RESEARCH ARTICLE OPEN ACCESS

Enhancement of Gene Regulation of ATP7A by Flavanoids from *Macrotyloma Uniflorum*: Computer-Aided Investigation in Menkes Disease

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Abstract

Menkes Disease (MD) is a deadly multisystemic problem of copper metabolism. Moderate neurodegeneration and connective tissue aggravations, along with the exceptional 'kinky' hair are the primary appearances of Menkes syndrome. MD happens because of mutation in the ATP7A gene and by far most of ATP7A mutations are intragenic mutations or partial deletion of ATP7A gene. ATP7A is an energy subordinate transmembrane protein, which is engaged with the delivery of copper to the secreted copper enzymes and in the export of excess copper from cells. Seriously impacted MD patients die on for the most part before the third year of life. A remedy for the disease doesn't exist; yet early copper-histidine therapy might treat a portion of the neurological symptoms. *Marcrotyloma uniflorum*, well known as horse gram is a lesser realized legume is profoundly nutritious and prominent for its ethano-therapeutic potential. Herein, the influence of flavonoids derived from *M. uniflorum* as the regulator of ATP7A genes of Menkes syndrome was evaluated using docking analysis. In the current investigation, we analyze the docking studies of flavanoids derived from *M. uniflorum* namely daidzein, genistein, kaempferol, myricetin and quercetin against the ATP7A protein. Using the AutoDock Vina tool the molecular docking studies was performed for drug components and target protein. As a result of molecular docking, *M. uniflorum* derived flavanoids myricetin and quercetin shows good binding energy scores of -6.4Kcal/mol at the active pockets of ATP7A protein and can be used as potent drugs against Menkes disease.

Keywords - **Menkes Disease, *Marcrotyloma uniflorum*, Flavanoids, ATP7A gene, Molecular Docking**

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I. INTRODUCTION

Menkes disease (MD) is a X-linked multisystemic deadly confusion of copper metabolism. Patients generally show a serious clinical course, with death in early childhood, yet factor structures exist and occipital horn syndrome (OHS) is the mildest structure. The faulty gene in MD (ATP7A) is anticipated to encode ATP7A, which is engaged with the conveyance of copper to the secreted copper enzymes and in excess copper export from the cells. Ordinary and strange copper metabolism in human and different organism has been the focal point of broad research, and enormous information has been gathered regarding this matter. Copper is the third most bountiful trace component in the body, after iron and zinc, and is needed for typical function of a many copper

enzymes taking part in significant metabolic processes. Copper is associated with neurotransmitter biosynthesis (dopamine b-hydroxylase); free-radical scavenging (superoxide dismutase); development of peptide hormones (peptidyl a-amidating enzyme); cell respiration (cytochrome-c oxidase (COX)); cross-linking of elastin, collagen (lysyl oxidase) and keratin (sulfhydryl oxidase); iron homeostasis (ceruloplas min and hephaestin) and melanin production (tyrosinase). Copper has additionally been ensnared in myelination in guideline of the circadian rhythm, and may likewise be fundamental for coagulation and angiogenesis [1, 2]. Although fundamental, attributable to its chemical properties, a similar metal might be exceptionally toxic. Copper can exist in two oxidation states, Cu(I) furthermore Cu(II), and reversible exchange between these two states is the premise of the enzymatic reactions. A similar property, notwithstanding, can bring about the production of free radicals, which have hindering impacts on cellular components. Fine guideline of copper homeostasis is, in this way, crucially significant for every living organism.

Copper homeostasis in Menkes disease

Elimination of copper from cells is the essential aggravation in MD, and practically every tissue aside from liver and brain will accumulate copper to strange levels. Although high, the copper level doesn't come to a toxic state in MD. This is partly because of a generally reduced intestinal copper retention, as a result of defective copper export from the mucosal epithelium, and halfway because of the scavenger function of metallothionein. In the MD patients liver, the low copper content is expected to prerequisite of the metal in different tissues, rather than distributed copper metabolism, as in the ordinary liver ATP7B, yet not ATP7A, is the principle copper transporter.

