Original Research Article

Prediction of Lead Molecule esculetin of Tridax procumbens against zika virus envelope protein

ABSTRACT

In the existing field of science, it is necessary for Siddha, Ayurveda therapists, to understand the interactions and to treat the target regions of the human system effectively. One of the most significant nightmares for scientists and medical professionals has been the Zika virus. Since the bite of the Aedes mosquito species has infected people worldwide with the Zika virus (Flavivirus). The human mechanism and infection control interactions of the Zika virus are still unclear. In addition, some herbal products may give a pathway to the treat the zika virus. Many herbs aim to regulate various diseases, but the herb Tridax procumbens has a key collection of pharmacological values for diseases. Traditional health practitioners have struggled to grasp the precise mechanism of linking existence and how to treat the disease, so the current Ayurinformatics approach and the evolving field are an idea of a modern medical science system. Here we examine the interaction of flavonoids procumbenetin and esculetin of Tridax procumbens with zika virus in silico docking and molecular simulation and ADME and toxicity profile for the drug candidate..

Keywords: Tridax procumbens, Zika flavivirus, in silico docking, Esculetin, Ayurinformatics

1. INTRODUCTION

Tridax procumbens a perennial, annual creeper herb that lives only for a short period and it was globally distributed. Stems are hairy and branched, ascending with the simple opposite petiole leaves. Flower heads were long stalks yellow rounded three toothed ray florets [1]. Several biological active compounds of herbs were isolated and involved in various pharmacological activities. Secondary metabolites have various medicinal activities like dysentery, wound healing, stomachache, malaria, blood pressure and some viruses [2] used in the Indian traditional medicine system [3]. Alkaloids, carotenoids, flavonoids, saponins, and tanninsm [4] from Tridax procumbens played a major role in drug discovery.

Nowadays, Zika viruses are a major threat of full disease in India, which persists in the human body for several days to a week, and once the individual was affected, they are protected from other infections such as neurologic complications [5]. So far, no vaccine or drugs to cure ZIKA virus-infected individuals. Zika virus is a mosquito borne flavivirus [6]. The flavivirus has several proteins responsible for the infection, but envelope glycoprotein is responsible for entering the virus for the infection and targets of neutralizing antibodies for other flaviviruses [7]. In the medical system field, medical informatics cannot be given to all emerging areas of Ayurveda because it varies from the modern system's concepts, though the aim was like that to give relief from pain or disease. So, the use of the recent emerging Ayurinformatics approach will help to bridge a link between the ancient system and the modern system of medicine. The docking tool was used to predict the preferred orientation of one molecule with respect to another molecule when they bound each other for a stable complex with a minimum energy level [8]. Here, the aim of the present study was to investigate the interaction of Tridax procumbens flavonoids procumbenetin and esculetin with (ZIKV) Zika flavivirus viral envelope protein using in silico docking, molecular simulation and insilico toxicological profile prediction for the initial stage of drug development.]

2. MATERIAL AND METHODS

Tridax procumbens flavonoids compounds as ligands selected for the study were esculetin and Procumbenetin. The esculetin ligand structure and physiochemical properties were retrieved from Pubchem database CID: 5281416 (Figure 1). PubChem was a universal public database used to search a broad range of properties [9]. The Procumbenetin chemical structure was designed based on the chemical formula [10] using CHEMSKTECH software. The physical properties were identified, such as molecular formula, weight, composition, molar refractivity, molar volume, parachor, index refraction, surface tension, density, dielectric constant, polarity, and mass.

The 3-Dimentsional structure of Zika virus (ZIKV) envelope glycoprotein was retrieved from the protein data bank (PDB) database [11]. The structure of Procumbenetin and Esculetin was represented in Figure 1 and Figure 2 respectively. The RCSB PDB operated by US data center for the global PDB archive enables the 3D structure with functional properties. The PDB ID used was 5JHM (Figure 3).

