Nirma Univ J Pharm Sci; 2021, 8(1&2) 59-70

ISSN 2348 –4012

ARTICLE

IN-SILICO PREDICTION OF THE NEURO-INFLAMMATION MECHANISM OF CAPPARIS SEPIARIA

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ABSTRACT

Neuroinflammation or neural dysfunction is a major risk factor that can initiate multiple intracellular signaling cascades to release different proinflammatory cytokines, chemokines and various reactive oxygen species leading to multiple neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. The adverse effects associated with the long-term use of conventional non-steroidal anti-inflammatory drugs is attracting herbal medicines as potential therapeutic candidates worldwide. Capparis sepiaria L (C. sepiaria) belonging to Capparaeae is therapeutic medicinal plant used to relieve various ailments including skin diseases, tumours, blood purification, toxaemia, snakebite and disease of the muscles. The objective of this study is to determine the pharmacokinetic and pharmacodynamic properties of C.sepiaria phyto-constituents as therapeutic molecules against neuro inflammation by using in-silico docking analysis and drug disposition. Six phyto- constituents identified from the leaves of C. sepiaria were docked against six pro-inflammatory markers of neuroinflammation followed by the prediction of their safety and bioavailability using GOLD 5.2, admetSAR softwareS respectively. The docking scores obtained were comparable and even better than the five standard marketed drugs. β-amyrin and quercetine present in the leaves of C.sepiaria showed highest fitness score with P38 MAP kinase, NF-kB, mTOR, TACE AChE, BChE

markers which are also the targets of the drugs like Galantamine, Donepazil and Rivastigmine. To our understanding this is the first study investgating the inhibitory effect of C. sepiaria in the neuroinflammation. Thus, Capparis sepiaria may prove to be a potential anti-neuroinflammatory agent and may be further explored as a potential therapeutic candidate for the management of neurodegenerative diseases.

Keywords: *Neuroinflammation, Alzheimer's disease, Capparis sepiaria, GOLD 5.2, admetSAR*

INTRODUCTION

Capparis sepiaria L. an important medicinal plant belonging to family, Capparidaceae is a prickly, evergreen shrub indeginously used as food, medicine and fuel. It is commonly known as "Himsara or Indian caper" and is traditionally used for treatment of asthma, allergy, jaundice, rheumatism and dysentery. It has also been reported to possess anti-inflammatory, analgesic, hepato-protective and anti-oxidant activity [1-4].

The medicinal potency presented by this herb is attributed to terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins that have several therapeutic effects. The leaves of C.sepiaria contain naturally occurring phytochemicals viz. α- amyrine, β-amyrine, quercetine, β-sitosterol, erythrodiol.

Neuroinflammation play a critical role in neurodegenerative diseases such as Alzheimer disease, Parkinson disease, traumatic brain injury, and stroke leading to their higher prevalence [5-7]. Previously the diseases that were largely believed to be based on dysregulation of neurotransmitter systems such as mood disorders/depression/anxiety, schizophrenia, and chronic pain now seem to have emerged due to inflammation of the nervous system [5].

The uncontrolled over activation of brain immune cells, especially microglial cells, lead to self-perpetuating inflammatory responses, accompanied by secretion of various inflammatory mediators like cytokines, reactive oxygen and nitrogen species, damaged protein products 'etc' [8-9]. Due to its complex multi-factorial mechanisms, therapeutic intervention in neurodegenerative diseases is a major challenge. Conventionally, anti-inflammatory drugs like Galantamine, Donepazil and Rivastigmine are generally prescribed in such condition, but, their long term usage is also associated with several side effects like gastrointestinal problems, dizziness, headache 'etc'[10]. Nowadays natural products like terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins have emerged promising therapeutics for neuroinflammation and neurodegenerative diseases because of their pleiotropic nature, and least side effects

