Diabetes mellitus (DM) is a complex disorder incorporating severe insulin dysfunction in conjunction with gross variations from the norm in glucose homeostasis, lipid and protein digestion system. In the World, number of people with type II DM and its complication is assumed to increase three times by the end of 2025 [1]. Type II DM predominantly influences more established people in developed countries, while in developing nations like Turkey; it is affecting the youthful populace in the prime of their working lives and subsequently represents a considerably more prominent risk to the wellbeing of these people [2]. There are different target receptors involved in the regulation of glucose and fatty acid metabolism reported by number of researchers which include aldose reductase, γ-receptor, activator of peroxisomal proliferator (PPARγ).

In silico validation of microalgal metabolites against Diabetes mellitus
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Aim. Present study aimed to evaluate the efficiency of microalgal metabolites as ligands for anti-diabetic target proteins viz., glucokinase, fructose-1, 6-bisphosphatase, glycogen synthase kinase, cytochrome P450, multi drug resistant protein, and peroxisome proliferator-activated receptor-γ (PPARγ) via computational approach.

Materials and methods. Three-dimensional structures of microalgal metabolites were retrieved from PubChem database and were energy minimized. The active site of target protein was predicted through PDB sum. Molecular docking was performed with microalga metabolites by using Hex 8.0 and DockThor server.

Results. Hex docking revealed that the binding interaction of fucoxanthin was higher with fructose 1.6 bis-phosphatase (298.31), human multidrug resistant protein 1 (369.67), and PPARγ (404.18). DockThor docking indicated that zeaxanthin with fructose 1.6 bis-phosphatase, human multidrug resistant protein, glycogen synthase kinase, PPARγ and cytochrome P450 produced higher total energy and interaction energy.

Conclusion. Further studies will assess the anti-diabetic effect of carotenoids of microalgae especially lutein, zeaxanthin and fucoxanthin.

Key words: carotenoid; diabetes mellitus; DockThor; glucokinase; microalgae
cytochrome P450, fructose-1, 6-bisphosphatase, glucoki-
nase, multidrug resistant protein and PPAR γ. The inhibi-
tory action of these receptors is an alternative treatment to
diabetes mellitus [3].

Microalgae are rich source of high value added com-
ounds including pigments, carotenoids, fatty acids, sterols, and
proteins. These metabolites were identified from differ-
ent microalgae and cyanobacteria including Phaeodactylum
tricornutum, Arthrospira, Porphyridium, Dunaliella salina,
Haematococcus pluvialis, Chlorella protothecoides, Prorocen-
trum minimum, Lyngbya majuscula, and Synechococcus [4].
Microalgal metabolites exhibit various pharmacological ac-
tivities viz., anti-inflammatory, analgesic, anti-viral, dietary
supplement antioxidants and anti-tumour agents [5]. To the
best of our knowledge, there is so far no information on micro-
algae specific metabolites in the treatment of diabetes mellitus.

Structure-based drug design is an essential study to scrutinize
the lead compounds to prevent the drug withdrawn from the
clinical and development process [6]. Predicting the target
sites of molecules using bioinformatics tools would be highly
beneficial and time efficient in pharmaceutical applications
to make a confident elimination avoid costly late-stage pre-
clinical and clinical failures. It covers the identification of lead
candidate, binding pocket, determination of target structure,
and evaluation of the potential lead candidate [7]. The present
study aimed to evaluate inhibitory action of microalgae me-
tabolites to some target protein related to glucose metabolism
and diabetes mellitus.

Materials and methods

Tools and software

The present study was performed by using bioinfor-
matics tools, biological databases like Protein Data Bank
gov/), Chimera, 3DLigandSite (http://www.sbg.bio.ic.ac.
.uk/3dligandsite/) and software’s like Open Babel 2.3.1.,
DruLiTo, Hex 8.0 and DockThor (http://dockthor.lncc.br/).

