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In Silico Proteomics EVOO Therapy for Lipid Lowering in the Patients of Diabetes Mellitus

Muhamamd Suhail and Samreen Riaz

Abstract

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by ineffectiveness of the insulin produced. Diabetes is a life-long disease marked by elevated levels of sugar in the blood. It is considered as the second progressing cause of color blindness and kidney defects throughout the world. There are four times higher chances of getting heart disease and strokes suffering with diabetes than other ailments. Type-2 diabetes has approximately 90% cases in Pakistan and need to be checked out for its therapy. Olive oil could be helpful in diabetes via many of ways, as prolonged inflammation plays a role as an enhancer of diabetes and other diabetes problems. This study reports identification of some of the cheapest ways to lower the cholesterol in the diabetes mellitus in the local population by using advanced technologies. In this work, the main focus of the research was to use olive oil as a therapy, and as a lipid lowering agent, to improve and reduce the chance of blood pressure, lipids, and hence diabetes and cardiovascular diseases.

Keywords: in silico, EVOO, diabetes, proteomic, dyslipidemia

1. In silico proteomics

Bioinformatics is a science of intellectual biology with respect to molecule recognition as it uses informatics principles to sort out the information of molecules on large scale. Applied math, statistics and computer laid down its foundation. In simple words, bioinformatics work as a platform for management of data in molecular biology field. It has many other applications too in molecular biology [1].

Bioinformatics has many applications in different fields of biology like genomics, biotechnology, molecular biology, and many areas of biomedical sciences. For instance, it is used in DNA fingerprinting, agriculture and drug designing approaches [1]. Drug designing is done in silico by the comparative association of ligand protein interactions; leads obtained this way provide innovative medicines. This type of association is the base of synthetic drug designing. The 3-D structure of proteins is studied via pyMOL or VMD softwares. These softwares clearly demonstrate the receptor ligand relationship in drug designing process. The drug developed by this way requires less budget with greater efficiency and less or no harmful side effects. Drug development is done utilizing informatics principles (Lagunin *et al.*, 2014).

Now a days criminal are captured by using bioinformatics tools like phylogenetic analysis provide a proof against the criminal in court of law. In forensic analysis Bayesian statistics are utilized to detect DNA of culprit [2]. Bioinformatics has made of healthcare system modernize with the introduction of personalized drugs etc. [3].

Now it has become possible with the advancement in the knowledge of bioinformatics that genetic defects are pin pointed on time and are tried to be eliminated before their expression. In this way better treatment has become possible in this era (Takeichi *et al.*, 2015). Bioinformatics has revolutionized the agriculture system with the introduction of new and better crops varieties with increased production potential and resistant against crop damaging agents (Katam *et al.*, 2015).

Bioinformatics is not only limited to Allied health fields but it is also play important role in many other fields too as given below;

- Energy sources
- Antibiotic resistance
- Biotechnology
- Bio-weapon manufacturing
- Climate change analysis
- Comparative studies
- Crop betterment
- Development of drought resistant types of plants
- Drug designing
- Evolutionary analysis
- Forensic studies
- Gene therapy
- Genome assembly
- Nutritional quality improvement
- Insect resistance
- Molecular medicine
- Personalized medicine
- Preventative medicine
- Re-sequencing
- Veterinary science
- Waste cleanup process

1.1 *In silico* drug designing

Discovery of new drug is lengthy and expensive process and require lots of mass efforts too. Many of the times the year’s hard work in drug discovery is wasted away due to certain factors like toxic effects, poor drug metabolism etc. (Grinter and Zou, 2014).

So with the advancement in the field of informatics, the drug discovery process has been completely changed as drugs are developed in silico with the help of latest Bioinformatics tools and applications. Today many fields use in silico drug design- ing (**Figure 1**) method like biochemistry, nanotechnology and molecular biology. This method has major advantage on others is its cost effectiveness and quickness (Grinter and Zou, 2014).

Many outstanding tools and softwares are used for drug development by in silico method, those includes;

- PBPK/PD modeling software
- PKUDDS
- Grid computing
- JAVA
- APIS
- Python and Perl
- MEGA
- Clustal W

