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COMPARATIVE BINDING ANALYSIS OF ESCULETIN AND GLYCYRRHIZIN TO HUNTINGTIN N-TERMINAL FRAGMENT

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder caused due to increased polyglutamine (CAG) repeats in huntingtin (HTT) protein. Increased CAG repeats cause misfolding of HTT and leads to aggregate formation. These aggregates cause the progression of HD giving rise to neurodegeneration and classical HD symptoms. There is no treatment for HD till now due to the large size of HTT which had made it difficult to crystallize it and elucidate its complete structure. Drug tetrabenazine is the only drug approved by FDA and that is also meant for only controlling chorea, the classical symptom of HD. Many molecules have been successful in vitro and in animal studies but fail in human trials due to poor blood brain barrier penetration or bioavailability. One approach to treat HD may be to find drugs which inhibit CAG repeats and inhibit aggregate formation, thus slowing down the progression of disease. In this study, we have done a comparative analysis of ADMET (Administration, Distribution, Metabolism, Excretion and Toxicity) and druglikeness properties of esculetin and glycyrrhizin by in silico approaches. Also, we have done comparative docking of esculetin and glycyrrhizin to Huntingtin N-terminal fragment, exon 1 by taking polyglutamine inhibitor C2-8 as a standard. Our results showed that esculetin was found to have better blood brain barrier penetration, HIA absorption, followed the Lipinski's rule and had a better dock score than glycyrrhizin.

KEY WORDS

Docking, ADME, huntingtin N-terminal fragment, esculetin, glycyrrhizin

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder. It was first described in 1872 by George Huntington [1]. The most obvious symptom of HD is chorea [2]. HD is classically associated with progressive emotional, psychiatric, and cognitive disturbances [3]. HD is characterized by GABAergic neuronal degeneration in striatal region [4]. The gene responsible for causing HD is IT15 gene which codes for 'huntingtin' (Htt) protein having molecular weight of 348kDa. HD is caused by expansion of CAG repeats in the N-terminal of IT15 gene [1]. CAG expansion, referred as Htt^{ex}, lies in exon 1[5]. Disease symptoms emerge when the number of repeats increases to more than 35, with the increase in repeat number being directly proportional to the age at onset of symptoms [6]. People with less than 35 repeats do not develop HD; people with 35-39 CAG repeats might or might not develop HD; people with 40-60 polyglutamine (PolyQ) repeats develop HD when they become adult; people with more than 60 repeats develop HD before the age of 20 [5].

Mutated Htt undergoes many post-translational modifications such as proteolytic cleavage, phosphorylation, glycosylation. Proteolytic cleavage produces N-terminal fragments which are accumulated

in the cells as inclusion bodies. A variety of N-terminal fragments are produced with different length due to cleavage by various caspases and calpains. The most prominent of these N-terminal fragments is the one with 100 amino acids. It is termed as Htt exon 1 because it is encoded by the first exon of Htt [7]. Htt is cleaved by a variety of caspases and calpains. Htt is cleaved by caspase-2, -3, -6 and -7 [8; 9;10; 11]. It is evident that cleavage of Htt by caspase-6 and release of proteolytic fragments containing exon 1 is an obligatory step in causing cellular toxicity [12; 13]. Cleavage of Htt at 552 amino acid residues by caspase has been shown to precede the neurodegeneration and the onset of HD phenotype. It is hypothesized that Htt fragments generated by cleavage of normal Htt are cleared by ubiquitin-proteasome pathway whereas those containing extended polyglutamine are accumulated [14]. PolyQ is contained in this cleaved fragment which tends to misfold and form a stable three-dimensional conformation enriched in β-sheet structure with self-assembly resulting in fibrillar aggregates [15; 16, 17]. Previous studies have shown that mHtt aggregates are detected earlier than pathogenic symptoms in human patients and mouse model of HD [18].

PolyQ aggregates have an ability to continuously elongate by self-assembly [19]. Aggregation of the extended PolyQ triggers disease by recruiting and sequestering other proteins. This deprives the cell of many important functions leading to neuronal death. Proline containing PolyQ sequences inhibit he elongation of PolyQ repeats by recruiting further glutamine monomers [20].

It was reported that if Htt exon 1 is blocked, the insoluble Htt aggregates disappear as well as the motor dysfunction [21]. Transgenic mice containing exon 1 of Htt has been reported to cause neurodegeneration along with the symptomatic chorea [22]. However, some studies suggest that insoluble aggregates seem to play a protective role, leading to autophagy-mediated clearance [23; 24]. Although it is controversial whether insoluble aggregates or soluble oligomers are more toxic, both contribute to toxicity of HD is reported. Thus, one therapeutic option to inhibit aggregation is needed in HD.