There are several factors viz. aging, air pollution, oxidative stress, infection,

neurotoxins, tissue damage and mutation which resulted in to the microglial activation. The microglial activation resulted into the abrupt excessive expression of several pro-inflammatory mediators viz. IL-1β, Reactive Oxygen Species (ROS), TACE; COX-2; mTOR; NF-kB, p38 map kinase. Consequently, this abrupt excessive expression of these pro-inflammatory mediators resulted into the neuronal dysfunction and death. In case of chronic neuroinflammation excessive damage of the neuronal cells were take place which resulted into the neurodegenerative disorders like Alzheimer disease. Extensive literature search revealed that many synthetic and natural drugs were reported for the treatment of neuro-inflammation although treatment with these drugs showed some side effects as shown in the Table 1. Additionally, these reported drugs were not found to be so much effective against the neuro-inflammation.

Table 1: Marketed drugs for the treatment of neuro inflammation with their side effects

Marketed Drugs	Targets	Side Effects
Galantamine	Acetylcholinesterase, Neuronal acetylcholine receptor subunit α-7, Muscle nicotinic acetylcholine receptor, Cholinesterase	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Donepezil	Acetylcholinesterase, 5-hydroxytryptamine receptor 2_A, Cholinesterase, Tumor necrosis factor-inducible gene 6 protein, Interleukin-1 β, Nuclear factor NF-kB, NMDA receptor	Nausea, vomiting, loss of appetite, muscle cramps and increased frequency of bowel movements.
Rivastigmine	Acetylcholinesterase, Cholinesterase	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Memantine	5-hydroxytryptamine receptor 3_A, α-7 nicotinic cholinergic receptor subunit, Dopamine D_2 receptor, Glutamate receptor ionotropic, NMDA 1, Glutamate (NMDA) receptor (Protein Group), GABA(A) Receptor (Protein Group), Glycine receptors (Protein Group)	Headache, constipation, confusion and dizziness.

Hence in the present study, research efforts were made to identify the important chemical classes of the phyto constituents responsible for the attenuation of the neuronal inflammation. Detailed literature search indicated that broadly terpenoids, phenolic derivatives, alkaloids, glycosides, and steroidal saponins were found to be effective in neuroinflammation and neurodegenerative diseases. Additionally, few published scientific reports described the activity of naturally occurring phyto-constituents viz. α- amyrine, β-amyrine, quercetine, β-sitosterol, erythrodiol against the neuro-inflammation. Hence in the present research, the plant *Capparis sepiaria* was selected for the evaluation of its activity against the neuroinflammation as this plant consist of all the above-mentioned chemical constituents which are important for the activity against the neuroinflammation.

Capparis sepiaria L is a profusely branched hedge plant with slender prickly shrubs, zigzag stems. Traditionally, *C. sepiaria* is used as blood purifier, stomachic, tonic and appetizer. It2 s flowers, leaves and roots are used in cough and toxaemia and root powder is also used as a cure for the snakebite. It also possesses febrifuge properties and is used to treat skin diseases, tumours, inflammation and diseases of the muscles.

Phytoconstituents reported from the leaves of *Capparis sepiaria* consisted of the phytoconstituents 6 major are from the class of flavonoids and alkaloids (Figure 1). List of these phytoconstituents along with their structure are shown in Figure 1. There are many studies on the anti-neuroinflammatory activity of these individual phytochemicals reported from different plant sources individually. Thus, C. sepiaria is an ideal candidate for prediction potential anti neuoinflammatory drug source as it consists of all of the important reported phytocontituents. In the present study, an attempt has been made to identify and compare the inhibition of neuroinflammation between C. sepiaria phytoconstituents and the synthetic drugs Galantamine, Donepazil and Rivastigmine by targeting the key receptors in the neuroinflammation pathway to determine anti-neuroinflammatory potential of *Capparis sepiaria.*