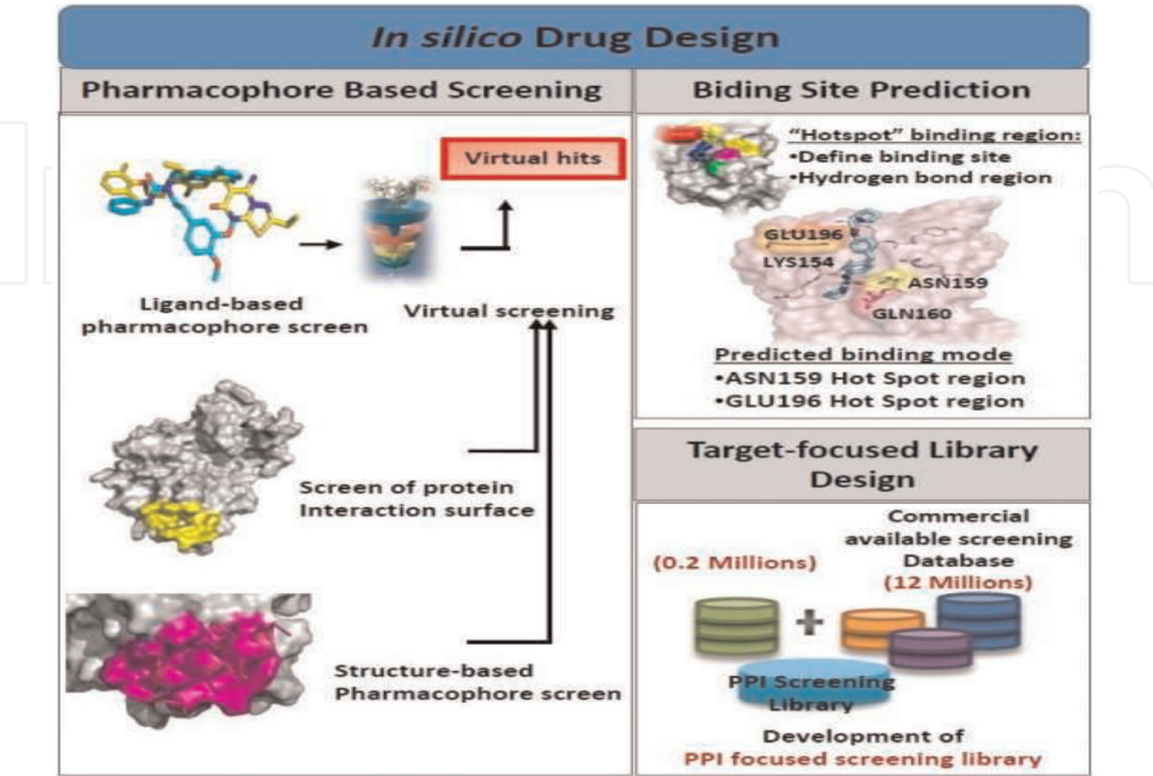


Figure 1.
In silico drug designing.

- Clustal Omega
- JALVIEW
- FATCAT server
- MN convert
- VMD
- Lipinski’s rule
- Patch dock &
- AutoDock Vina

This in silico based approach is used in every stage of drug discovery starting from preclinical phase to terminal phase of drug development process [4].

1.2 Phytochemicals

Olea europaea (extra virgin olive oil EVOO) was selected as an anti-diabetic agent as it has many beneficial effects like improvement of hyperglycaemia and lowering of lipid profile of diabetic patients. That is why it was selected and its docking was augmented against certain proteins genetically linked to diabetes.

1.3 Proteins involved in diabetes

There are following proteins which are genetically linked to diabetes, these proteins with their characteristics and available commercial drugs against these protein markers have been shown in **Table 1**.

Protein name	Mol. mass (Da)	Amino acids	Available drugs	Structure
Calcium/calmodulin-dependent protein kinase type 1D	42,914 Da	385	No drug found	Quaternary structure
A disintegrin and metalloproteinase with thrombospondin motifs 9	216,491 Da	1935	No drug found	Quaternary structure
Calpain-10	74,952 Da	672	No drug found	Quaternary structure
Cell division cycle protein 123 homolog	39,135 Da	336	No drug found	Quaternary structure
Threonylcarbamoyladenosine tRNA methylthiotransferase	65,111 Da	579	No drug found	Quaternary structure
Hematopoietically expressed homeobox protein HHEX	30,022 Da	270	No drug found	Quaternary structure
Insulin-degrading enzyme	117,968 Da	1019	6bK inhibitor	Quaternary structure Homodimer
Insulin-like growth factor 2 mRNA-binding protein 2	66,121 Da	599	No drug found	Quaternary structure

Protein name	Mol. mass (Da)	Amino acids	Available drugs	Structure
Juxtaposed with another zinc finger protein 1	27,079 Da	243	No drug found	Quaternary structure
ATP-sensitive inward rectifier potassium channel 11	43,541 Da	390	Sulfonylurea	Quaternary structure
Potassium voltage-gated channel subfamily KQT member 1	74,699 Da	676	Incretin	Quaternary structure Tetramer
Leucine-rich repeat-containing G-protein coupled receptor 5	99,998 Da	907	No drug found	Quaternary structure
Melatonin receptor type 1B	40,188 Da	362	Melatonin supplements, DPP4 inhibitors	Quaternary structure
Peroxisome proliferator-activated receptor gamma	57,620 Da	505	Thiazolidinediones, pioglitazone	Quaternary structure
Transcription factor 7-like 2	67,919 Da	619	Sulfonylurea, linagliptin, incretin	Quaternary structure
Thyroid adenoma-associated protein	219,607 Da	1953	No drug found	Quaternary structure
Tetraspanin-8	26,044 Da	237	No drug found	Quaternary structure
Wolframin	100,292 Da	890	Incretin	Quaternary structure

Table 1.
Proteins involved in diabetes along with their properties, structure and available drugs has been demonstrated.

1.4 Significance of *in silico*, drug designing

In silico medical practitioner to select best suitable drug for the treatment of disease. The drug designed by this way prove to be less toxic, fewer or no side effect and more capable than any other drug available. In silico drug designing has application in all the phases of new drug development process. All these in silico analysis are done with the help of different tools and application softwares of bioinformatics (Schmidt *et al.*, 2014; Tian *et al.*, 2015).