The justification behind the low copper content in the brain of MD patients is anyway unique. The mammalian brain is one of the most extravagant copper-containing organs in the body. Regulation of brain copper level isn't surely known, however ATP7A should participate in this process, since MD prompts low copper levels in the brain. In MD patients, copper is logical caught in both the blood–brain barrier and the blood–cerebrospinal liquid barrier, while the neurons and glial cells are denied of copper [3]. This likewise upholds the function of ATP7A in brain copper take-up. Neuronal demyelination is likewise seen in MD patients because of ATP7A inactivation [4]. Intervention of ATP7A-related copper release through NMDA-receptor activation recommends another function of this protein in brain dysfunction other than through hardship of copper-dependent enzymes. It is, hence, probable that seizures and neuronal degeneration observed in MD patients may likewise be related with an upset neuronal transmission through impaired action of NMDA receptors [5].

Macrotyloma uniflorum

Medicinal plants are utilized as a source of drugs for treatment of different ailments everywhere, from ancient period to the present day. They fill in as a source of significant natural substances for producing traditional and current medications. Food legumes, a fundamental part of balanced human diet are perceived as the second most significant group of crops later cereals [6]. *Macrotyloma uniflorum* (horse gram) is one of the legumes which are having high nutritious just as ethno-therapeutic values in the developing nations. These days, to meet the ever expanding interest for vegetable protein there is an increase demand for the underutilized vegetables as new substitute protein sources [7]. *Macrotyloma uniflorum* a vegetable species of the Fabaceae family and famously known as "horse gram" is found in the tropics and subtropical areas, native to African and Asian nations. It is the storage house of proteins and is basically wealthy in amino acids like lysine, arginine, histidine, valine, leucine, and so on. Restricted measures of tryptophan and methionine have been reported [8]. *M. uniflorum* consist of a variety of bioactive molecules such as phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, sinapic acid, and gallic acid),

flavonoids (daidzein, genistein, kaempferol, myricetin, and quercetin) and anthocyanins (Cyanidin, delphinidin, malvidin, and petunidin) [9, 10, 11]. It also exhibits various medical benefits such as anti-microbial [12, 13, 14], anti-obesity [15, 16], anti-diabetic [17, 18], anti-inflammatory [19], anti-oxidant [20, 21, 22, 23] and antihistaminic [24].Horse gram contains the highest calcium content among the pulses [25, 26] and has rich micronutrient content. The horse gram also contains various nutritional values in the seeds and 19% of copper content is present in 100g of dry seeds [27, 28]. Hence, the flavanoids from horse gram were rich in copper content and play a vital role in regulation of ATP7A gene that involves copper metabolism in Menkes disease.

Computer-Aided investigation helps to analyse the molecular interaction between the *Macrotyloma uniflorum* derived flavanoids and ATP7A gene. The current investigation aims to evaluate the docking scores and the type of interaction between the ligand and target protein, bond distances and the binding affinity of daidzein, genistein, kaempferol, myricetin and quercetin against active sites of the ATP7A protein using molecular docking technique.

II. METHODOLOGY

Retrieval of Protein structure

The database of RCSB Protein Data Bank (PBD) (https://www.rcsb.org/), was used to retrieve the 3-dimensional structure of the target protein ATP7A (PDB ID: 7LU8). The 3D structure was saved from database in ".pdb" format.

Protein processing

The retrieved from was then processed using the BIOVIA Discovery studio tool. The target protein was first loaded on the workspace, then the water molecules, hetatoms and other ligand groups were removed and the processed protein was finally saved in ".pdb" format.

Ligand Retrieval

The retrieval of ligand molecules was done by using the online database PubChem (https://pubchem.ncbi.nlm.nih.gov/). In this database consists of vast information about components such as chemical formula, IUPAC, smiles, molecular weight, 2D, 3D and crystalline structures. The 3-dimensional structure of flavanoids from M. *uniflorum* namely daidzein, genistein, kaempferol, myricetin and quercetin (PubChem ID: 5281708, 5280961, 5280863, 5281672 and 5280343) were retrieved in ".sdf" format.

Docking using PyRx

PyRx tool was used to perform the molecular docking of ligands with the active sites of target protein. The ligand molecules were first loaded in ".sdf" format PyRx window. Then all the ligand molecules were minimized and were converted into ".pdbqt" format. Once the ligand molecules were converted to ".pdbqt" file the target protein was loaded using the option load molecule. Then the target protein was made as a macromolecule and ligand molecules were selected. In the AutoDock vina window ligands and target protein were selected and the grid box was made. The docking was performed and finally, the docking values were stored in Comma Separated Values (.CSV) format.

Building Protein-Ligand complex

The complex was constructed using PyMol tool. The protein and ligand complex was constructed from the output file out PyRx that was automatically stored in ".pdbqt" format. The output protein and output ligand file were imported in the PyMol work space. The complex was built and was exported as a single file molecule in ".pdb" format.