Selection of ligands

The bioactive metabolites of microalgae such as carot-
enoids, PUFA, sterols, alkaloids, and proteins were used as
ligands (Table 1). The two-dimensional (2D) chemical struc-
tures of the ligands were downloaded from the PubChem da-
atabase as .sdf format. The 2D structures of the selected ligands
were converted into their 3D formats using Chem Sketch and
it saved as .mol format. Further, the selected .mol format of
lead structures were converted into a .pdb format using Open
Babel 2.3.1. Sub-atomic adaptability was taken into account
considering every ligand as a gathering of conformers com-
municating to various zones of the conformational space
available to the particle inside of a given energy range. This ap-
proach helped to explore for adoption of the best conformer in
Chimera, which is based on the generalized CHARMM force
field implementation with default parameters. This program
uniformly identifies the best three-dimensional arrangements
of ligands, exploring the activity variations across the target
receptors.

Preparation of receptors

The PDB was used to download the target proteins Gluco-
 kinase (PDB ID: 1V4S), Fructose 1, 6 bisphosphatase (PDB
ID: 2JKJ), Human multidrug resistance protein (PDB ID:
2CBZ), and Cytochrome P450 (PDB ID: 3LC4), PPARγ
(PDB ID: 1ZGY), glycogen synthase kinase (PDB ID: 1H8F).
The structure was visualized by using a molecular graphics
program PyMol for the structural visualization of proteins.

Drug-likeness predictions

DruLiTo was used to determine selected microalgae me-
tabolites as a lead like candidate based on eight filters namely
Lipinski’s rule, MDDR-like rule, Veber rule, Ghose filter,
BBB rule, CMC-50 like rule, weighted and unweighted Quan-
titative Estimate of Drug-likeness.

Active sites prediction

3DLigandSite is an online tool to predict the binding site
of a protein. It utilizes the idea of interaction energy between
the protein and Vander Waals test to find enthusiastically good
binding pockets. Energetically favourable probe sites clustered
according to their spatial proximity and clusters then ranked
according to the sum of interaction energies for sites within
each cluster. These clusters were placed in rank request of the
probability of being a binding site as indicated by total binding
energies for each cluster.

Docking via Hex

The docking analysis of target proteins with microalgae
metabolites was carried out by using HEX 8.0, which calcu-
lates and displays possible docking poses of protein and ligand.
Docking determines the ligand with best scores and identifying
the drug-receptor complex with lowest free energy. The generated
metabolites were docked with the receptor by using follow-
ing parameters.

1. Correlation type – Shape + Electrostatics
2. FFT Mode – 3D
3. Post Processing – MM Energies
4. Grid Dimension – 0.6
5. Receptor range – 180
6. Ligand range – 180
7. Twist range – 360
8. Distance Range – 40

Docking using DockThor server

The best scores and lowest free energy of the metabolite
of Hex docking was further studied with DockThor program.
DockThor® employs a multiple solution genetic algorithm as
the search method [8] and the MMFF94S force field as the
scoring function for ranking the generated poses (http://dock-
thor.lncc.br/). The main steps of the ligand and protein set up
are available on DockThor Portal, being possible to change the
amino acid residues protonation states and include cofactors
(e.g. structural water molecules, metals, organic molecules) as
rigid entities. Grid size 34 A°, dimension x-17; y-17; z-17 and
discretization 0.35 was used. Hydrogen bond contacts, lipoph-
philic interactions and non-bonded contacts were calculated
using LIGPLOT [9].
**Results**

**Prediction of physicochemical and Drug–likeness properties of ligands**

The physicochemical property includes molecular weight, number of hydrogen bond acceptor and donor of selected microalgae metabolites are shown in Table 1. The drug likeness properties such as compound’s hydrophilicity, the polar surface area prediction, molecular refractivity, number of rotatable bonds, number of atom, number of acidic groups, rotatable bond count, number of rigid bond, number of atom ring, number of hydrogen bonds, structure alerts are explained in Table 2.