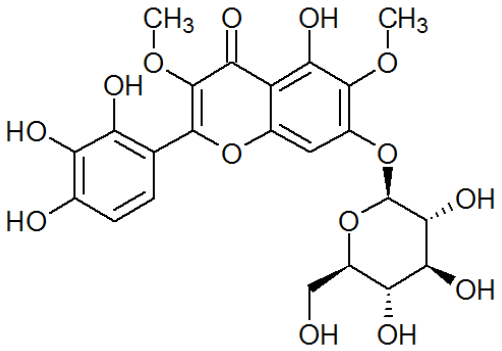

Fig.1 Procumbenetin

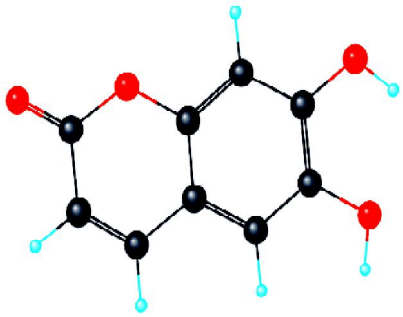

Fig.2 Esculetin

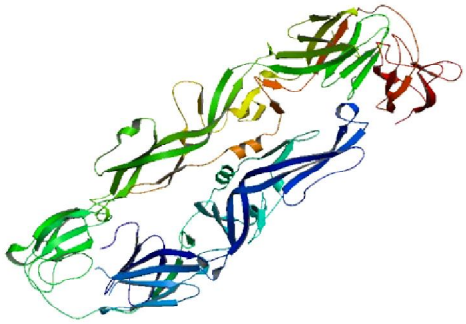

Fig.3 5JHM
(ZIKV- Envelope Protein)

Molecular docking studies were performed using commercial software suite SYBYL-X 1.3 software. This software expedites molecular modeling/computational chemistry and other discovery works from high throughput screening to late lead optimization. Here we used to predict the small molecules such as drug candidate interaction and receptor binding. The ligands esculetin and procumbenetin were predicted for the binding orientation against the target Zika virus to estimate the affinity and activity of the molecule.

Discovery studio visualizer 3.1 software suite created by Accelrys to visualize, analyze, and share biological and chemical active substances. The software was used to view 3D graphic sequences, SMILES, Structure binding, editing and analysis functionally. This suite was very useful for drug discovery. The suite generates receptor-ligand binding interaction plot and patterns between ligand and the protein. The Molecular dynamic and simulation annealing was performed to determine the ZIKV protein and esculetin complex's stability. The simulation was carried out for 150ns to

identify the structural transition in envelope protein. The analysis was performed using g_rms, g_rmsf, and g_hbond GROMACS, root mean square fluctuation (RMSF), the root mean square deviation (RMSD), and the number of H-bonds between the ligand and the zika was obtained.

The insilico toxicological profiles were predicted using online software tools SwissADME and ProTox-II. The SwissADME was used to evaluate Physical chemical properties, Lipophilicity, water solubility, Pharmacokinetics, Drug likeness and medicinal chemistry12. ProTox-II for the prediction of toxicities like LD50, Toxicity Class, Organ toxicity, Carcinogenecity, Immunotoxicity, Mutagenicity, Cytotoxicity and Androgen receptor13).

3. RESULTS AND DISCUSSION

Molecular docking of Esculetin and Procumbenetin showed interaction with ZIKV 5JHM envelope protein using SYBYL Software, which predicted the interactions between each other through some parameters. The total score was expressed as –log (Kd) and it includes the crash score between ligand atoms that are separated by rotatable bonds (Table 1 & 2).

Table 1: Properties Of Esculetin

Properties	Values	Properties
Molecular weight (g/mol)	178.143	Molecular weight (g/mol)
Hydrogen bond donor count	2	Hydrogen bond donor count
Hydrogen bond acceptor count	4	Hydrogen bond acceptor count
Complexity	248	Complexity
Topological polar surface area	66.8 A	Topological polar surface area

Table 2: Properties Of Envelope Protein

Chain	Residue		Carbon	Nitrogen		Oxygen	Total	Water molecules
A	391	1867	521	575	2988	128		
B	390	1860	519	571	2975	145		

The binding affinity was represented in which procumbenetin interacted with the residues like LYS-110, ASP-98 and ARG-99. LYS-110 was an essential compound involved in the formation of a double trimmer complex (Figure 4 a & b).