1.5 Aims of study

In silico bioinformatics analysis were used to screen and check the therapeutic potential of *Olea europaea* to treat type two diabetes mellitus. For this purpose certain proteins responsible for diabetes were selected and in silico analysis were done to check the reversion effect of *Olea europaea* against these diseases causing proteins expression. The purpose of the study was to screen and isolate potential therapeutic agent present in the *Olea europaea* which could be the best treatment option of diabetes.

1.6 Future prospective

The *in silico* drug development could be meaningful in the certain aspects:

- Reduction of drug invention time and cost
- Harmless, effective and target specific drug development
- To improvement in result analysis
- Develop efficient bioinformatics ways and algorithms for
- Lead identification
- Lead optimization
- Target identification
- Target validation

2. Diabetes mellitus

Diabetes is a persistent ailment which happens because of the raised blood glucose due to the lack of the body's ability to produce insulin secretion or sufficient insulin secretions. Insulin secretion is obtained from the pancreas gland in the body and is prerequisite for the transport of glucose from bloodstream to the living cells where it is utilized to obtain energy for the body. Deficit of insulin or impotence of cells to response to insulin results in high level of blood glycaemia or hyperglycaemia which is distinctive feature of diabetes. Hyperglycaemia, if remained uncontrolled over long period, can cause severe damage to different body parts leading to development of disabling or many life-threatening health issues like cardiovascular disorders, neuropathy, nephropathy and eye diseases leading to retinopathy and blindness. Proper management of diabetes helps prevent or delay those complications (Atlas 2017 intro 1).

The term diabetes was initiated from Greek word "syphon" or "mellitus" means Honeyed proposed by Rollo in late eighteenth century [5]. Matthew Dobson for the first time reported the hyperglycemic nature of diabetes in his article during 1776; he observed elevated glucose level in urine and plasma of patients. Holt et al. [6] reported the raised level of ketone bodies in diabetes.

Million people die of diabetes each year, mostly from cardiovascular disease exacerbated by lipid disorders and other complications. Identification of new treatment strategies using olive oil for lipid lowering in diabetes through proteomic analysis was needed. With the increase in the world population the proportion of diabetes is also increasing with great speed so, the more health care budget is needed to cope with this problem. As without proper primary strategies and care it will take the face of epidemic which will kill millions of lives in the world. With this speed it is possible that diabetes would become the number one killer of the world within next coming 25 years. Abrupt actions are required to root out the diabetes from the world.

Olive oil supplementation with daily food intake might play a protective role to reduce lipid profile in diabetes due to the beneficial effect of olive oil exerted by its antioxidant constituents.

In most of the cases when the disease is detected it is already too late to be cured. The percentage of fatalities in patients is therefore very high. Not only there is a vast number of precious lives are lost but the treatment and maintenance of such patients is a heavy financial burden on the individual families and the national

resources. It is therefore necessary that cheap and quick methods are made available which can be used to screen population so that the diseases can be detected at a very early stage. Research and development work based on the samples from our population groups is essential because findings have been reported to be dependent on race variations, and socio-economic and other factors. Thus the studies reported on the basis of population groups in other parts of the world may not be valid at least in some cases.

This study reports identification of some of the cheapest way to lower the cholesterol profile in the diabetes mellitus in the local population by using advance technologies. In this work, the prime focus was to the use of olive oil as therapy for cholesterol lowering agent to improve and reducing the chance of blood pressure, lipids and hence diabetes and cardiovascular disease will be discussed. The findings from present research work would prove better treatment options for diabetic patients by introduction and characterization of new therapy methods.

DM is one out of many lethal melodies. It is ranked fourth deadliest malady, and the rate of prevalence is increasing sharply. Olive oil a liquid fat captured from olives tree crop cultivating in the Mediterranean Basin. Complete olives are pressed to gain olive oil.

Results generated from this research will help devise for the novel diagnostic procedures for early and control of diabetes complications in our population. These findings will provide a base in planning better treatment strategies for diabetic patients by suggested dose of olive oil nutritional supplement. The research work proposed to be undertaken under this project shall elucidate the roles of olive oil in diabetes mellitus in our population. Therapy for the diabetes to be investigated should lead to not only saving lot of precious lives but also ensure saving of huge numbers of man-hours and colossal amount financial resources spent on treatment and patient maintenance. The other financial gains accruing from this project is the application of the project results in developing therapy methods locally for routine use in our clinical laboratories.

2.1 Classification

Diabetes has been grouped into two types since 600 BC but the clear classification into two types was done in nineteenth century. Diabetes of young before ketoacidosis and diabetes of obese people, later these two types were named as MODY by WHO. By the discovery of human leukocyte antigen (HLA) and antibodies in 1970, it was become clear that all the patients on insulin treatment were suffering with autoimmune disorder [6]. WHO revised the classification into two types of diabetes in 1980 and 1985 based on the clinical findings, which were insulin-dependent diabetes mellitus (IDDM) and the noninsulin-dependent diabetes mellitus (NIDDM). Classification of 1999 was accepted and revised with small changes and diabetes was grouped into (T1DM), (T2DM), gestational diabetes mellitus, and some other types [7].