High molecular weight of HTT has made it impossible till now to crystallize it and elucidate its three-dimensional structure. Recently crystal structure for HTT N-terminal fragment, exon 1 containing 36 polyglutamine (36Q) repeats i.e. mutant huntingtin (mHTT) has been determined by insertion of three histidine residues with X-ray diffraction technique. It has been shown that there is a transition of N-terminal fragment from α-helix to loop to β-hairpin which causes the polyQ strands to be in β-sheet conformation. α-helix and loop are inert structures whereas β-sheet conformation might cause pathogenic interactions of mHtt in cells [25].

Development of structural biology has made it possible to elucidate 3D structures of proteins. The 3D structures of proteins are saved in a database named Protein Data Bank (PDB) which holds structures of more than 27000 proteins. These structures give insights into their biological function and molecular biology of the disease. This elucidation of protein structures has been very useful in finding out the molecular pathogenesis of various neurodegenerative diseases [26].

In silico techniques like homology modelling have also made it possible to predict the 3D structures of proteins. Homology modelling or structural modelling refers to generation of 3D structures of protein from amino acid sequence by using computer algorithms and programs [27]. Recent research indicates that Htt and PACSIN1 interact together and aggregate in the cytoplasm. This study elucidated the 3D structure of mutant Htt with 0Q, 17Q and 36Q repeats by homology modelling using I-TASSER server and then docking two proteins by HADDOCK [28]. I-TASSER is a computer program available online for hierarchical protein structure modelling based on approach based on the secondary-structure enhanced Profile-Profile Threading Alignment (PPA) and and the iterative implementation of the Threading ASSEmbly Refinement (TASSER) program [27]. HADDOCK (Hight Ambiguity Driven DOCking) is a docking approach which uses non-experimental data for docking based on combination of energetics and shape complementarity during energy minimization and water refinement stages [29;30]. Another study also used the same methodology for analysing the binding of Htt to SH3GL3. It is a Grb2- like protein with an important role in CNS and signal transduction. This research indicated that SH3GL3 interacts with 17Q and 36Q which implies that there is an increased interaction between mutant Htt and SH3GL3 which causes an abnormal distribution of Htt-SH3GL3 complex leading to leading neuronal death. Molecular docking is many a times combined with molecular simulations. It is a recent computational

technique which is used to view motion of atoms and molecules [31].

Many small molecules like Congo red, trehalose, C2-8, PGL-135 have been shown to inhibit aggregate formation [32;33]. PGL-135 inhibits aggregation of HDQ51 protein i.e. Htt protein containing 51 repeats. Antibody mAb1C2, thioflavine S, chrysamine G and Direct Fast yellow inhibit HD exon 1 aggregtion in a dose dependent manner in an *in vitro* model [33]. But congo red cannot cross blood-brain barrier. It failed to ameliorate the symptoms of HD in a R6/2 mouse [34]. C2-8 is a polyglutamine inhibitor which is known to inhibit polyglutamine aggregation in HD *in vitro* [35] and in vivo [36]. There is a need for developing such a drug against HD which can cross BBB and can also inhibit aggregate formation.

We have investigated the polyglutamine aggregation inhibition by Esculetin and Glycyrrhizin by docking both the molecules with HTT N-terminal fragment, exon1 structure containing 36Q repeats (HTT 36Q) using C2-8 as a standard and its docking ability with HTT N-terminal fragment has been analysed. The structure of Htt36Q in beta conformation was chosen as it is known to cause pathogenicity.

Pharmacodynamics and pharmacokinetic studies for Esculetin and Glycyrrhizin has also been carried out. Esculetin is a coumarin found in many plants which is shown to have neuroprotective properties. Glycyrrhizin (or Glycyrrhizic acid or glycyrrhizinic acid) is the chief sweet-tasting constituent of Glycyrrhiza glabra (liquorice) root. C2-8 is a cell-permeable amido sulphonamide compound that potently inhibits polyglutamine (poly-Q)-aggregation in vitro and in vivo.

MATERIALS AND METHODS

Target Preparation

The structure of N-terminal of Htt i.e. exon 1 containing 36Q was retrieved from Protein Data Bank (PDB) with PDB ID 4FE8. Stereochemistry of the protein structure was checked by RAMPAGE. Z score was analysed using What if server.

Ligand Preparation

The structures for Esculetin and C2-8 were downloaded from PubChem database in SDF format. The structure of glycyrrhizin was downloaded from Chemspider in SDF format.

ADMET Studies

Pharmacokinetic studies for drugs were done using PRE-ADMET server [37]. Pharmacokinetic properties include absorption, distribution, metabolism, excretion and toxicity. 2-D structures of glycyrrhizin and esculetin were subjected to analyses for blood brain barrier penetration, solubility, intestinal absorption, cytochrome P450 inhibition, human epithelial colorectal adenocarcinoma cell line (Caco-2) cell permeability, apparent Madin-Darby Canine Kidney (MDCK) cell permeability, skin permeability, plasma protein binding ability and Ames test.

Druglikeness

Druglikeness was assessed using Pre-ADMET server. The two compounds were screened for druglikeness according to Lipinski's rule (Rule of Five), CMC like rule, Lead like rule, MDDR like rule and WDI like rule.