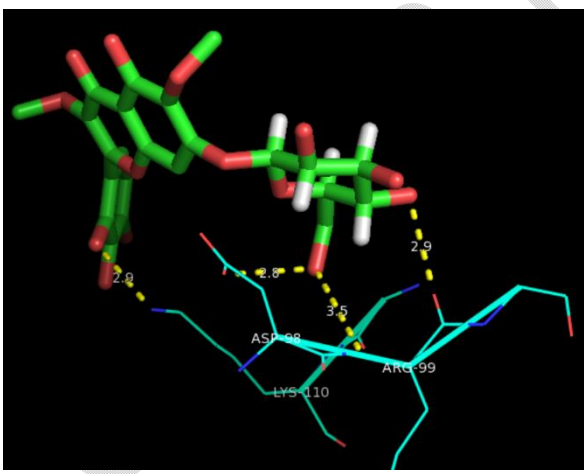

Fig 4a: Docking interaction of procumbentin vs Zika

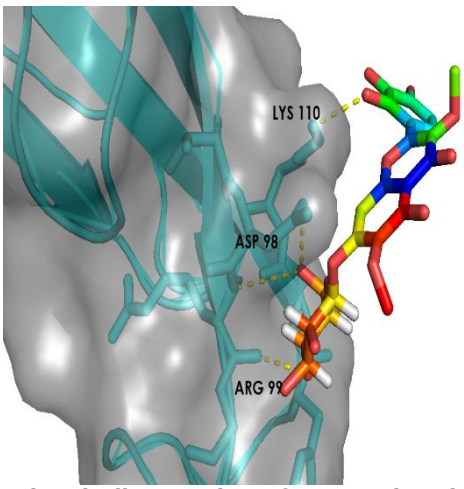

Fig 4b: Binding pocket of procumbentin vs Zika

The observed bond length was 2.8, 2.9, 2.9 and 3.5. This shows the hydrogen bonding affinity with some close interaction. It was reported that LYS-110 is a crucial proliferating cell nuclear antigen (PCNA) target sequence for replication and repair foci. LYS-110 was located near the nuclear localization region. The activation of LYS-110 was

known to cause mutations in the heterochemical region of PCNA. PCNA was involved in a wide spectrum of cellular functions, including DNA replication, repair, and epigenetic maintenance [14]. The docking result has shown the potential activity of procumbenetin in binding with LYS-110, predicting the inhibiting capacity of procumbenetin on LYS-110. ASP-98 structurally analogous to the proton-abstracting residues found in the zinc-containing enzymes, suggesting a related role for this residue. ASP-98 has been suggested to act as part of a proton shuttling pathway to this residue. ASP-98 is reported to alter nitrite binding. The activation of ASP-98 would result in the formation of mutation at the extracellular boundary of transmembrane helix 2 that reduces gonadotropin-releasing hormone activity. Nitrite was responsible for cell damage, increasing the methemoglobin level and leading to the development of cancer [15]. The docking result has shown the interaction of procumbenetin with ASP-98. They must be further analyzed to find its potential or inhibiting activity so that the clear role of procumbenetin on ASP-98 can be explained. ARG-99 was a derivate of ARG that has a crucial role in the protein-protein interface. It can enhance hydration, maintain access to water, and contact polar head groups to stabilize the charged form. It has a unique capacity to retain its charge in environments expected to promote the neutral form. It was the right choice when a biochemical function requires the presence of charge in hydrophobic environments [16]. The interaction of procumbenetin with ARG-99 was observed in our study that could be further clarified to find its potential or inhibiting activity. The nitrating binding affinity of procumbenetin with LYS-110, ASP-98 and ARG-99 could be positive or negative, which must be further analyzed. Its interaction could help enhance the potential activity of the residues on interaction or the harmful activity of the residue. The pharmacological activities of procumbenetin include insecticidal, hypotensive, leishmanicidal, wound healing, hair growth-promoting, anti-inflammatory, immunomodulatory and hepatoprotective [17].Procumbens was a very promising species that produce secondary metabolites reported to have a variety of medicinal uses. Its properties also include high blood pressure, anti-anemic, gastrointestinal, anti-diabetic respiratory infections, and anesthetic properties. This species has a long history of traditional use by different communities [18].