Visualization of Protein-Ligand interaction

The constructed complex structure was opened in BIOVIA Discovery studio tool. After the complex structure was opened in the receptor-ligand interaction window the receptor and ligand were defined. Once the receptor and ligand were defined the ligand interaction was performed. Then the amino acids were labelled and type of interaction and bond distances were analysed. Finally the 2D

and 3D interactions were visualized and were saved in ".png" or ".jpeg" format.

III. RESULTS AND DISCUSSION

Docking Scores

The molecular docking was performed for flavanoids against the target protein ATP7A and the docking score results were tabulated in table1.

Table 1: Docking Scores of flavanoids against ATP7A protein

ID	Ligand	Binding Score (Kcal/mol)
5281708	Daidzein	-5.9
5280961	Genistein	-6.1
5280863	Kaempferol	-6.2
5281672	Myricetin	-6.4
5280343	Quercetin	-6.4

M. uniflorum derived flavanoids were docked on the binding pockets of the target protein ATP7A. Depending on the negative value and the lowest value of energy (DGbind) the best docking orientation was selected among the five flavanoids. Based on the docking scores it was revealed that daidzein, genistein and kaempferol show less binding affinity when compared to myricetin and quercetin. Myricetin and quercetin has better interaction at the active sites of ATP7A protein with the binding score of -6.4kcal/mol.

Protein-Ligand Interaction

The ligand and the target protein interactions were visualized and the types of bond interaction were analysed. The amino acid with hydrogen bond interaction was tabulated in table 2. The ligand molecule daidzein with target protein ATP7A formed one (1) Pi-Donor hydrogen bond interaction with amino residue Leu73 at bond distance 2.71956 Å and three (3) Pi-Alkyl type of hydrophobic interaction with Leu73, Pro51 and Lys75 at 5.23862 Å, 4.65013 Å and 5.2394 bond distances. with genistein formed two (2) conventional hydrogen bond with Ala56 (donor) and Asn55 (acceptor) at 2.33393 Å and 2.3856 Å bond distances.

Table 2: H-Bond interactions flavanoids with ATP7A protein

ID	Ligand	H-Bond interaction
5281708	Daidzein	Leu73
5280961	Genistein	Ala56, Asn5 (2)
5280863	Kaempferol	Lys75, Val54
5281672	Myricetin	Thr7, Val54
5280343	Quercetin	Thr7

It also formed one (1) carbon hydrogen bond with Asn55 at 3.56597 Å and three (3) Pi-sigma hydrophobic bonds with Leu8, Pro51 and Ala56 at bond distances 3.65542 Å, 3.9007 Å and 3.63296 Å respectively. Other interactions include one (1) Amide-Pi-stacked hydrophobic and one (1) Pi-Alkyl hydrophobic with amino residues Asn55 and Pro51 at 5.40899 Å and 5.44799 Å bond distances.