For the diagnosis of diabetes, the diagnostic criterion has been adopted and revised over and again by world health organization (WHO). The current criteria states that diabetes is diagnosed by the elevated level of glucose in the bloodstream of diabetic patients (Table 2).

2.2 Type 1 diabetes mellitus

T1DM is an autoimmune ailment caused when the body's immune system attack on the Beta-cells in the islets of pancreas gland. As a conclusion body produce very

DIABETES should be diagnosed if ONE OR MORE of the following criteria are met	IMPAIRED GLUCOSE TORELANCE (IGT) should be diagnosed if BOTH of the following criteria are met	IMPAIRED FASTING GLUCOSE (IFG) should be diagnosed if BOTH of the following criteria are met
Fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL)	Fasting plasma glucose < 7.0 mmol/L (126 mg/dL)	Fasting plasma glucose 6.1-6.9 mmol/L (110 to 125 mg/ dL)
or	and	and
Two-hour plasma glucose ≥ 11.1 mmol/L (200 mg/dL) following a 75g oral glucose load	Two-hour plasma glucose $\geq 7.8 < 11.1$ mmol/L (≥ 140 to < 200 mg/dL) following a 75g oral glucose load	Two-hour plasma glucose < 7.8 mmol/L (140mg/dL) following a 75g oral glucose load
or		
A random glucose > 11.1 mmol/L (200 mg/ dL) or HbA1c ≥ 48 mmol/mol (equivalent to 6.5%)		

Table 2.
Diabetes diagnostic criteria.

little or none insulin with a relative or absolute deficiency of insulin in the body. The reason of this deadliest process are not completely understood but it is thought that genetic susceptibility and environmental factors like viral infection, toxins and some dietary factors could be the cause of the problem (Chapt1 type1 10). Type 1 diabetes occurs mostly in children and adolescents. It is managed with proper daily injections of insulin otherwise the survival of the patient would be impossible. Type 1 is diagnosed by raised level of glucose and with the specific symptoms (**Figure 2**). Type 1 diabetes incidence rate in Karachi, the largest city of Pakistan is 1.02/100,000 in comparison to type 2 diabetes mellitus [8].

2.3 Type 2 diabetes mellitus

Type 2 diabetes is commonest type of diabetes throughout the world accounting around 90% of all the diabetic patients. It is occurred due to insufficient production of insulin or insulin resistance. During insulin resistance insulin become ineffective as a result, body produce excessive insulin initially to control the elevated level of

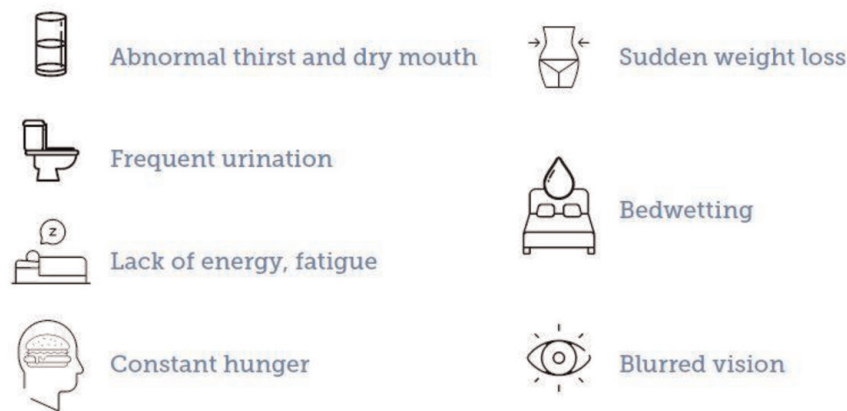


Figure 2.
The symptoms of type 1 diabetes.

glucose but as the time passes productive ability of insulin producing cell is lost ultimately a state of inadequate insulin production is developed. Usually type 2 diabetes occurs in older adults but it is increasingly seen in children, adolescent and young adults due to physical inactivity, poor diet increasing level of obesity (13–15). Type 2 diabetes alarming incidence in Pakistan is associated with poor diet etc. [9]. The estimated prevalence of Pakistan ranges from 7.6 to 11% in 2011 [10]. The symptoms of T2DM are like T1DM (Figures 3 and 4).

2.4 Gestational diabetes mellitus (GDM)

GDM is a non-pathophysiological condition and one out of seven new born has gestational diabetes according to [11]. GDM caused by glucose intolerance which was first diagnosed in pregnancy period and may remain after pregnancy (Gavin III *et al.*, 1997). The development of undiagnosed asymptomatic T2DM and T1DM during pregnancy is referred as GDM [12]. The occurrence of GDM is reported during the last 6 months of the pregnancy while the development of diabetes in women during first trimester is termed as T2DM [13].

2.5 Type 1.5 diabetes

Latent Autoimmune Diabetes in Adults (LADA) non-officially called as Type 1.5 diabetes. The term 1.5 indicates that disease is a type 1 diabetes mellitus but it shares



Figure 3.
The symptoms of typ. 2 diabetes.