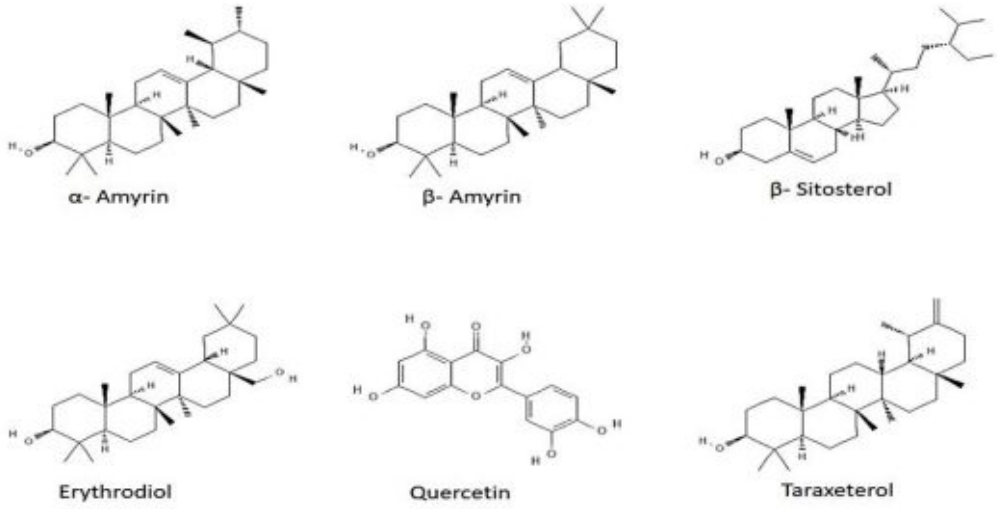

Figure 1. Important phyto-constituents of the leaves of *Capparis Sepiaria*

MATERIAL AND METHODS

In-silico ADMET prediction

The admetSAR is an online reliable freeware utilised widely for the prediction of Adsorption, Distribution, Metabolism and Excretion along with the acute oral toxicity of any compound. This online free tool stores the data based upon the previously reported library of the compounds and predicts the *in-silco* ADMET property of the desired compounds. In the present research SMILE format of the desired phytoconstituents were generated and ADMET properties of these phytoconstituents were predicted. Major advantage of this tool is of *in-silico* toxicity prediction in the form of class-I chemicals (LD50 d" 50mg/kg), class-II chemicals (500mg/kg > LD50 > 50mg/kg), class- III chemicals (5000mg/kg > LD50 > 500mg/kg) and class-IV chemicals (LD50 > 5000mg/kg). It also gives an idea about the carcinogenicity of the chemical.

Target Identification

Through the extensive literature search molecular targets for the neuro-inflammation were identified. Most of the literature revealed the exploration of molecular targets viz. COX-2, TACE, P38 MAP Kinase, NF-kB in the area of the neuroinflammation. Overall based upon literature search; COX-2, TACE, P38 MAP Kinase, NF-kB, AChE, & BChE were explored for the molecular docking study to identify the mechanism of action of the *Capparis Sepiaria*.

Molecular docking study

The co-crystallized structures of different proteins viz. COX-2 (PDB ID: 3LN1), TACE (PDB ID: 1ZXC), P38 MAP Kinase

(PDB ID: 3GCS), NF-kB (PDB ID: 5T8P), acetyl choline esterase (PDB ID: 2X8B) and butyl choline esterase (PDB ID: 2WIL) were downloaded from the protein data bank. Prior to molecular docking study, both, ligand and receptor were prepared. Water molecules and co-crystallized ligands were removed from the protein. The binding position of the co-crystallized ligand was considered to be the binding pocket of the protein. After the extraction of these co-crystalized ligand from the binding pocket, all the desired phytoconstituents were docked on their respective protein.

RESULTS AND DISCUSSION

In-Silico ADMET prediction

In-silico ADMET of the phytoconstituents of Capparis sepiaria were evaluated in terms of partition coefficient (AlogP), molecular weight, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), carcinogenicity, acute oral toxicity, human intestinal absorption (HIA) and water solubility (Log S) shown in Table 2.

