As a result of restrictions in vivo the production of GLA, supplementation with preformed GLA is becoming substantial. This has led to attention in developmentand commercialization of the sources of GLA[13]. Evening primrose oil may have an ability to reduce body weight by lowering cholesterol level, reduce symptomatic diabetic neuropathy, reduce blood pressure, and also reduce inflammation associated with diabetes[10].

Due to the lack of studies to evaluate the effect of evening primrose oil in reducing the complications of T2DM, this study was designed to track the efficacy of primrose oil in T2DM in glycemic control and lipid reducing effect, consequently ameliorating complications of T2DM.

2 Materials and Methods

2.1 Study design

This study was a prospective randomized -controlled interventional open- label study to evaluate the efficacy of primrose oil in T2 diabetic patients. The study was conducted between February 2015 up to October 2015.

Patients

Newly diagnosed type 2 diabetes patients with age ranges between (35-60) years. Sixteen patients were female and 10 patients were male. Most of patients are either overweight having a BMI ranging from (25-29.9) kg/m² or obese with a BMI (≥30) kg/m². Apparently healthy control subjects were included in the study. The eligible patients and subjects were allocated into three main groups; Group 1 include 13 patients who are assigned to receive metformin 500 mg tablets twice daily alone (as a conventional therapy) for 3 month therapy (as an active comparator).Group 2 include 13 patients who are assigned to receive metformin 500 mg plus evening primrose oil 2 gm capsule twice daily for 3 month therapy. Group 3 include 14 apparently healthy control subjects. The ethics approval was obtained from the Institutional Scientific Committee**.**

2.2 Methods

Serum fasting glucose is measured using Hexokinase/G-6-PDH[14]**.** The fasting total cholesterol levels has been measured using enzymatic assay, triglycerides were enzymatically measured using Glycerol Phosphate Oxidase, andthe HDL-C was measured using Accelerator Selective Detergent15**.** Low density lipoprotein cholesterol can be calculated mathematically using Friedwald's method[16]. Similarly very low density lipoprotein cholesterol concentration is calculatedaccording to method of Fiancis and David[17]. Fasting serum insulin (FSI) was determined by the DEMEDITEC Insulin ELISA Kit[18]. Insulin resistance and B-cell function was determined by using homeostatic model assessment (HOMA) depending on fasting insulin concentration and fasting glucose concentration and calculated as19:

HOMA-IR = fasting insulin (microU/L) x fasting glucose (mmol/L)/22.5

HOMA-B% =20 × fasting insulin (microU/L) /fasting glucose (mmol/L)− 3.5

2.3 Statistical analysis

The statistical analyses were carried out using the computer program SPSS version 20(Statistical Package for Social Sciences-version 20).The results were expressed as mean ±SD. Data were statistically evaluated using paired *t*-test to compare between pre and post treatment results among the study groups. Values with $P<0.05$ were considered significantly different.

3 Results and Discussions

3.1 Patients demographic characteristics

The demographic data of the 40 patients are presented in table 1, were 22 female patients (55%) and 18 male patients (45%) with no statistical difference were found between study groups in respect to both genders. The mean age of the study groups were as follows: control group (42.5±5) years, group 1 (48.6±7.1) years, and group 2 patients (49.3±6.6) years. The BMI value of control subjects were (26.7±0.8) kg/m^2, while patient groups were (29.2±1.9**)** kg/m^2 in group 1, and (29.2 ±1.6) kg/m2 with and group 2 with. Statistical differences were found between patient groups and control subjects ($P< 0.001$).

Changes in metabolic markers in type 2 diabetic patients

In the present study, there were significant elevation in baseline level of fasting serum glucose and HbA1cin both patient groups 1 and 2 compared to control subjects, with statistically high difference were found between patient groups and control subjects (*P*< 0.001).Also highly significant reduction in FBG after 3 months of treatment was found in both patient groups compared to pre treatment level (*P*< 0.001), but no significant difference in fasting serum glucose reduction between patient groups (Table 2).

Table 1: Patients demographic characteristic

Variables		Study groups			*p* value
		Control n %	Group 1 n %	Group 2 n %	
Gender	(Female)	6 42.9%	8 61.5%	8 61.5%	0.526NS
	(Male)	8 57.1%	5 38.5%	5 38.5%	
Age (Years)		42.5±5.0	48.6±7.1	49.3±6.6	0.013*
BMI (kg/m2)		26.7±0.8	29.2±1.9	29.2±1.6	<0.001**

Data presented as mean ± SD, and number (n) and percentage (%) were:

NS: Not significant (p>0.05), * Significant difference (p<0.05), ** Highly Significant difference (p<0.001)

Table 2: Fasting serum glucose and glycosylated hemoglobin (HbA1c) in type 2 diabetic patients treated for three months

Variables	Study groups	Pre treatment	Post treatment	*P* value
Glucose (mg/d)l	Control	97.29± 7.19	.	
	Group 1	231.85±27.14a**	178.23±24.34	<0.001**
	Group 2	226.62± 20.31a** bNS	164.92±21.75bNS	<0.001**
HbA1c (%)	Control	5.22±0.32	.	
	Group 1	8.54±0.67 a**	7.05±0.64	<0.001**
	Group 2	8.5±0.54 a** bNS	6.88±0.56 bNS	<0.001**