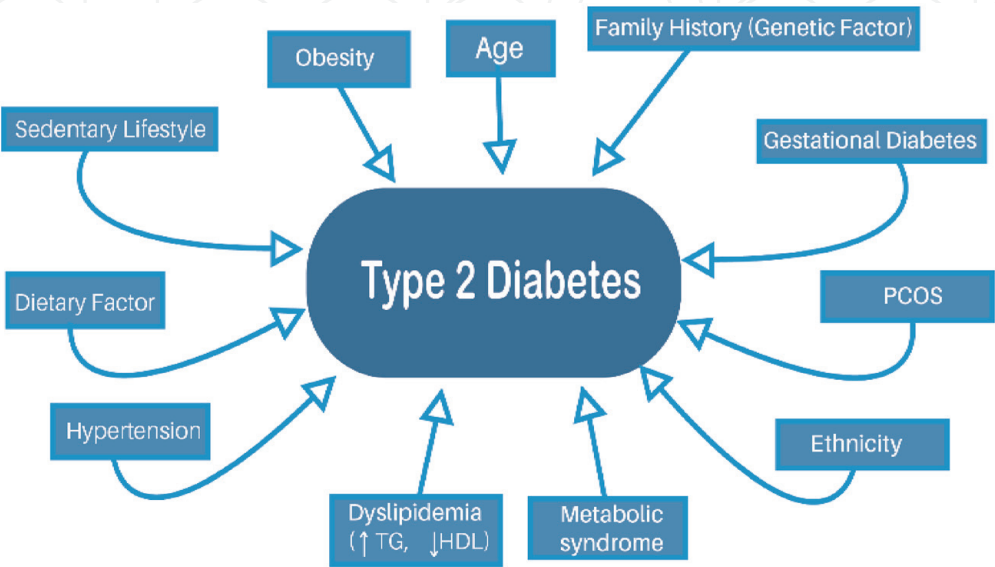


Figure 4.
Different risk factors of typ. 2 diabetes mellitus (T2DM).

some feature with type 2 diabetes mellitus that is the reason it is called as Type 1.5 diabetes means in between T1DM and T2DM. It is also known as Type 3 diabetes. Type 1.5 diabetes is diagnosed during adulthood and has slow onset similar to T2DM however, it is type 1 diabetes mellitus and will need insulin therapy in future. Around 15–20% patients with type 2 diabetes are actually suffering with type 1 diabetes. They are treated with oral medication initially but will need insulin therapy after some period of oral treatment. Type 1.5 diabetic patients do not have standard symptoms of type 2 diabetes and have reduced risk of heart problem if their blood sugar is kept in control (Diabetes, UK).

2.6 Other specific types of diabetes

Some other types of diabetes occur due to the genetic malfunctions, impaired secretory activity of pancreas and over production of certain hormones. For instance MODY situation affects certain genes which are responsible for beta-cells development, functioning and regulation of glucose. Likewise excessive production certain growth hormones, cortisol, epinephrine and glucagon enhance the glucose production in liver cells and increase insulin resistance in muscle cells resulting in the progression of diabetes mellitus [14]. Cystic fibrosis-related diabetes (CFRD) occurred in cystic fibrosis patients due to insulin resistance and genetic dysfunction of beta-cell linked with inflammation and infection [15].

2.7 Epidemiology

With every passing decade individual with diabetic ailment are increasing tremendously, currently there are 425 million people suffering with diabetes and 352 million people have falsified glucose tolerance (IGT). IGT is also named as impaired fasting glucose (IFG) or “Pre-Diabetes”. The individual with IFG has been at peak