Pharmacodynamic studies

These studies were carried by Prediction of Activity Spectra for Substances (PASS) ONLINE server [38]. PASS is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule. *PASS* provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. This study is done to assess the physiological and biochemical effect of drug on an organism.

Docking Studies

Docking was carried out using Ligand fit module in Accelerys Discovery Studio 4.5 which is an automated tool for protein – small molecule docking/scoring [39]. Steps are as follows

- Applying CHARMm forcefield to protein structure for energy minimization.
-All water molecules were removed; Hydrogen atoms were added to the protein and clean geometry was done for stabilization of ligand.
- Define binding site (ligand-based or cavity-based).
- Dock ligand to each binding site of receptor.
-Save the top docked structures (diverse poses).

RESULTS AND DISCUSSION

ADME studies

Pharmacokinetics refers to the absorption, distribution, metabolism and excretion of a compound during the time course [40]. There is no guarantee that the compound having the best binding interactions for the desired target might prove to be the best drug. A

compound can be classified as a drug, if it is entirely and quickly absorbed from the gastrointestinal tract, distributed specifically to its target, metabolized in a way that does not eliminate its activity, and removed in an appropriate manner without causing any damage [41]. The prediction of blood-brain partitioning for drugs is expressed in terms of log BB value. Compounds are distributed across blood brain barrier (BBB) in terms of their lipophilicity. High lipophilic compounds cross the BBB by diffusion, whereas low lipophilic compounds cross BBB by specific carriers. Therapeutic drugs designed for CNS should be able to cross BBB for their effectiveness [42; 43]. Compounds having logBB value more than 0.3 have high absorption to central nervous system (CNS), 0.3~-1.0 have middle absorption to CNS and less than -1.0 have low absorption to CNS [44]. Log BB is the ratio of concentration of drug between brain and blood [45]. In our study Esculetin is found to have high absorption to CNS and Glycyrrhizin is having middle absorption. Thus, Esculetin has better BBB penetration than Glycyrrhizin. As HD is a disorder affecting brain, so it is important for any drug to have good BBB permeabilty to be able to cure HD.

Human intestinal absorption (HIA) is the process by which drugs are absorbed from intestine into the bloodstream [46]. HIA is very important in the design, optimization and selection of new molecules as drugs [47]. HIA data are the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile and faeces [48]. Compounds having 0-20% are poorly absorbed, 20-70% are moderately absorbed and 70-100% are well absorbed compounds [49]. In our results, Esculetin has far more HIA values than Glycyrrhizin.

Protein binding affects the performance of a drug. Drugs bind to a variety of proteins in plasma like albumin. Binding of drugs to proteins in the plasma affect their half-life [50]. A drug should have less plasma protein binding for its diffusion and transport across the body. Generally, only the unbound drug is available for interaction with a pharmacological target [51]. Drug-protein complexes are too large to cross the plasma membrane [52]. In our study, Esculetin shows very less plasma protein binding than Glycyrrhizin. The difference in the plasma protein binding of two drugs is in the range of 11-82. Above 80-85% range, plasma protein binding affects drug's efficacy.

It has been reported that excretion process that eliminates the compound from human body depends on the molecular weight and octanol–water partition coefficient (logP) [53]. Log P is used to express lipophilicity. It is an important parameter related to membrane permeability [54]. Higher the lipophilicity of a compound more is the metabolism and poor absorption. Also, there is an increased probability of its binding to undesired hydrophobic macromolecules, thereby increasing the potential for toxicity [53]. Increased hydrophobicity in a drug renders it to be less soluble in the gut and increasingly soluble in fat globules. As a result, it will have poor absorption [55]. Esculetin has been reported with lower logP value than Glycyrrhizin which states that Esculetin has less lipophilicity and better absorption than Glycyrrhizin. (Table 1).

Body tries to eliminate the drugs or xenobiotics. This metabolism of drugs is carried out in liver by enzymes Cytochrome P450s. CYP3A4, CYP2D6, CYP2C9 and CYP2C19 are important for the metabolism of drugs. Each drug acts with CY450 differently. Drugs may act as inhibitor or induced for cytochrome P450 enzymes. Drugs may not necessarily inhibit or induce all the types of CYP450 enzymes. Only one CYP450 is enough for metabolism [56].

Caco-2 and Madin-Darby canine kidney (MDCK) monolayers are used to assess oral absorption. They also contain transporter proteins.

Table 1. ADME predictions for Glycyrrhizin and Esculetin using Pre-ADMET.