Data presented as mean ± SD were: a Comparison with control group.;b Comparison with group 1

NS: Not significant (p>0.05), *Significant difference (p<0.05), ** Highly Significant difference (p<0.001)

The addition of evening primrose oil to metformin therapy in newly diagnosed T2DM patients non significantly reduced the fasting serum glucose level compared to patients on metformin alone with same regimen, this is obviously due to the potent pharmacologic effect of metformin on glycemic control. In a preliminary study done by Takahashi *et al* where that administration of 4 g of EPO, 2.4 g of sardine oil and 200 mg of vitamin E among diabetic patients for 4 weeks showed no effect on glycemic control[20]. Experimentally, a controversial effect of evening primrose oil in ameliorating blood glucose in animals, so that Azra *et al* found that evening primrose oil produce significant reduction in blood glucose at different doses[21]. On the other hand, Nishikant *et al* stated that gamma linolenic acid (active substance in evening primrose oil) did not alter blood glucose in Streptozotocin diabetic rats[22]. Meanwhile, other study showed that administration of evening primrose oil to diabetic patients produced a significant decrease in blood glucose. Very recently, Mehri*et al* in 2016 found thatsupplementation of evening primrose with vitamin D to women with gestational DM for 6 weeks had beneficial effects on glycemic control, which was considered the first trial in evaluating the effects of vitamin D and EPO supplementation in hyperglycemia and in gestational DM[23].

Additionally, in the current study, the high level of HbA1c in newly diagnosed T2DM patientsshowed no significant reduction after the addition of evening primrose oil to metformin, and this result is compatible with the previously mentioned study[20]. Although Takashige *et al* found a significant reduction in HbA1c % in patient treated with evening primrose oil after twelve weeks of treatment[24].

There were significant elevation in a baseline level of total cholesterol and LDL-C inboth patient groups 1 and 2 compared to control subjects, with statistically high difference was found between patient groups and control subjects (*P*<0.001). The highly significant reduction after 3 months of treatment was found in both patient groups compared to pre treatment level(*P*< 0.001), but no significant difference in both parameters

between patient groups (Table 3). Both triglyceride and VLDL-C showed no significant difference in baseline levelof in patient groups 1 and 2 compared to control subjects, but highly significant reduction after 3 months of treatment was found in both patient groups compared to pre treatment level (*P*<0.001), still no significant difference in TG and VLDL-C reduction between patient groups. The level of serum HDL showed lower levels in a baseline in both patient groups 1 and 2 compared to control subjects, significantly in patient groups 2 only (*P*<0.05). Also significant increase in serum HDL after 3 months of treatment was found in both patient groups compared to pre treatment level (*P*< 0.05), again, no significant difference in serum HDL increase between patient groups.

Table 3: Lipid profile in type 2 diabetic patients treated for three months

Variables	Study groups	Pre treatment	Post treatment	*P* value
TC mg/dl	Control	181.71± 6.87	-	-
	Group 1	190±8.11 a*	173.54±8.14	<0.001**
	Group 2	191.46±8.36 a** bNS	171.85±8.91 bNS	<0.001**
TG mg/dl	Control	108.36±19.34	-	-
	Group 1	103.08±13.38 aNS	84.92±11.06	<0.001**
	Group 2	105.77±14.23 aNS bNS	87.92±13.48 bNS	<0.001**
HDL-C mg/dl	Control	48.07±4.43	-	-
	Group 1	46±3.11 aNS	50±3.51	0.004*
	Group 2	43.46± 2.22 a* bNS	47.62± 3.23 bNS	0.003*
LDL-C mg/dl	Control	111.97± 8.76	-	-
	Group 1	123.38± 8.29 a**	106.55± 8.97	<0.001**
	Group 2	126.83± 8.31 a** bNS	106.65± 7.97 bNS	<0.001**
VLDL-C mg/dl	Control	21.67± 3.87	.-	-
	Group 1	20.63± 2.66aNS	16.98± 2.21	<0.001**
	Group 2	21.15±2.85 a NS bNS	17.58±2.7 bNS	<0.001**

Data presented as mean ± SD were; a Comparison with control group.; b Comparison with group 1
NS: Not significant (p>0.05), *Significant difference (p<0.05), ** Highly Significant difference (p<0.001)

It is well known that the newly diagnosed with T2DM presented with abnormalities in lipid profile because insulin regulates several steps of lipid metabolism. The addition of evening primrose oil to metformin in the current study did not show significant reduction in lipid profile comparedto metformin treated patients at thesame dose and duration. An expected result due to well established effect in all the utilized approaches of treatment with different doses of metformin in newly diagnosed T2DM patient. While many other studies stated that EPO containing GLA provides beneficial effect on lipid profile like the reduction of TG, VLDL, and the elevation of the HDL, namely the one done by Sayantani *et al* where the addition of evening primrose oil in a high doses for short term to groundnut oil fed animal produced a significant reduction in TC, TG, LDL, VLDL and significant increase in HDL[25].