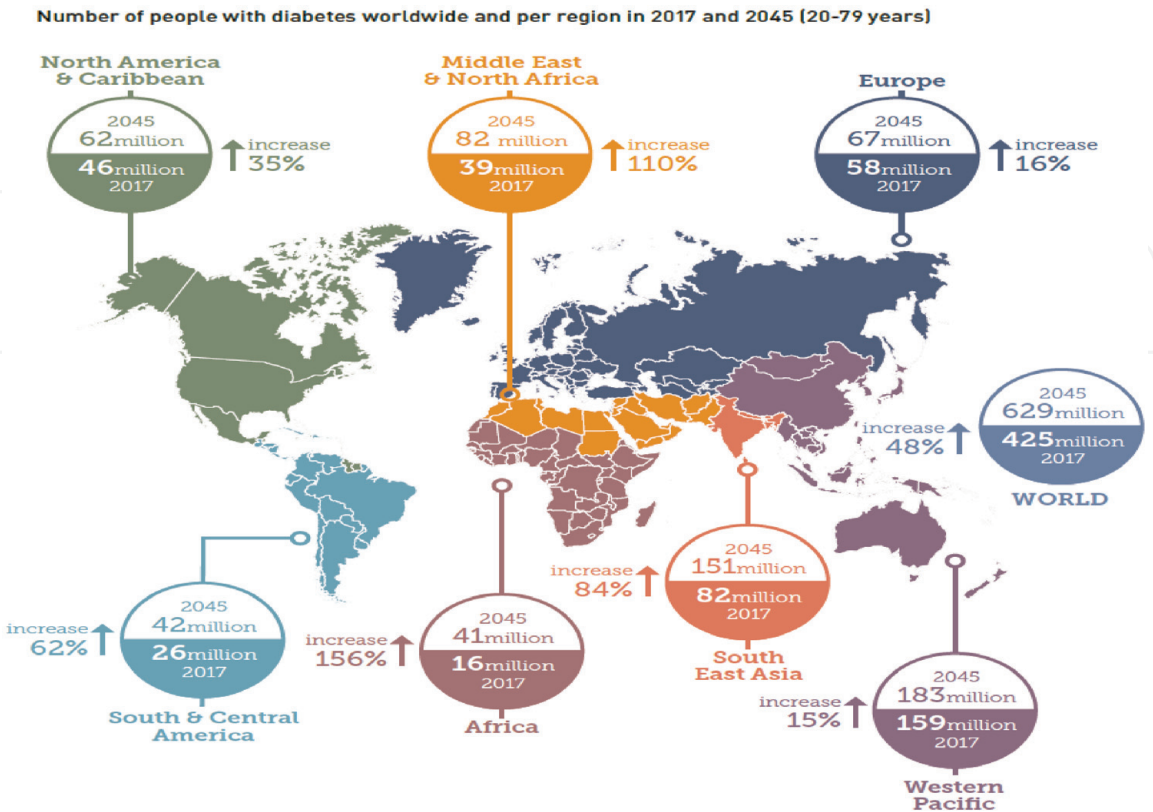


Figure 5. Worldwide distribution of diabetes. Adapted from: IDF diabetes (IDF 2017).

risk of diabetes in near future. According to IDF, 1 in 11 people has diabetes till now and it is projected to be 1 in 10 till 2040 (IDF 2017).

Pakistan is among the top 10 most affected diabetic countries of the world and is projected to be at 8th position until 2045 (20–79 years of age. Other major countries on the top of the list are China, India, United States, Brazil, Mexico, Indonesia, Russian federation, Egypt, Germany and Pakistan respectively. According to latest estimates Pakistan has 7.2 million population of diabetes and projected to be 16.1 million by 2045 (Atlas 2017). Previous studies reported 5.2 million diabetic population of Pakistan in 2000 and projected to be 13.9 million till 2030 [16]. The world-wide prevalence of diabetes is shown in **Figure 5**. In urban areas the ratio of diabetes is 22.04% and in rural areas it is 17.15%. In Punjab, diabetes in female is 19.3% and in male it is 16.6%. In KPK it is 11.1% in both male and female in Balochistan it is 10.8% in both [17, 18]. But in Sindh female proportion is 11.7% and in male is 16.2% (Hussain and Ali, 2017) **Figure 6** demonstrate the distribution of diabetes in all the provinces of Pakistan (**Figure 7**) [19–39].

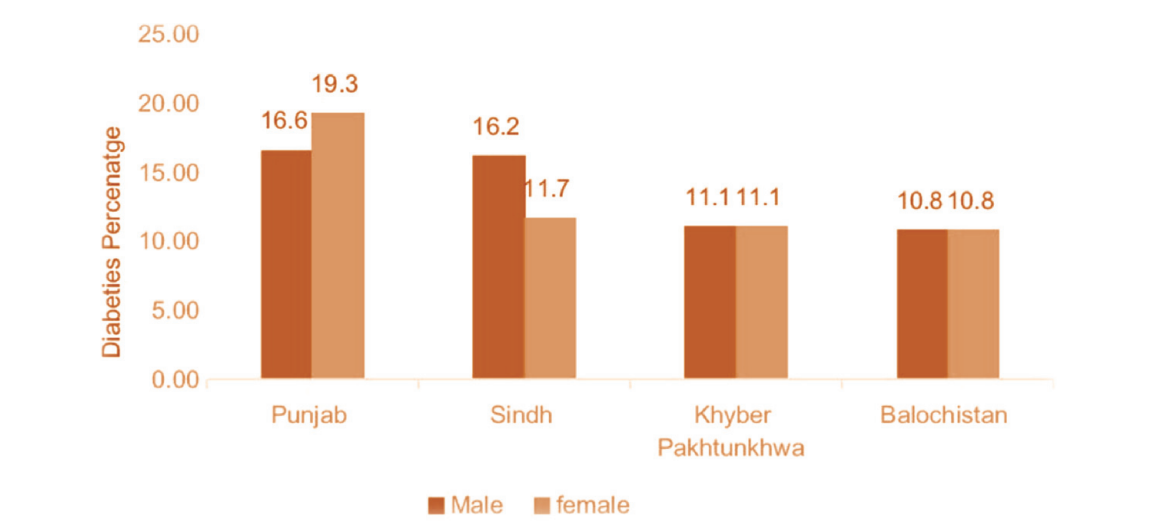


Figure 6.
Distribution of diabetes in different provinces of Pakistan [40].

2017			2045		
Rank	Country/territory	Number of people with diabetes	Rank	Country/ territory	Number of people with diabetes
1	China	114.4 million [104.1–146.3]	1	India	134.3 million [103.4–165.2]
2	India	72.9 million [55.5–90.2]	2	China	119.8 million [86.3–149.7]
3	United States	30.2 million [28.8–31.8]	3	United States	35.6million [33.9–37.9]
4	Brazil	12.5 million [11.4–13.5]	4	Mexico	21.8 million [11.0–26.2]
5	Mexico	12.0 million [6.0–14.3]	5	Brazil	20.3 million [18.6–22.1]
6	Indonesia	10.3 million [8.9–11.1]	6	Egypt	16.7million [9.0–19.1]
7	Russian Federation	8.5 million [6.7–11.0]	7	Indonesia	16.7million [14.6–18.2]
8	Egypt	8.2million [4.4–9.4]	8	Pakistan	16.1 million [11.5–23.2]
9	Germany	7.5 million [6.1–8.3]	9	Bangladesh	13.7 million [11.3–18.6]
10	Pakistan	7.5 million [5.3–10.9]	10	Turkey	11.2 million [10.1–13.3]

Figure 7.
List of top 10 countries having diabetes (IDF Atlas 2017).