The predicted active sites are A/ASP98, A/ARG99, A/GLY100, A/TRP101, A/GLY102, A/ASN103, A/GLY104, A/CYS105, A/GLY106, A/LEU107, A/PHE108, A/GLY109. Our docking research article has discussed the importance of interacting residues with esculetin like GLY 109, ASP-98 and ARG-99. It has other potential active sites like ASP98, GLY100, TRP101, GLY102, ASN103, GLY104, CYS105, GLY106, LEU107 and PHE108. ASP98 was a protein trimmer present at 3 Å sphere [19]. GLY100 has the role in destabilizing the pyrophosphatases globular structure [20]. TRP101 plays a vital role in maintaining a defined phosphoserine aminotransferase microenvironment essentially required for optimal enzymatic activity [21]. A study has confirmed GLY 104 in the interaction, making it stable and emerging as hotspots in the antigen–EDE antibody association related to dengue fever [22]. The role of other residues is not yet clearly known A study on Zika virus stated that the decoding LYS28, ASN17, PHE24, ARG213, LYS29, GLY81, SER150, SER215, SER56, GLY106, ARG84, TRP87, THR104, ASP146, GLY107, HIS110, ILE147 and GLY148 might be the potential target for zika virus drug [23].

The Esculetin showed a total score of 2.8065 and a crash score of -0.7647 than procumbenetin of 1.7476 and -1.1283. This was due to the degree of penetration by the ligand into the protein. A crash score close to 0 was favorable and a negative number indicates good penetration with the ligands Esculetin molecule. The D score of -42.8704 and -12.8443 chemscore of Esculetin denotes the charge and van der Waals good interactions between the protein and the ligand and hydrogen bonding, metal-ligand interaction, lipophilic contact, rotational entropy, along with an intercept than procumbenetin molecule. Esculetin had the interaction with the residues like GLY 109, ASP-98 and ARG-99 with the following bond length 3.5, 3.1, 3.2 and 3.0 (Figure 5).

GLY-109 residue may forbid the formation of this bond due to steric hindrance [24]. It can also lead to its replacement by one of eight bulkier residues that could result in collagen disease termed osteogenesis imperfect [25]. Our docking result has shown the binding affinity of esculetin with GLY-109 that might have the potential in supporting the activity of GLY-109. Like procumbenetin, esculetin had also shown the interaction with ASP-98 and ARG-99. Its potential activity must be further analyzed. The bond length represents that the interaction was within the hydrogen bond length. The typical hydrogen bond length was between 2.8 to 3.5 Å, where above 3.0 was the critical configuration barrier. Lower Van der Waals energy denotes the impact of hydrogen bond activity of these compounds during protein or enzyme interaction. Hydrogen bond with the active site of the target macromolecule will result in a high Glide score. The formation of hydrogen bonds in docking results could predict its potential binding affinity that can not be easily broken by external force [26]. Multiple molecular analysis has stated that the incidence of hydrogen bonds and ranges are key factors affecting the binding interactions of the ligand-receptor interaction [27]. In our docking result, the bond length varies from 3.0, 3.1, 3.2 and 3.5 (Figure 5a, b & c). The simulated results show the bond length of 3.0, 3.0, 3.3 and 3.6. The bond length of 3.0 was difficult to compare to the bond length of 3.5. This shows the potential binding affinity. As discussed above, the bind could enhance the potential activity of the compound or could suppress the harmful effect of the compound that must be studied further. A recent study on esculetin has shown its anti-tumor efficacy on loading to Polylactide-co-glycolide nano-micelles and nano-encapsulation against its cytotoxic activity on in vitro model [28]. Esculetin was reported to be an anti-inflammatory drug for treating bowel disease inflammation that was studied on the rat model through colon cytokine

analysis [29]. It was also reported to have potential activity against anti-proliferative and cell death with other pharmacological properties like anti-edema, anti-inflammatory and anti-tumor [30].