PARAMETERS	ESCULETIN	GLYCYRRHIZIN
BBB	0.57039	0.036543
Caco2	18.5799	20.7977
Buffer solubility mg/L	3379.18	173820
CYP2C19 inhibition	Inhibitor	Non
CYP2C9 inhibition	Inhibitor	Inhibitor
CYP2D6 inhibition	Non	Non
CYP2D6 substrate	Non	Non
CYP3A4 inhibition	Inhibitor	Inhibitor
CYP3A4 substrate	Non	Substrate
HIA	88.197	9.458664
MDCK	39.7188	0.0434155
Plasma protein binding	11.46962	82.484315
Pure water solubility mg/L	1169.29	0.0554931
Skin permeability	-3.97242	-1.89676
SKlogD_value	1.537220	0.444740
SKlogP_value	1.537220	2.940740
SKlogS_buffer	-1.721960	0.675270
SKlogS_pure	-2.182850	7.171130

Data represents values for Blood brain barrier penetration (BBB), CYP- cytochrome P450 inhibition, Human intestinal absorption (HIA), octanol-water partition coefficient (logP), solubility (logS).

Table 2. Druglikeness values for Esculetin and Glycyrrhizin using Pre-ADMET tool

DRUGLIKENESS RULE	ESCULETIN	GLYCYRRHIZIN
CMC like Rule	Not qualified; No total atoms	Not qualified
CMC like Rule Violation Fields	No total atoms	Molecular weight, AMolRef, No total atoms
CMC like Rule Violations	1	3
Lead-like Rule Violation Fields		Molecular weight
Lead like Rule	Suitable if its binding affinity is greater than 0.1microM	Violated
Lead like Rule Violations	0	1
MDDR like Rule	Mid- structure	Mid structure
MDDR like Rule Violation Fields	No rings, no rotatable bonds	No rotatable bonds
MDDR like Rule Violations	2	1
Rule of Five	Suitable	Violated
Rule of Five Violation Fields		Molecular weight, No hydrogen bond acceptors, no hydrogen bond donors,
Rule of Five Violations	0	3
WDI like Rule	In 90% cut off	Out of 90% off
WDI like Rule Violation Fields		Molecular weight, No H bond acceptors, No H bond donors.
WDI like Rule Violations	0	15

Data represents values for various druglikeness rules. MDDR- MDL Drug Data Report; CMC- Comprehensive Medicinal Chemistry; WDI- World Drug Index.

Table 3. Ames test and carcinogenicity predictions for Esculetin and glycyrrhizin using Pre-ADMET tool.

PARAMETERS	ESCULETIN	GLYCYRRHIZIN
Ames test	Mutagen	Non- mutagen
Carcinogenicity in rat	Positive	Positive
Carcinogenicity in mouse	Negative	Positive

Data represents predicted values for Ames test and carcinogenicity for rats and mouse. Positive indicates negative test and vice versa.

Table 4. Pharmacodynamics results for Esculetin

Pa	Pi	Activity
0,900	0, 002	Antimutagenic
0,847	0,004	Peroxidase inhibitor
0,683	0,004	Free radical scavenger
0,688	0,017	Anti-inflammatory
0,585	0,010	Lipid peroxidase inhibitor
0,574	0,013	Antineoplastic(breast cancer)
0,568	0,008	Antimetastatic

Pa- probability "to be active" estimates the chance that the studied compound is belonging to the sub-class of active compounds (resembles the structures of molecules, which are the most typical in a sub-set of "actives" in PASS training set).

Pi- probability "to be inactive" estimates the chance that the studied compound is belonging to the sub-class of inactive compounds (resembles the structures of molecules, which are the most typical in a sub-set of "inactives" in PASS training set).

Table 5. Pharmacodynamics results for Glycyrrhizin.

Pa	Pi	Activity
0,995	0,000	Hepatoprotectant
0,994	0,001	Chemopreventive
0,993	0,001	Antitoxic
0,861	0,005	Anti-inflammatory
0,609	0,004	Dementia treatment

Pa- Probability to “be active”, Pi- Probability to “be inactive”

Table 6. Docking analysis of esculetin, glycyrrhizin and C2-8

LIGAND	BINDING SITE	POSES	PMF	DOCKING SCORE
C2-8	4	10	-59.36	66.363
ESCULETIN	22	10	-33.72	144.812
GLYCYRRHIZIN	2	10	-17.75	135.334

Data represents binding analysis on the basis of binding site, poses, potential of mean force (PMF) and docking score.

Druglikeness

Drug likeness is a concept that optimises various pharmacokinetic and pharmaceutical properties of a drug like solubility, chemical stability, distribution profile and bioavailability, and, thus can define whether a particular molecule is similar to known drugs. It is comprised of various molecular descriptors which make different rules [57]. Descriptors are physicochemical or structural property of a molecule which are used to assessment of properties like lipophilicity, solubility and absorption in humans [58]. Lipinski’s rule states that the absorption of a drug will be impaired if logP > 5; Molecular weight >500; Number of hydrogen donor groups >5; Number of hydrogen acceptor groups >10. MDDR like rule, CMC like rule and WDI like rule are based on molecular descriptors other than those described in Lipinski’s rule, for compounds recorded in MDL Drug Data Report (MDDR) database,

Comprehensive Medicinal Chemistry (CMC) database and World Drug Index (WDI) respectively [59]. Esculetin qualified Lipinski's rule or Rule of five, WDI like rule and Lead like rule, whereas Glycyrrhizin did not qualify any of the rules stated for druglikeness (Table 2).