Another study indicates that addition of evening primrose oil to cheddar cheese not produce a significant effects in both HDL and triglyceride (TG) concentration and produce significant reduction in total cholesterol (TC)[26]. Similar improvement in lipid profile was noticed in gestational diabetic women when treated with vitamin D plus EPO supplementation led to significant reductions in serum TAG, VLDL, TC, LDL and TC/ HDL compared with the placebo, but did not influence serum HDL concentrations[23].

The mechanism of the TAG-lowering effect of GLA may be associated with inhibition of hepatic TAG synthesis, and the decrease in serum TC and LDL concentrations is through a reduction in VLDL synthesis, which in turn may limit the amount of TC converted to LDL. In addition, upregulation of LDL receptor activity and an increase in clearance of LDL from the plasma may result in decreased levels TC and LDL levels[27].

The effect of EPO on metabolic parameters in the present study is expected to be underestimated because of the small sample size and the programmed metformin therapy for the newly diagnosed patients.

3.2 Changes in insulin resistance and β cell function markers in type 2 diabetic patients

The significantly high baseline level of fasting serum insulin (FSI) was found in both patient groups 1 and 2 compared to control subjects, and highly significant decline in FSI level post treatment was found in group 2 patients only compared to pre treatment level (*P*< 0.001), but no significant reduction was found in group 1 patients compared to pre treatment level. Additionally, both insulin resistance (HOMA- IR) and β cell function (HOMA- B) showed statistically significant opposing difference between patient groups and control subjects (*P*< 0.001), with highly significant elevation in β cell function and decrease in insulin resistance after 3 months of treatment in both patient groups compared to pre treatment level (*P*< 0.001), and no significant difference between them (Table 4).

Table 4: Insulin resistance and β cell function markers in type 2 diabetic patients treated for three months

Variables	Study groups	Pre treatment	Post treatment	*P* value
FSI (μIU/ml)	Control	9.31±1.91	-	-
	Group 1	34.83±2.88 a**	29.21±2.56	<0.001**
	Group 2	35.51±1.39 a** bNS	27.29±2.25 b*	<0.001**
HOMA-IR	Control	1.23±0.27	-	-
	Group 1	5.51± 0.73 a**	4.22± 0.44	<0.001**
	Group 2	5.49±0.39 a** bNS	3.98± 0.47bNS	<0.001**
HOMA-B (%)	Control	92.42±4.75	-	-
	Group 1	55.55±5.79 a**	72.32±13.68	<0.001**
	Group 2	57.85±6.15a** bNS	77.68±13.57bNS	<0.001**

Data presented as mean ± SD were: a Comparison with control group.; b Comparison with group 1
NS: Not significant (p>0.05), *Significant difference (p<0.05), ** Highly Significant difference (p<0.001)

The elevation in the level of fasting serum insulin is the major metabolic consequences of hyperglycemia in T2DM patients. Highly significant reduction in the level of fasting serum insulin concentration after the addition of evening primrose oil to the conventional metformin therapy was found in the present study T2DM patients compared to those treated with metformin alone (*P*<0.001). This result is compatible with previously, reported study in gestational diabetes mellitus where reduce serum insulin and other glycemic parameters was clear after co-administration of evening primrose oil[23]. Another study showed that treatment with GLA not produce significant reduction in insulin level but addition of GLA to the α-lipoic acid produced significant reduction in insulin level in the treatment of obese zuckerrats[28].

Significant increase in the insulin resistance (due to hyperglycemia) and significant reduction in β-cell function in newly diagnosed T2DM patients compared to control was clear in the present study. Adding evening primrose oil to metformin therapy did not produce significant reduction in insulin resistance or significant improvement in B-cell function compared to the conventional metformin alone, despite the highly significant reduction in the level of fasting serum insulin concentration after the addition of evening primrose oil. Incompatible result when EPO administration for 6 weeks reduces FPG, serum insulin levels, HOMA-IR, HOMA-Band increases QUICKI compared with a placebo[23].

4 Conclusion

To our knowledge, we are aware of no randomized controlled trial that assessed the effect EPO administration on metabolic markers in type 2 diabetic patients, particularly among Iraqi population. The significant reduction in FSIlevel after three months of combining evening primrose oil supplement to the conventional metformin therapy, and the non significant improvement in insulin resistance and β cell function give no doubt that GLA played a pivotal role in a disease process, and the dietary control of fatty acid intake would be expected to modify the disease progression. From the results, it can be concluded that early supplementation with natural oil rich in gamma linolenic acid with hypoglycemic drugs can improve therapeutic outcome and slows the progression of diabetes complications.

5 Conflict of interest

The authors declared none

6 Author's contributions

MKA (the corresponding author) brings the study design into it's applicable state along with drafting the manuscript. And the literature review, result discussion, lab work, and data collection was carried out by MSH. Finally MSK arranged the data into tabular form. All authors read and approved the final manuscript.

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