Table 2. In-Silico ADMET prediction of phytoconstituents of leaves of Capparis Sepiaria

ADMET Properties of the chemical constituents	ALogP	Molecular Weight	HBA	HBD	Rotatable Bonds	Carcinogenicity	Acute Oral Toxicity	Human Intestinal Absorption	Water Solubility (LogS)
α-amyrine	8.02	426.73	1	1	0	No	Class-III	More than 30%	-4.251
β-amyrine	8.17	426.73	1	1	0	No	Class-III	More than 30%	-4.251
β-sitosterol	8.02	414.72	1	1	6	No	Class-I	More than 30%	-4.703
Erythrodiol	7.14	442.73	2	2	1	No	Class-III	More than 30%	-4.307
Quercetin	1.99	302.24	7	5	1	No	Class-II	More than 30%	-2.999
Taraxeterol	8.02	426.73	1	1	0	No	Class-III	More than 30%	-4.123

ALogP value usually indicates the hydrophilic capacity of the chemical component. Higher the ALogP lesser is the hydrophilcity of the given chemical component. Most of the *capparis sepiaria* phytoconstituents indicated the AlogP value more than 5 except quercetin which proved that these phytoconstituents are in hydrophobic in nature. This hydrophobic nature of phytoconstituents of *capparis sepiaria* responsible for the activity in neuroinflammation as these phytoconstituents able to cross the blood brain barrier (BBB).

Almost all the phytoconstituents of capparis sepiaria are small molecules and having molecular weight less than 500 which is one of the drug like property. Similarly as per the Lipinski rule of five molecules must possess hydrogen bond acceptor (HBA) less than 5 and hydrogen bond donor (HBD) not more than 10. In lieu of this all the phytoconstituents of capparis sepiaria following this Lipinski rule where all the phytoconstituents does not have the HBA and HBD more than 5 and 10, respectively.

All the phytoconstituents are non-carcinogenic in nature. In case of acute oral toxicity β-sitosterol and quercetin falls in the category of class-I and class-II chemicals, respectively. While on the other hand all the remaining phytoconstituents are falling in the class-III chemical clearly indicated the non-toxic nature of these phytoconstituents. In addition to this, most of the phytoconstituents revealed the human intestinal absorption more than 30% which proved the drug ability of all the phytoconstituents. As it is clearly observed from the hydrophobicity (ALogP) of the phytoconstituents that these components were less soluble in the water.

Molecular docking study

Various molecular targets viz. COX-2, TACE, P38 MAP Kinase, NF-kB, acetyl choline esterase (AChE) and butyl choline esterase (BChE) which are involved in the pathogenesis of the neuroinflammation were taken for the detailed molecular docking study as shown in the Table 3.

Table 3. Molecular docking score of the important phytoconstituents of leaves of *Capparis Sepiaria*

	Docking Score (GOLD FITNESS)						
Phyto Constituents	**P38 MAPK (PDB ID: 3GCS)**	**NFkB (PDB ID: 5T8P)**	**mTOR (PDB ID: 4JT6)**	**COX-2 (PDB ID: 3LN1)**	**TACE (PDB ID: 1ZXC)**	**AChE (PDB ID:2X8B)**	**BChE (PDB ID:2WIL)**
α-amyrine	98.62	118.2	121.58	116.79	110.03	102.08	105.7
β-amyrine	98.88	119.88	123.97	116.66	112.09	109.4	104.5
β-sitosterol	40.97	2.001	35.98	-4.08	42.86	30.34	39.42
erythrodiol	30.06	-106.03	15.27	-152.18	41.66	40.21	30.23
quercetin	52.56	60.11	58.99	59.27	62.22	113.2	114.5
taraxeterol	-93.44	-57.42	28.18	-156.19	26.94	32.21	33.45

β-amyrin was found to be best among all the other phytoconstituents of *capparis sepiaria* in terms of docking score as it shown highest GOLD Fitness against P38 MAP kinase, NF-kB, mTOR, TACE. In addition to this it showed same binding interactions as that of the respective co-crystallized ligand of various proteins shown in the Figure 2.