Toxicity and Pharmacodynamic Studies

Toxicity studies predicted Esculetin to be a mutagen but non-carcinogenic in rats. Glycyrrhizin was non-mutagenic (Table 3). Pharmacodynamics states the relationship between the between the drug concentration at the site of action and the effects- beneficial or adverse- it produces in the time course [40].

Inspite of toxicity predictions, Pharmacodynamic studies showed that Esculetin was antineoplastic, antimetastatic (Table 4). Glycyrrhizin was found to be effective in dementia treatment (Table 5).

Ramchandran Plot

Ramchandran Plot analysis of Htt N-terminal fragment gave a Z-score of -0.707 and showed 96.8% (1168) residues in favoured regions, 2.9% in allowed regions and 0.35% residues in outlier regions (Fig 1 & 2). RAMPAGE is used for structure validation of protein structures. Ramchandran Plot is an indicator of intrinsic quality of the protein structure. Z-score is evaluating the quality of a Ramchandran Plot. Z-score of less than -4 is an indicator of serious problem with the protein structure (more negative score indicates lower quality [60].

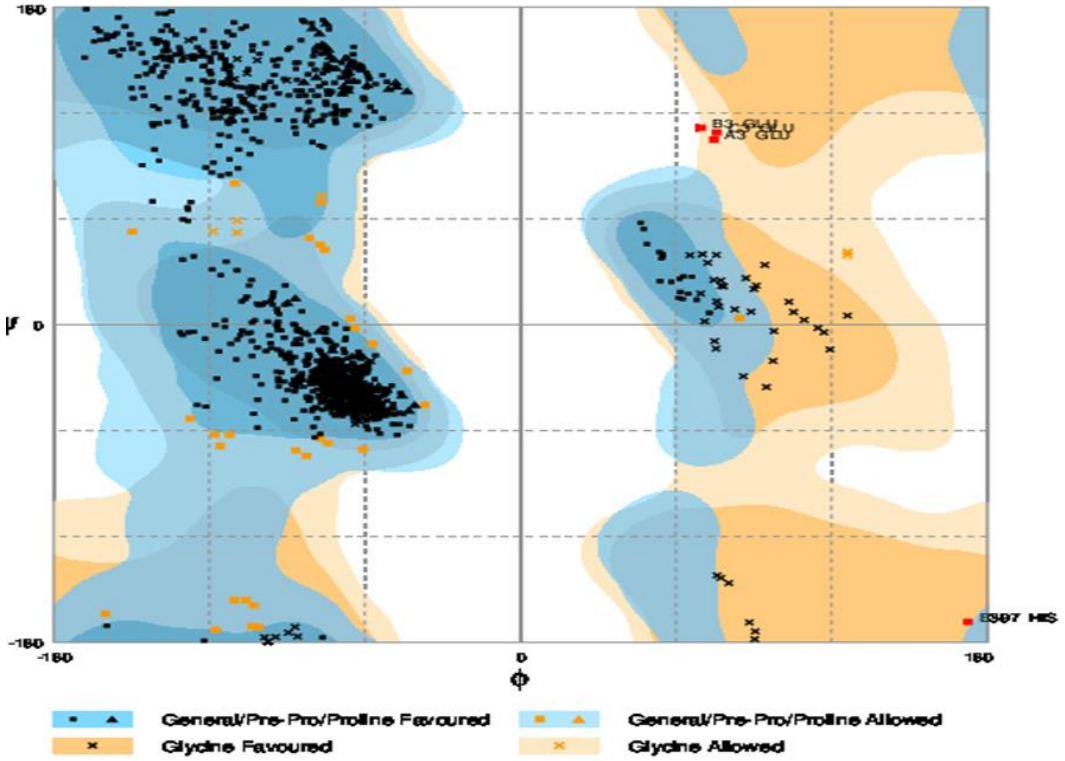

Fig.1 Ramchandran Plot analysis of HTT N-terminal fragment showing all the amino acids together in general and glycine and proline in favoured and allowable regions.