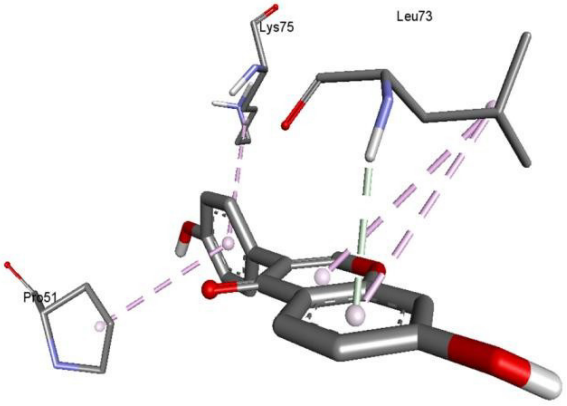

Figure 1: 3D interaction of Daidzein with ATP7A

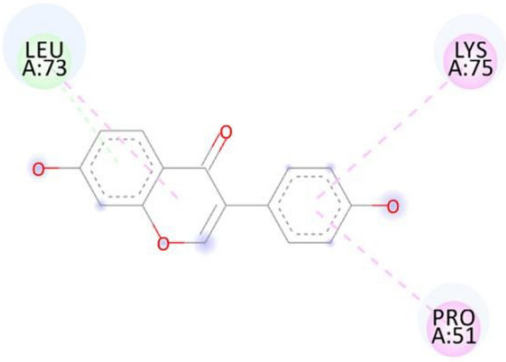

Figure 2: 2D interaction of Daidzein with ATP7A

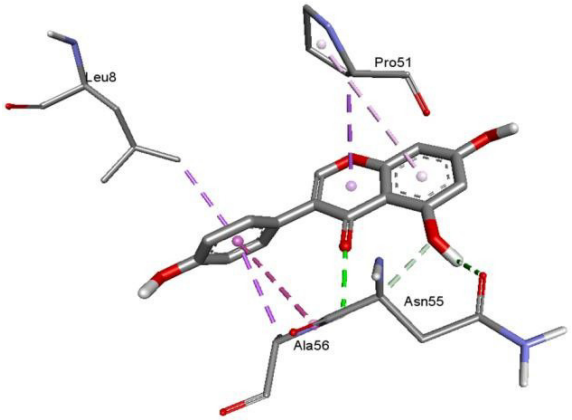

Figure 3: 3D interaction of Genistein with ATP7A

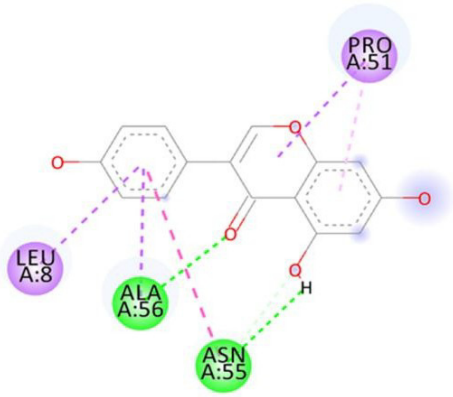

Figure 4: 2D interaction of Genistein with ATP7A

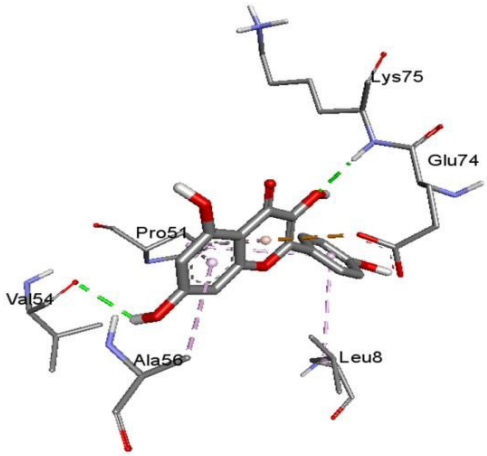

Figure 5: 3D interaction of Kaempferol with ATP7A

Kaempferol with ATP7A protein formed one (1) donor conventional hydrogen bond and one (1) acceptor conventional hydrogen bond interaction with Lys75 and Val54 at bond distances 2.0539 Å and 2.46244 Å. Also one (1) Pi-Anion electrostatic interaction with Glu74 at 3.70861 Å and five (5) Pi-Alkyl hydrophobic interactions with Pro51 (3), Ala56 and Leu8 at 4.20422 Å, 4.63886 Å, 5.41071 Å, 5.27457 Å and 5.0911 Å bond distances.