Stable interaction was found in the ZIKV protein residues ASP 98 and ARG 99. Outcomes showed that esculetin has remained stable at 310 K with residues in ZIKV protein ASP 98 and ARG 99 for 150ns. No modifications are produced between the ZIKV protein and tannic acid in the bond distance and angle. Although the protein undergoes confirmation change during annealing, it does not impact the interacted residues between the tannic acid-protein complexes. Also, the polar range did not go beyond 3.9 Å. Thus, the simulation analysis suggests that the structure was stable and not affected. The above Graph of RMSD and Radius of Gyration retained its values where RMSD did not cross 2Å from its initial configuration, and gyration was maintained at 20Å. So, the molecule was stable. Root mean square deviation (RMSD) of atoms ZIKA protein – Esculetin docked complexes at 310K (Figure 6 2, b & c).

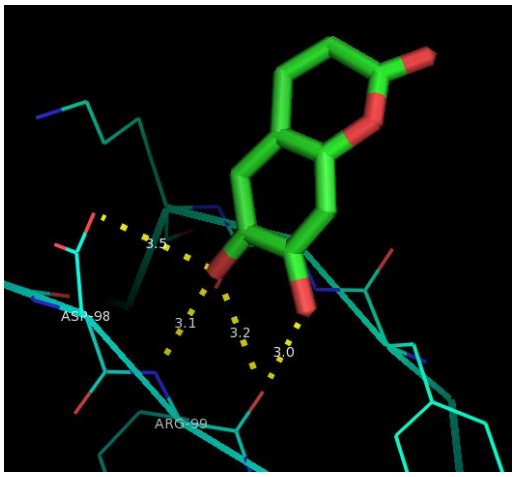

Fig 5a: Docking interaction of esculetin vs Zika (Annealing result)

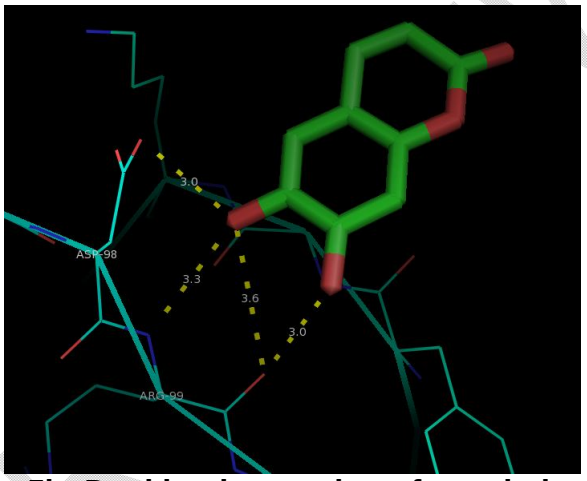

Fig 5b: Docking interaction of esculetin vs Zika (Simulation result)