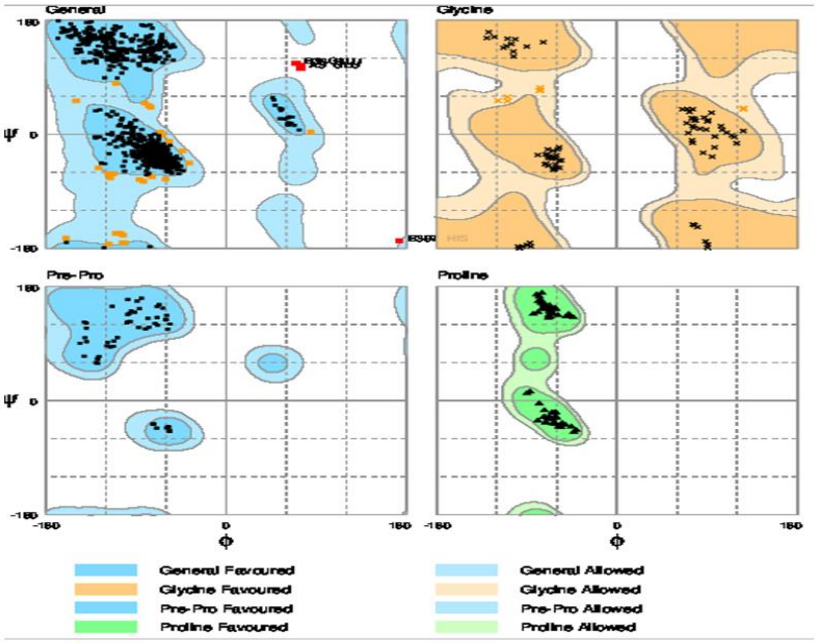

Fig.2 Ramchandran Plot analysis showing separate graphs for general amino acids, Glycine and Proline in favoured and allowable regions.

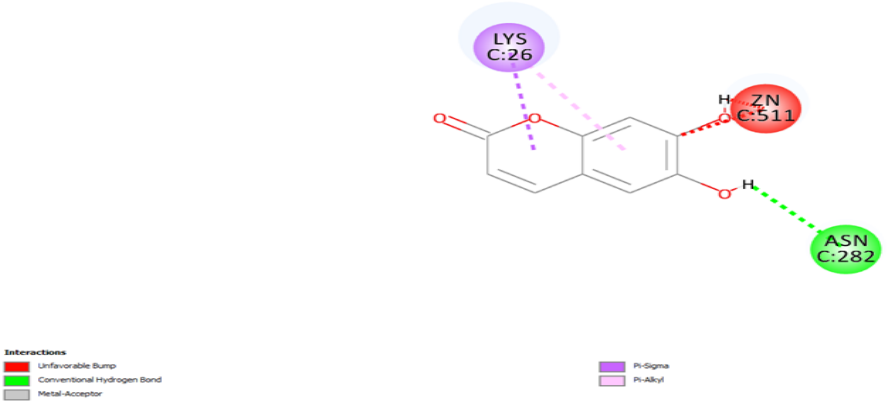

Fig. 3 Docking of Esculetin to N-terminal fragment represents the best docked pose of esculetin to N-terminal fragment i.e. pose in 22nd binding site showing amino acids involved in interaction.

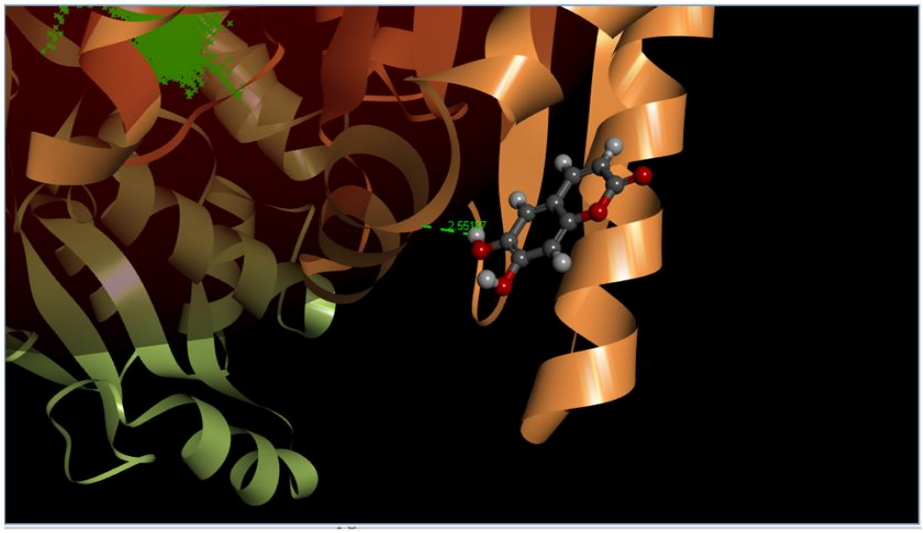

Fig. 4 Bond lengths of hydrogen bonds involved in interaction of esculetin and HTT N-terminal fragment.