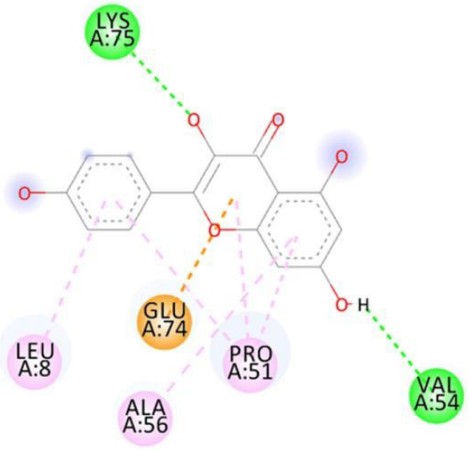

Figure 6. 2D interaction of Kaempferol with ATP7A

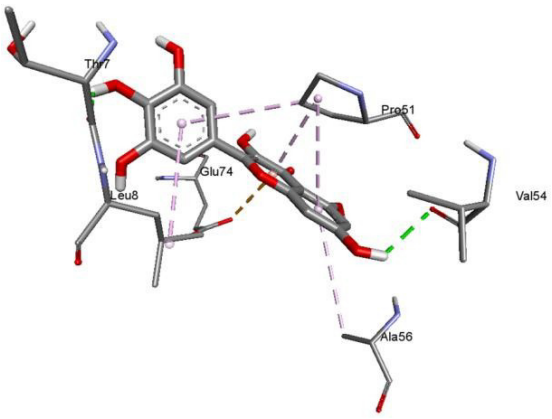

Figure 7: 3D interaction of Myricetin with ATP7A

Drug compound myricetin with ATP7A formed two (2) acceptor conventional hydrogen bond interaction with Thr7 and Val54 at distance 2.43728 Å and 2.4585 Å. Other interactions include one (1) Pi-Anion electrostatic with Glu74 at 3.70861 Å and five (5) Pi-Alkyl hydrophobic interaction with amino residues Pro51 (3), Ala56 andLeu8 at 4.65417 Å, 4.21692 Å, 5.3852 Å, 5.26142 Å and 5.15355 Å bond distances. Quercetin with target

protein formed one (1) acceptor conventional hydrogen bond with amino residue Thr7 at 2.41189 Å, one (1) Pi-Anion electrostatic interaction with Glu74 at bond distance 3.71044 Å and five (5) Pi-Alkyl hydrophobic interaction with Pro51 (3), Ala56 and Leu8 at bond distances 4.21821 Å, 4.68909 Å, 5.31004 Å, 5.222 Å and 5.15076 Å.

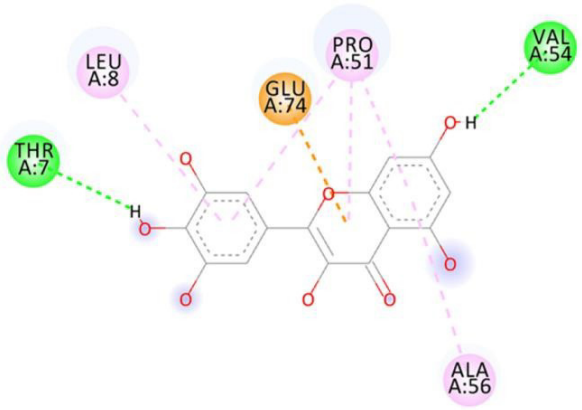

Figure 8: 2D interaction of Myricetin with ATP7A

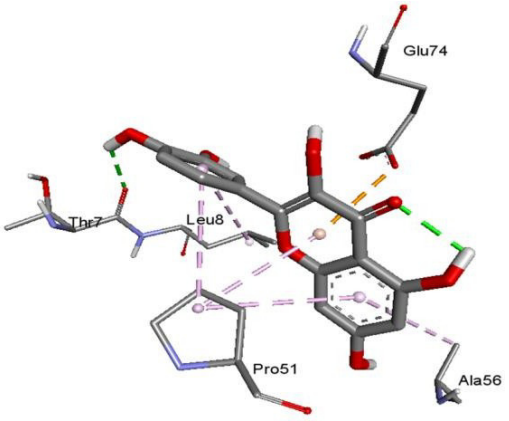

Figure 9: 3D interaction of Quercetin with ATP7A

The protein and ligand interaction depends on the binding affinity, number of hydrogen bond interaction and distance of the interaction bond [29, 30]. The docking results reveal that of our ligand molecules shows better affinity towards the binding site of ATP7A protein that leads to the gene regulation of the target protein. Therefore, based on binding scores with ATP7A protein makes our compounds myricetin and quercetin more potential drugs against Menkes disease.

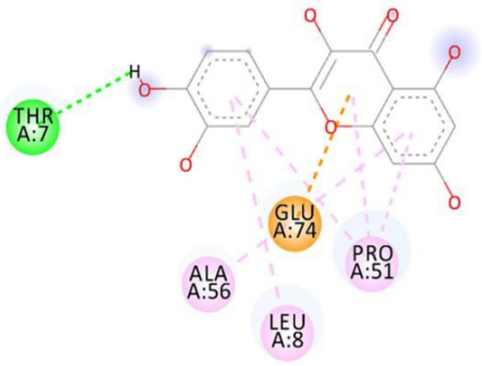

Figure 10: 2D interaction of Quercetin with ATP7A

IV. CONCLUSION

In this computer-aided investigation the effect of *Macrotyloma uniflorum* derived flavanoids daidzein, genistein, kaempferol, myricetin and quercetin on the ATP7A gene were analysed using molecular docking tool. Among the five flavanoids myricetin and quercetin exhibits better binding score of -6.4Kcal/mol. Based on the molecular docking scores, it was suggested that these pharmacologically active flavanoids are potential molecules to be tested against ATP7A gene, that leads to copper deficiency in Menkes disease and can be used to design and develop effective drugs for the treatment of Menkes disease.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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