**Prediction of active sites residues in receptor**

Computational approaches screen the possibilities of microalgae metabolites (ligand) to treat diabetes and its complication. Glucokinase have the following residues in the active sites GLU 256, PHE 152, PRO 153, THR 168, SER 151, GLY 229, GLU 290, ASP 205, GLC 500, LYS 169, ASN 204, and ASN 231. Fructose 1,6 bis phosphatase have THR 31, ALA 24, GLY 28, ARG 22, VAL 17, THR 31, LEU 30, GLY 28, and THR 27. Human multidrug resistant proteins have GLN 713, LYS 684, VAL 680, GLY 681, THR 660, SER 686, TRP 653, ATP 1873, CYS 682 and. Cytochrome P450 have ASN 367, PHE 470, PHE 429, HIS 370, GLY 438, THR 307, TRP 128, ARG 109, HIS 109.
residues in their active site. PPARγ have HIS 323, PHE 282, LEU 469, HIS 449, TYR 327, ILE 326, CYS 285, MET 364 and Glycogen synthase kinase have 28ILE, 33PHE, 36VAL, 49ALA, 51LYS, 76VAL, 99ASP, 100TYR, 101VAL, 104THR, 151GLN, 152ASN, 154LEU, 165CYS, 166ASP residues in their active site (Fig. 1).

Docking of microalgae metabolites with receptors

Hex server based docking results of the aldose reductase, cytochrome P450, glucokinase and fructose-1, 6-bisphosphatase, permeability glycoprotein, PPARγ with ligands of microalgae metabolites interaction energy are shown in the Table 3. The binding interaction of fucoxanthin simulated higher total binding energy with fructose 1,6 bis-phosphatase, multidrug resistant protein 1, and PPARγ. Lutein simulated more total binding energy with glycogen synthase kinase, and zeaxanthin simulated higher total binding energy with glucokinase and cytochrome p450. Amongst 16 major microalgae metabolites, fucoxanthin, lutein and zeaxanthin were simulated as higher binding energy with anti-diabetic target proteins. DHA, gamma linolenic acid, EPA and GABA exhibited least binding energy with target proteins compared to carotenoids. Microcolin A and okadaic acid were simulated higher binding energy with target proteins compared to fatty acids (Table 3).

DockThor simulation was carried out to confirm binding interaction of target proteins with fucoxanthin, lutein, zeaxanthin, microcolin A and okadaic acid. Table 4 indicates the results of total energy and interaction energy. Docking simulation of lutein with fructose 1,6 bis phosphatase produced higher total energy (145.66 kcal/mol) and interaction energy (-23.01 kcal/mol) on the first run. Lutein with multidrug resistant protein produced higher total energy (1, 48,085 kcal/mol) and interaction energy (-8.531 kcal/mol) on the 8th run. Zeaxanthin with glucokinase produced higher total energy (111.23 kcal/mol) and interaction energy (-2.99 kcal/mol) on the 25th run. Lutein with glycogen synthase kinase produced higher total energy (1, 59, 766 kcal/mol) and interaction energy (-0.018 kcal/mol) on the 11th run. Lutein with PPARγ produced higher total energy (135.38 kcal/mol) and interaction energy (-30.604 kcal/mol) on the 8th run. Lutein with cytochrome p450 produced higher total energy (137.113 kcal/mol) and interaction energy (-30.279 kcal/mol) on the 10th run. Fig. 1 indicated the molecular interaction of lead candidates with target receptor.

Discussion

Hex is an interactive modern molecular graphics program can calculate protein-ligand docking, protein –protein docking and protein– nucleotides docking modes. Assuming that the ligand is rigid, ligand docking can superpose pairs of three dimensional structures of molecules [10]. The superpose can be used as spherical polar fourier (SPF) correlation to accelerate the calculations. It encodes surface shape, electrostatic charge, and potential distribution. This feature allows each property to be represented by a coefficient vector. In the present study, the electrostatic charge distribution of microalgae metabolites with the surface of target receptors was calculated. The surface states of proteins utilizing a two-term surface skin property to be represented by a coefficient vector. In the present study, the electrostatic charge distribution of microalgae metabolites with the surface of target receptors was calculated. The surface states of proteins utilizing a two-term surface skin property to be represented by a coefficient vector.
Figure 2. Docking interaction of lutein and zeaxanthin with target receptors predicted by LigPlot (blue line – ligand bonds; red line – non-ligand bonds; dotted lines – hydrogen bonds and its length; half red circle – non-ligand residues involved in the hydrophobic contacts; black dots – corresponding atoms involved in the hydrophobic contacts).