2.8 Prevention of diabetes

As diabetes is multifactorial gene disorder, as genetic information and environmental effects both play a role in this disease. Type 1 diabetes is comparatively less affected with environmental factors but type 2 does. So, it is difficult to stop the onset of type 1 diabetes, but for type 2 diabetes there are many known preventive measures. These preventive measures include maintaining normal body weight, use of healthful diet and regular physical exercise. Changing in eating habits are known to reduce the risk of diabetes, such as taking whole grain diet, using diet with good fats (nut, fish) and reducing use of saturated fats (red meat). But above all, limit use of sweet edibles is the most important and effective preventive measure as well as in controlling the complication of diabetes. In addition, if an individual quits smoking, the risk of onset of diabetes reduces many folds (**Figure 7**) [41].

3. Extra virgin olive oil (EVOO)

EVOO is one of the most important components of Mediterranean diet having many health benefits. At first, excess of (MUFA) especially oleic acid, was thought



Figure 8.
Representing health benefits of extra virgin olive oil.

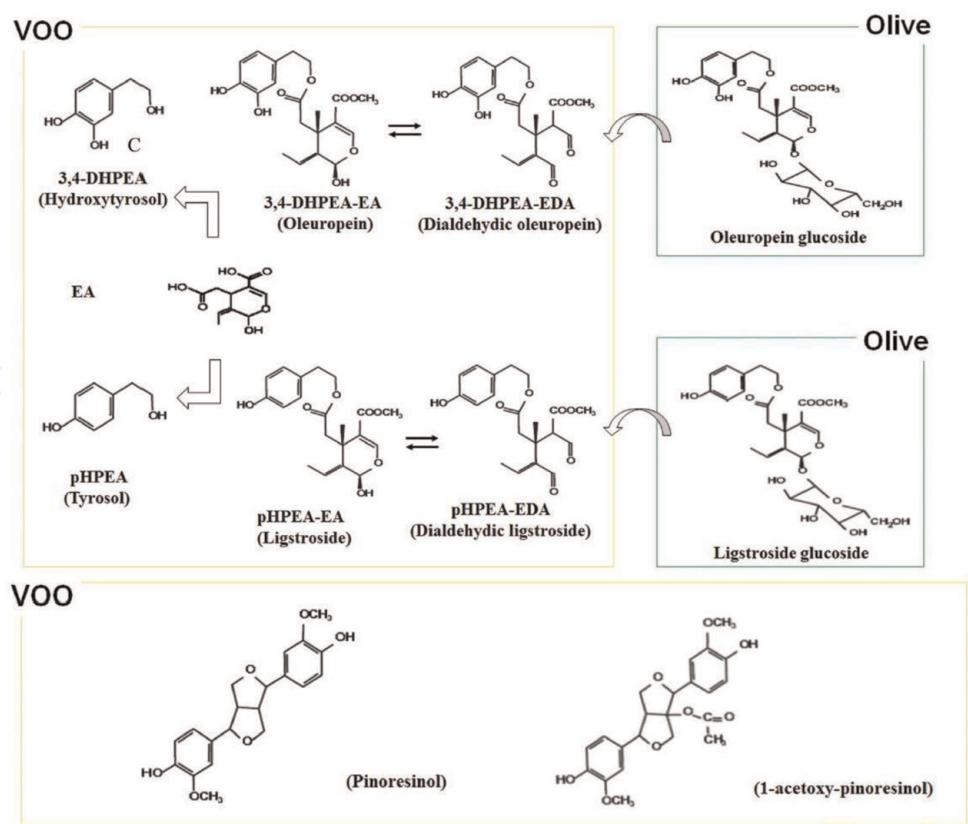


Figure 9.
Chemical structure of EVOO.