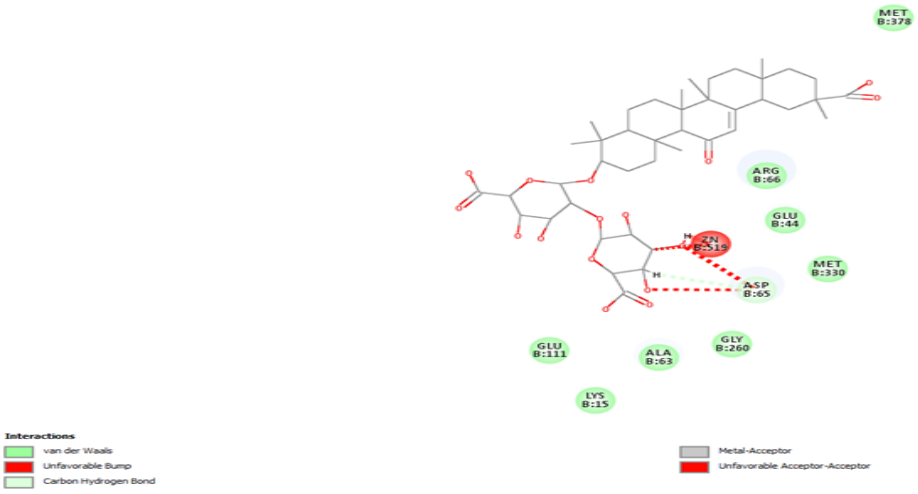

Fig. 5 Docking of glycyrrhizin to N-terminal fragment represents best docked pose of glycyrrhizin to HTT N-terminal fragment i.e. pose in 2nd binding site. It shows amino acids involved in interaction.

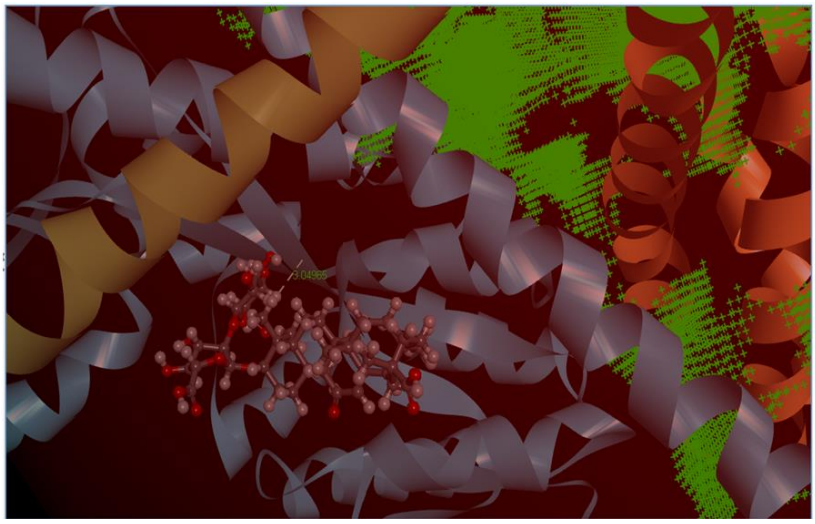

Fig. 6 Bond length of hydrogen bond involved in interaction of glycyrrhizin and HTT N-terminal fragment.

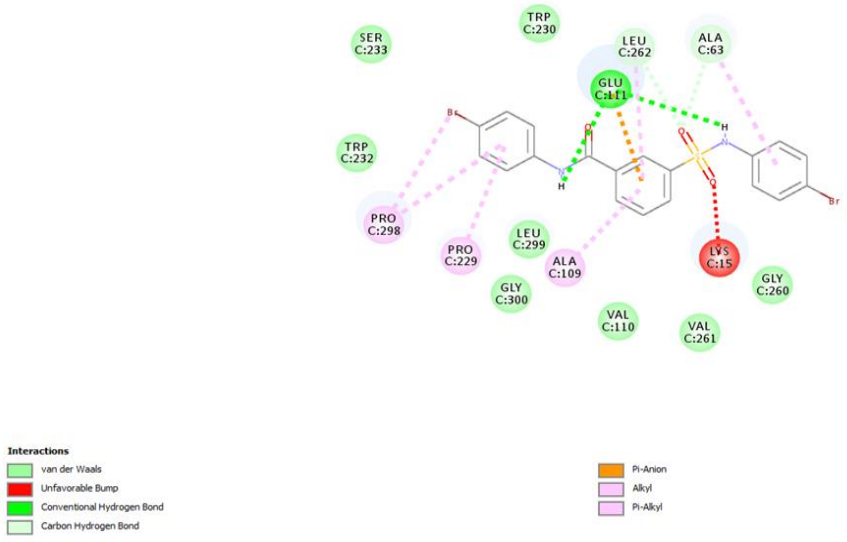

Fig. 7 Docking of C2-8 to N-terminal fragment represents best docked pose of C2-8 i.e. pose in 4th binding site. It shows amino acids involved in interaction.

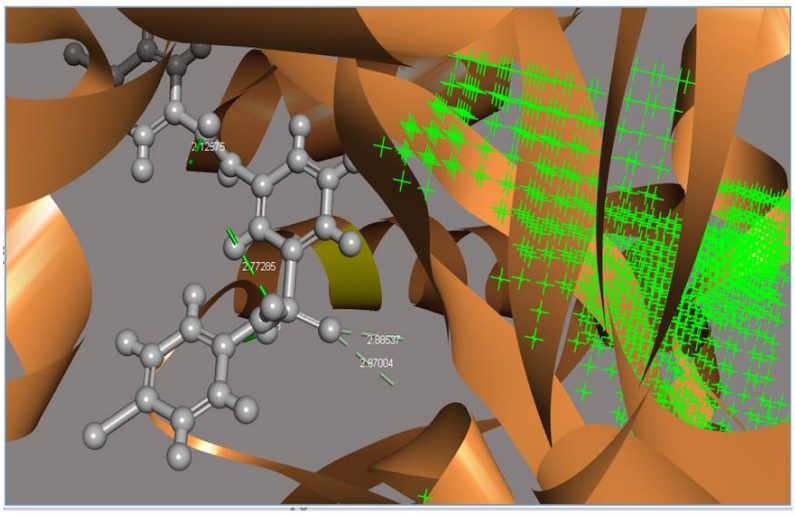

Fig. 8 Bond lengths of hydrogen bonds involved in interaction of C2-8 and HTT N-terminal fragment.