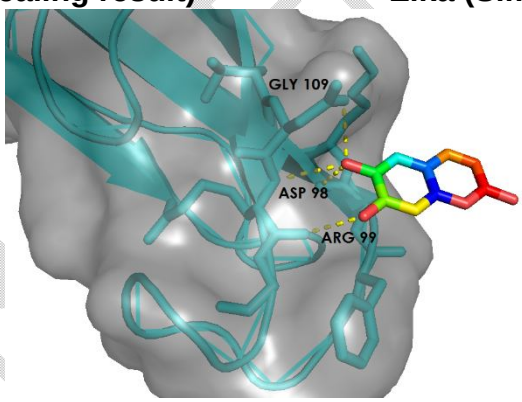

Fig 5c: Binding pocket of esculetin vs Zika

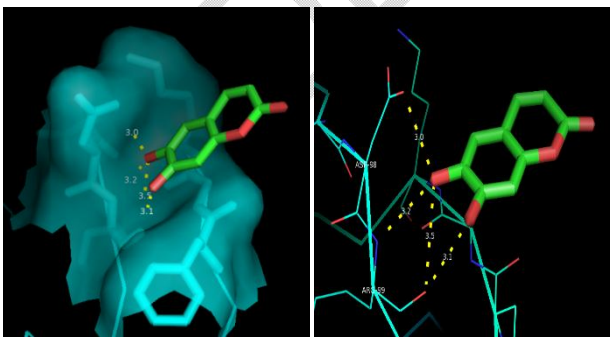

Fig 6a: Simulation Pocket

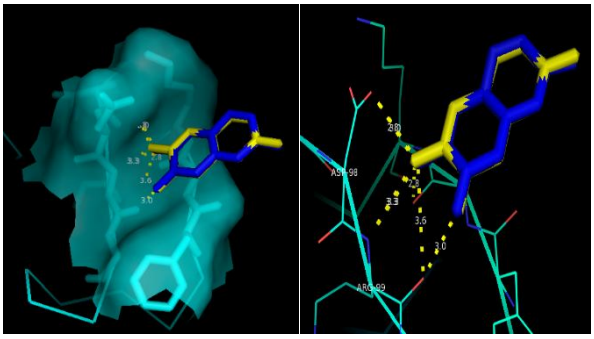

Fig 6b: Simulation with Esculetin

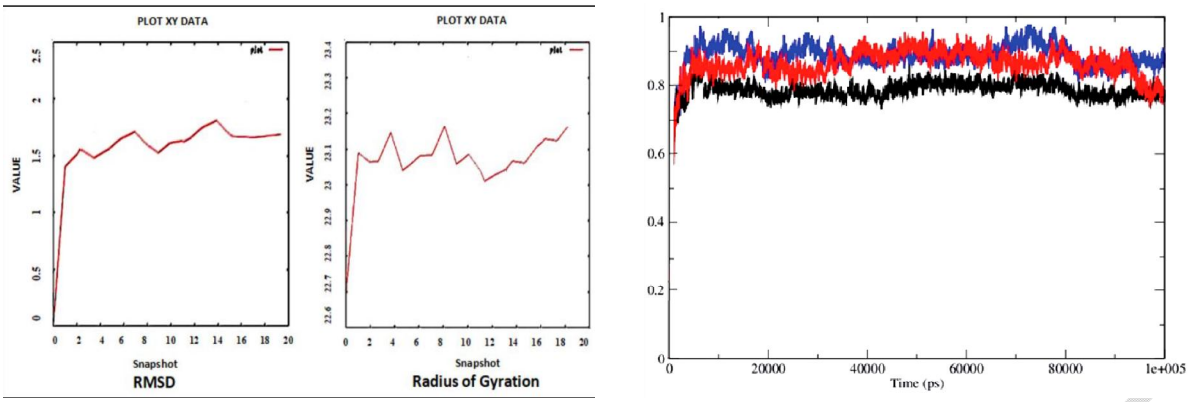

Fig 6c: Radius of Gyration and Retained value of RMSD all Ca atoms of the ZIKA protein and - ESCULETIN complex

The rise in the binding energy represents the possibility of a new type of binding in the energetically favorable region. The shift in the value leads to a stronger binding of ligands. This binding flexibility was due to the high hydrophilic nature of the ligands and binding site [31]. The docking of a molecule in a protein-binding domain was a robust technique to elucidate the appropriate binding pose between a numbers of predicted compound poses. Molecular recognition of viral protease-ligand complex at the atomic level was also determined by analyzing the bound conformation of the ligands [32]. The compounds were well positioned at the active site, fully occupying the binding pocket. The docking study led to the prediction that the molecules interact with the zika hydrolase binding domine through the residues ASP 98 and ARG 99. Intermolecular H-binding between ligand and protein plays a crucial role in stabilizing protein-ligand compounds [33]. The stability of the hydrogen bond network formed at room temperature was calculated throughout the 150 ns simulation period.

ADME, which stands for Absorption, Distribution, Metabolism, and Excretion, refers to the pharmacokinetic processes that a drug or substance undergoes within the body. Understanding the ADME profile of a compound was essential for assessing its potential therapeutic uses, as well as its toxicity. The physico-chemical properties indicated the molecular weight (524.43g/mol); number of heavy atoms (37); number of aromatic heavy atoms (16); number of rotatable bonds (6); number of hydrogen bond acceptors (14); number of hydrogen donors (8); molar refractivity (123.14). Water solubility showed 3.28e-01mg/Ml and soluble in nature. The pharmacokinetics exhibited the results on GI absorption was too low; no BBB permanent; with P-gp substrate and no CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibitors. The drug likeness bioavailability score showed 0.17.