(a) The atomic interaction between HE21 atom of the GLN267 (red colour) in the cytochrome p450 receptor and an oxygen atom of lutein; (b) The atomic interaction between OD2 atom of the ASP199 (red colour) in the fructose 1,6 bisphosphatase and oxygen atom of lutein; (c) The atomic interaction between oxygen atom of the PRO312 and PHE 62 (red colour) in the glucokinase receptor and a hydrogen atom of zeaxanthin; (d) The atomic interaction between OGN267 (red colour) in the fructose 1,6 bisphosphatase and oxygen atom of lutein; (e) The atomic interaction between oxygen atom of the ARG 780 (red colour) in the human multidrug resistant protein and a hydrogen atom of zeaxanthin; (f) The atomic interaction between OD2 atom of the ASP199 (red colour) in the cytochrome p450 receptor and an oxygen atom of zeaxanthin.

Table 4

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Run</th>
<th>Total energy (Kcal/mol)</th>
<th>Interaction energy (Kcal/mol)</th>
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<tr>
<td></td>
<td>Ligand 16</td>
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<td>96.11</td>
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<td>Glucokinase</td>
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<td>-3.97</td>
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<tr>
<td></td>
<td>Ligand 16</td>
<td>25</td>
<td>111.23</td>
<td>-2.99</td>
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<tr>
<td>Human Multidrug resistant protein</td>
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<tr>
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<td>88.041</td>
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</table>

Depending on the stress condition applied, a wide range of polyunsaturated fatty acids, carotenoids, carbohydrates, and sterols were produced from microalgae in a non-toxic manner [12]. Taouis et al. showed that food supplements enriched with omega 3-unsaturated fats expanded the cell plasticity and reduced insufficient insulin action caused by the accumulation of high fatty acids [13]. There is a strong relationship between controlling blood glucose level and prevention rate of microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) (Zoungas, 2014). In the present study, sixteen different microalgae metabolites including Astaxanthin, Arachidonic acid, Brassicasterol, β-Stigma sterol, β-Carotene, Canthaxanthin, Docosahexaenoic acid, Eicosapentaenoic acid, Fucoxanthin, γ-linolenic acid, γ-amino butyric acid, Lutein, Lycopene, Microcolin A, Okadaic acid and Zeaxanthin were evaluated in their inhibitory action against target proteins.

Glucokinase and fructose-1, 6-bisphosphatase are the most important enzymes to regulate blood glucose level in human liver. The activities of these enzymes enhanced production of glucose through glycolysis or gluconeogenic amino acids [14]. The constant formation of glucose affected serious non-insulin dependent diabetic conditions. The analogues of lutein and zeaxanthin reported to have significant binding affinity with ligands.
enzyme involved in the regulation of ADME properties of endogenous and exogenous compounds through activating or deactivating drug molecules [17]. Surprisingly, a severe hyperglycemic condition associated with free radical formation leads to hepatocellular damage and elevated level of CYP450 dependent monoxygenase enzyme in diabetic rats [18]. The dietary fucoxanthin showed greater decrease in blood glucose level, plasma insulin concentration and increase in the activity of enzymatic antioxidants in diabetic/obese KK-A mice model [19]. It showed more potential DPPH free radical scavenging activity than other carotenoids under anaerobic condition [20]. In our study, docking of fucoxanthin with cytochrome P450, glucokinase and MDRP-1 showed potential binding interaction. Liu et al. reported that fucoxanthin purified from an edible marine seaweed Undaria Pinnatifida could diminish the rifampin-affected Cytochrome P450 3A4 and multiple drug resistance 1 expression through attenuation of Human pregnane X receptor mediated by CYP3A4 promoter activation [21]. Earlier reports showed that fucoxanthin and fucoxanthinol has the potential to reduce body fat and lipid accumulation via inhibition of 3T3-L1 adipocyte cells differentiation by down regulation of peroxisome proliferator-activated receptor A [22]. Combined effect of peroxisome proliferator-activated receptor (PPAR) gamma ligands such as fucoxanthin and troglitazone which potentially decreased the viability of colon cancer Caco-2 cells. Additionally the purified fucoxanthine ligand showed significant DNA fragmentation in Caco-2 colon cancer cells lines when compared to astaxanthin and beta carotene [23]. Kohno et al. reported that azoxymethane and dextran sodium sulfate induced colon tumorigenesis was significantly inhibited by troglitazone PPAR ligand molecules [24]. Therefore, fucoxanthin may represent a therapeutic target in the treatment of diabetes-induced oxidative stress and hyperlipidemic condition.