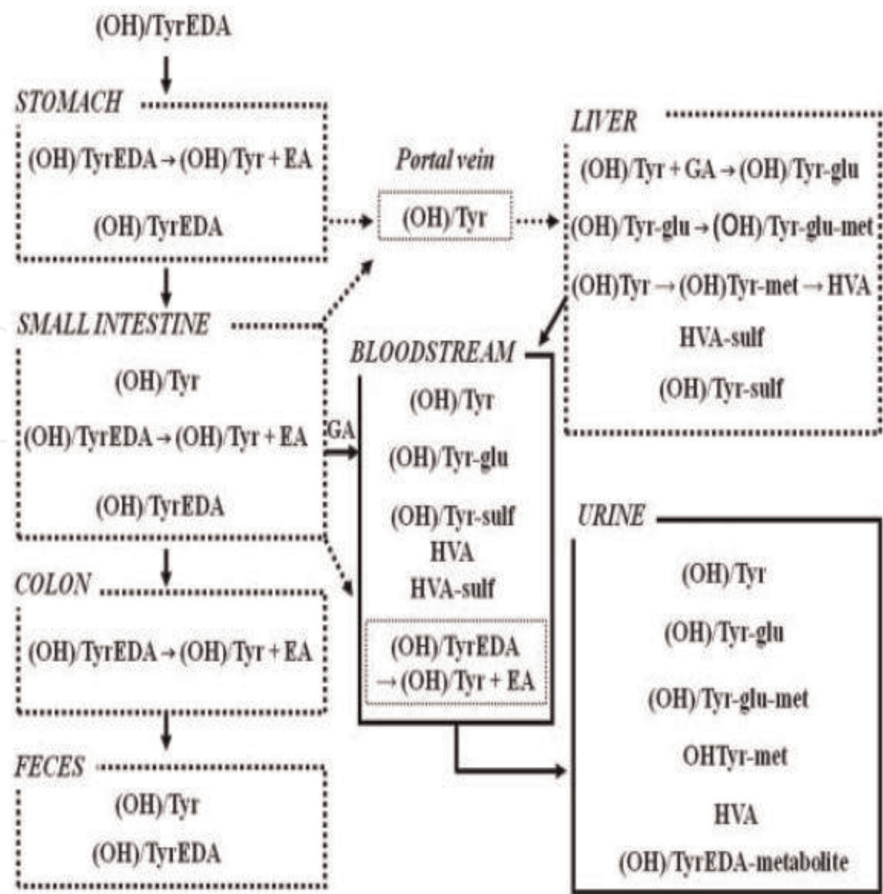


Figure 10.
Metabolism of EVOO.

as the major beneficial constituent of virgin olive oil. Most important health benefit of EVOO (**Figure 8**) is improved cardiovascular health, reduction of inflammation and oxidative stress. It is used in Mediterranean diet since many decades. Studies suggested that Mediterranean food specimen along with EVOO lowers the risk of heart disorders as compared to controls. Other studies reported that EVOO improve hyperglycemia and lipid level in patients with dysfunctional fasting glucose (Roberto *et al.*, 2016). Almost 200 'minor particles' in the un-saponifiable portion of olive oil comprise about 2% of the total weight of EVOO [42–63].

3.1 Nutritional effects of EVOO

One tablespoon of EVOO contains 14 g of total fat, 2 g of saturated fats and has no fiber, sugar, cholesterol etc. Vitamins E, K are also present in it also has phyto-sterols, which is structurally similar to cholesterol because it hinders cholesterol absorption from food and lower LDL values associated with heart disease. Major EVOO polyphenols include secoiridoids, phenols, and lignans. The chemical structural formula of EVOO is shown in **Figure 9** and metabolism of EVOO is shown in **Figure 10**.

3.2 Effects on cholesterol level

There is no cholesterol in the EVOO while other animal obtained fats contain different amount of cholesterol. Recently, the European Food Safety Authority (EFSA) has reported beneficial effect of EVOO on LDL oxidation process due to its phenolic (**Figure 10**).

4. Justification

Diabetes mellitus is one of the most lethal melodies of the world and ranked fourth in deadliest diseases. It is metabolic defect that results in hyperglycaemia. The distribution of diabetes is spreading with great speed. The diabetic mass of Pakistan is among the top 10 diabetic populations of the world and is projected to be at 8th position among most prevailing diabetic countries by 2045 (IDF 2017). With this rate 1 out of 4 persons in Pakistan is diabetic. It is not a single etiological condition but it is a multifactorial ailment that is affecting Pakistan population severely. Diabetes has no cure at the moment only preventive and supportive therapy is given to improve the living of people by means of oral medication and insulin injections [63–72].

Million people die of diabetes each year, mostly from cardiovascular disease exacerbated by lipid disorders and other complications.

Olive oil use has may a protective role to reduce the lipid profile in diabetes due to the beneficial effect of its antioxidant level. Since oral medications and insulin are the best possible treatment option but still these have some side effects like drugs toxicity, renal failure and inflammation and infection at the point of injection of insulin to minimize the effect and to obtain a better alternative treatment option which should be natural, harmless, cheap and could prove a permanent cure of diabetes we chose EVOO as a therapy of the type 2 diabetes.

Since there are some recent studies available which support the use of EVOO as a lipid and glucose lowering agent in type 2 diabetes mellitus. EVOO improves the post prandial glycaemia by incretin like mechanism in which up-regulation of incretin took place in this way lipid and glucose levels are reduce by EVOO. EVOO also reduce oxidative stress, inflammation lower lipid level which is the

hallmark of the diabetes that is why EVOO is compound of choice for the therapy of diabetes. The phenols in the EVOO are the major components responsible for these beneficial effects. Due of the fact that the EVOO therapy for the treatment of diabetes has never been studied before in the diabetic population of Pakistan we step forward and to use this natural compound as a therapy for treatment of type 2 diabetes [72–80].

There is no study available in Pakistan that support the therapeutic effect of EVOO in type 2 diabetes so got the idea to prove the therapeutic value of EVOO in the Diabetic population of Pakistan, for this we selected the Lahore region that is one of the populated city of Pakistan.


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