Docking Studies

In recent times, molecular docking is frequently used for drug designing studies. Molecular docking can be defined as an optimization study, which gives the "best fit" orientation of a ligand which would bind to protein of interest. Ligand can be defined as the molecule which binds to the protein of interest [61].

In the present study Accelerys Discovery Studio 4.5 using Ligand Fit module was used to find the possible binding site of 4FE8. Ligand fit docking in Discovery Studio uses cavity detection algorithm. It uses Monte Carlo technique to generate ligand confirmations which are then docked to the active site of the protein [62].

From the binding site analysis total 33 binding sites were found in huntingtin N-terminal exon 1(4FE8). Esculetin and Glycyrrhizin were docked to all the 33 binding sites and the binding site in each molecule with the highest docking score was chosen. Dock score is a simple force field-based scoring function [63]. Esculetin showed binding to 12 binding sites with 22nd binding site giving the highest dock score. Glycyrrhizin showed binding to 2 binding sites with 2nd binding site giving the highest dock score. C2-8 showed binding to 4 binding sites with 4th binding site giving the highest dock score. The docking scores, number of binding poses and potential of mean force (PMF) are tabulated in Table 6.

Detailed analysis of the best docked pose of Esculetin to 4FE8 showed that amino acids involved in interaction were Lys26 (Lysine) by Pi-Sigma bond, V23 (Valine), Asp 282 (Asparagine) by conventional hydrogen bond (Fig 3). The bond length of the hydrogen bond involved was 2.55187 (Fig 4).

Detailed analysis of the best docked pose of glycyrrhizin to 4FE8 showed that amino acids involved in interaction were Met378 (Methionine), Arg66 (Arginine), Gly260 (Glycine), Glu111 (Glutamic acid), Glu44, MET 330, Lys 15 (Lysine) and Ala 63 (Alanine) by van der waals force. Asp65 (Aspartic acid) was bonded by carbon-hydrogen bond (Fig 5). The bond length of the hydrogen bond was 3.04965 (Fig 6).

Amino acids which were involved in interaction with C2-8 were Trp232 (Tryptophan), Ser233 (Serine), Trp 230, Leu299 (Leucine), Gly300, Val110 (Valine), Val261, Gly260 by van der waals forces. Pro 298, Pro 229, Ala 109 were bonded by alkyl and Pi-alkyl bond. Leu262 and Ala63 were bonded by carbon-hydrogen bond and Pi-alkyl bond. Glu111 was bonded by hydrogen bond (Fig 7). The bond length of hydrogen bonds involved were 2.12575, 2.77285, 2.88537, 2.87004 (Fig 8).

Docking of Esculetin, Glycyrrhizin and C2-8 to Htt N-terminal fragment showed higher dock score of Esculetin and Glycyrrhizin than C2-8.

Docking of Esculetin to 4FE8 shows the presence of a conventional hydrogen bond whereas Glycyrrhizin does not have any conventional hydrogen bonding, instead it has a carbon-hydrogen bond. Formation of a Hydrogen bond is important as it affects the solubility, permeability and receptor molecular recognition of a drug [64]. This implies that Esculetin and Glycyrrhizin may also act as polyglutamine inhibitors, but, Esculetin can inhibit polyglutamine aggregation better than glycyrrhizin. Our results also indicate that Esculetin has better ADME properties and docking score than Glycyrrhizin.

CONCLUSION

The present study showed a comparison of various parameters between Esculetin and Glycyrrhizin which are required for a molecule to be classified as drug. The findings of the present study indicate that Esculetin has better blood brain barrier penetration, higher intestinal absorption, low excretion, follows Lipinski's rule and a more docking score to HTT N-terminal fragment (4FE8) than Glycyrrhizin. Thus, our study shows that Esculetin may inhibit aggregate formation in HD and might be a therapeutic option. If Esculetin is successful in further studies, it can be good option as a cure for HD because of its being a product of natural origin. Many plant based products are being used as drugs in treatment of many days without having any toxic effects.

It can be further assessed for its use as a drug of choice in treatment of HD. This is an *in silico* study, so we suggest that further *in vitro* and *in vivo* studies in various animal models can be carried to test the ability of Esculetin to inhibit aggregate formation and decreasing the symptoms of Huntington's disease.

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Declaration of interest

There is no conflict of interest to declare.

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