The toxicity profile for the molecules revealed cytotoxicity and immunotoxicity. No hepatotoxicity. Carcinogenicity, mutagenicity, androgen receptor, estrogen receptor, peroxisome proliferator activated receptor gamma, heat shock factor response element, mitochondrial membrane potential. Further in-vivo studies fetch the exact scenario on the toxicity (Figure 7).

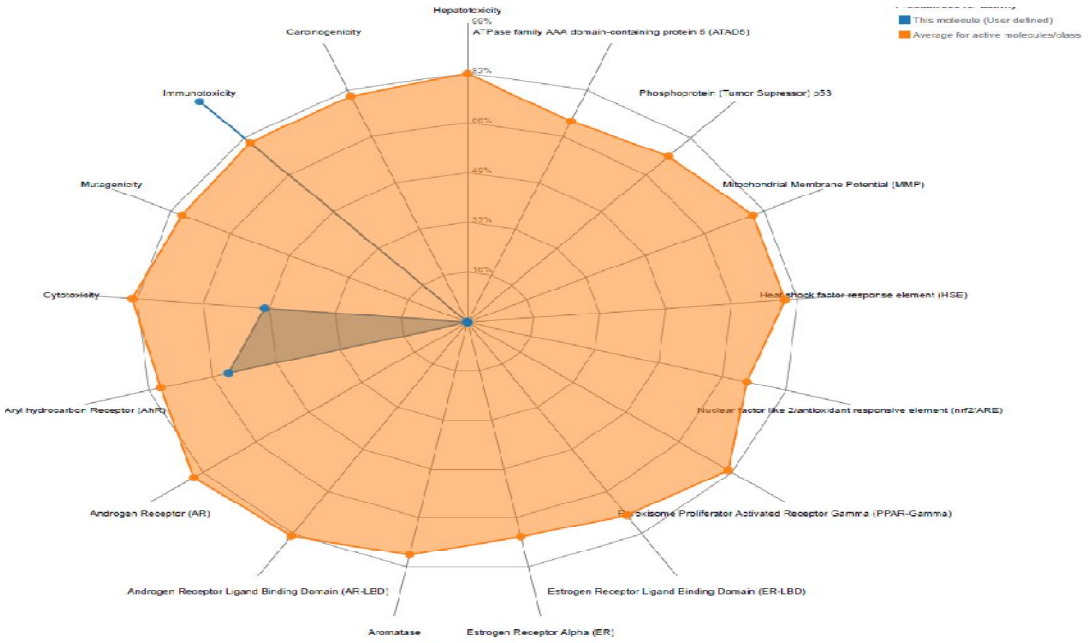

Fig 7 : Radar Chart for Toxicity

Specific impact on esculetin's absorption was uncertain. However, with specific in-vitro data on esculetin, was challenging to determine its distribution characteristics accurately. The specific isoforms involved and the resulting metabolites of esculetin remain largely unknown. The ADME properties and toxicity profile of Zika protein esculetin are well-established through computational approaches. Further research was needed to elucidate its ADME behavior and evaluate its toxicity, ensuring a comprehensive understanding of its safety and potential therapeutic applications

4. CONCLUSION

In the current study, esculetin showed better scores on the interaction parameters compared to procumbenetin molecule. However, both Procumbenetin and Esculetin bind with zika virus envelope region. The ZIKA envelope docked active region sites with Esculetin were on the region of GLY 109, ASP 98 and ARG 99. Hereby we conclude that these two compounds have the property to suppress the ZIKA virus and are stable and active with ZIKA virus. Molecular docking of Esculetin and Procumbenetin with ZIKV 5JHM, they bind with each other on active site pockets ASP98, ARG99, GLY100, TRP101, GLY102, ASN103, GLY104, CYS105, GLY106, LEU107, PHE108, GLY109. The interaction results are neutral, which means not too good interaction nor not too bad interaction. The molecular dynamics and stimulation studies demonstrated that the complex of esculetin with zika at the residues ASP-98 and ARG 99 was stable in the time of 150 ns. The toxicity profile for the molecules revealed the adverse effects on cytotoxicity and immunotoxicity. No hepatotoxicity. Carcinogenicity, mutagenicity, androgen receptor, estrogen receptor. Further need an in-vitro and in-vivo model to know the exact mechanism.

CONSENT

None to declare.

ETHICAL APPROVAL

None to declare.

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