Glycogen synthase kinase is a type of serine or threonine kinase enzyme which is involved in the glycogen and protein synthesis [25]. However over expression of glycogen synthase kinase leads to insulin inability which causes huge amount of glucose deposition in respective muscles. There are valuable reports on acceleration of insulin dependent glycogen synthase kinase inhibition and glucose metabolism in skeletal muscles of type II DM patients [26]. In the present study, lutein showed high binding energy with Glycogen synthase kinase. In silico findings might provide new insights into treatment of type II DM. Reduced level of lutein and zeaxanthin in the dietary supplement cause age related macular degeneration diseases in humans which generally affect the individual central vision. Bone et al. reported that the graded doses treatment of lutein (2.4 to 30 mg/d) and zeaxanthin significantly increased the level of serum concentration and macular pigment density in the human subjects [27]. Prolonged hyperglycemic conditions decreased the level of antioxidants, nitro tyrosine and increased apoptotic conditions in the retina cells. The vision loss in diabetic rats was significantly reduced by oral administration of 0.5 mg/kg of lutein up to 12 weeks [28]. Also the lutein adjuvant therapies need further studies to improve effective drug molecules. Lutein could diminish the deleterious outcomes of cerebral I/R in stroke patients [29]. The present study was supported by this information which explains the inhibitory action of aldose reductase by lutein and zeaxanthin. Overproduction of reactive oxygen species and oxidative stress are closely associated with various health issues such as progression of atherosclerosis, hypercholesterolemia, ischemic reperfusion, and diabetes with advanced glycation products, hyperlipidemia, foot ulcer complications, cardiovascular diseases and further endothelial dysfunction [30]. PPARγ is also called as glitazone receptor, which are involved in the regulation of fatty acid storage and glucose metabolism in humans. Remarkably, the PPARγ concerned in the pathology of various diseases including diabetes mellitus, obesity and atherosclerosis [31]. As keto-carotenoids, astaxanthin and canthaxanthin are abundant in algae while they are rarely seen in plants [32]. Previous studies showed that the antioxidant activity of astaxanthin is approximately higher than zeaxanthin, lutein, canthaxanthin, beta-carotene and alpha-tocopherol [33]. Oral administration of astaxanthin significantly reduces the plasma glucose level in alloxan-induced diabetic mice [34]. The dietary intake of 0.1% fucoxanthin significantly reduced lipid hydro-peroxide levels of the liver, abdominal white adipose tissue and blood glucose levels of KK-Ay mice [35].

In conclusion, this study reveals that some special microalgal carotenoids; particularly lutein, fucoxanthin and zeaxanthin represent excellent source for the development of the novel antidiabetic drugs. As revealed by docking analyses in this study, the binding interaction of fucoxanthin is considerably high with fructose 1,6 bis-phosphatase, human multidrug resistant protein 1, and PPARγ. Moreover, lutein with fructose 1,6 bis phosphatase, human multidrug resistant protein, glycogen synthase kinase, PPARγ and cytochrome p450 produce higher total energy and binding interaction. Lastly, zeaxanthin with glucokinase produces remarkably high total energy and interaction energy. Further experimental studies will confirm the therapeutic efficacy of these carotenoids for development of novel antidiabetic drugs.

**Additional information**

**Compliance with ethical standards**

No conflict of interested. The authors did not receive any fund for this work.

**Acknowledgments**

The authors are grateful to The Scientific and Technological Research Council of Turkey (TUBITAK -2216) for researcher grant and the Research fund of Istanbul Medeniyet University for financial support (Project#FBA-2012-185)


To cite this article:

Медицинская диетология

doi: 10.14341/DM8212