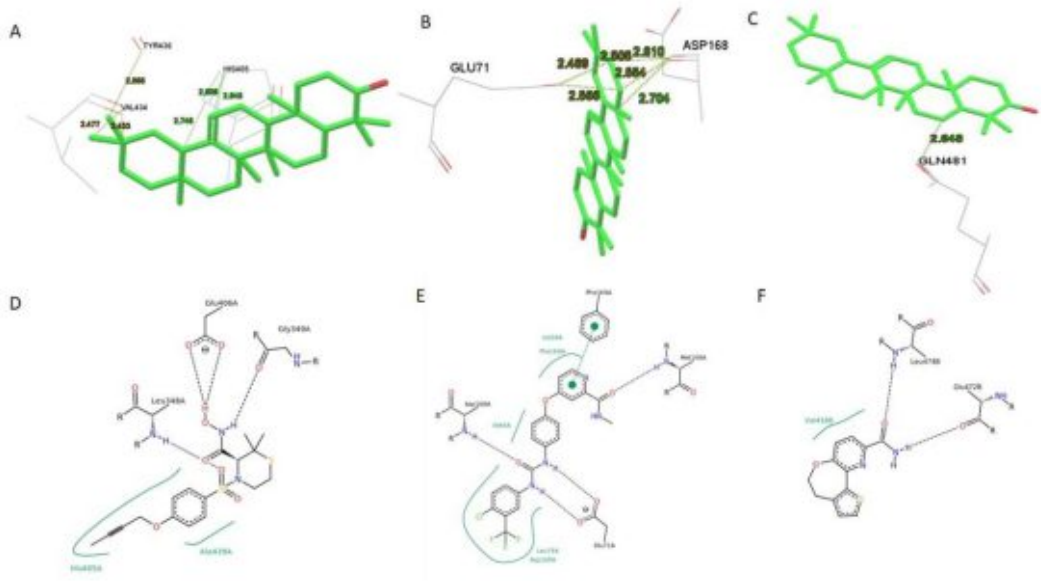

Figure 2: Molecular docking interactions of β-amyrin with (A) TACE (PDB ID: 1ZXC) (B) P38 MAP kinase (PDB ID: 3GCS) (C) NF kB (PDB ID: 5T8P). Docking interactions of standard co-crystallized ligands with the (D) TACE (PDB ID: 1ZXC) (E) P38 MAP kinase (PDB ID: 3GCS) (F) NF kB (PDB ID: 5T8P)

In case of COX-2, α-amyrine showed highest docking score. Overall molecular docking study revealed that α-amyrine and β-amyrine were acting predominantly on molecular targets which are involved in the pathogenesis of the neuro-inflammation. In case of TACE, double bond of β-amyrin at 12th position showed π-π stacking with His 405 which is considered to be the hydrophobic binding interaction which is similar to the binding interaction of the co-crystallized ligand of the TACE shown in the Figure 2(A). In case of P38 MAP kinase; β-amyrin showed binding interactions with the Glu71 and Asp168 which is similar to the binding interactions of the co-crystallized ligand of the P38 MAP Kinase shown in the Figure 2(B). In case of NF-kB, β-amyrin showed binding interactions with the Gln481 as shown in the Figure 2(C) although it didn't show any similar binding interactions as that of the standard co-crystallized ligand of the NF-kB. Molecular docking study revealed that β-amyrin was one of the important phyto constituent of the *capparis sepiaria*

which played an important role in the inhibition of the inflammation markers like NF-kB, TACE and P38 MAP kinase.

For this purpose the standard marketed drugs against the neuroinflammation were taken into the consideration. The marketed drugs Galantamine, Donepazil and Rivastigmine were showed interactions against the acetylcholine esterase and butylcholine esterase which is also evident in the literature. Based upon this phytoconstituents of *capparis sepiaria* were taken for the molecular docking study against the AChE and BChE. In case of AChE, molecular docking study revealed that Donepazil showed good docking score and binding interactions compared to the Galantamine and Rivastigmine. Additionally it also showed similar binding interaction as that of the co-crystallized ligand of the AChE. Interestingly the phytoconstituents of the *capparis sepiaria* also shown good binding interactions with the AChE. But among all the other phytoconstituents, quercetine showed highest docking score and similar binding interactions as that of the standard shown in the Figure 3.

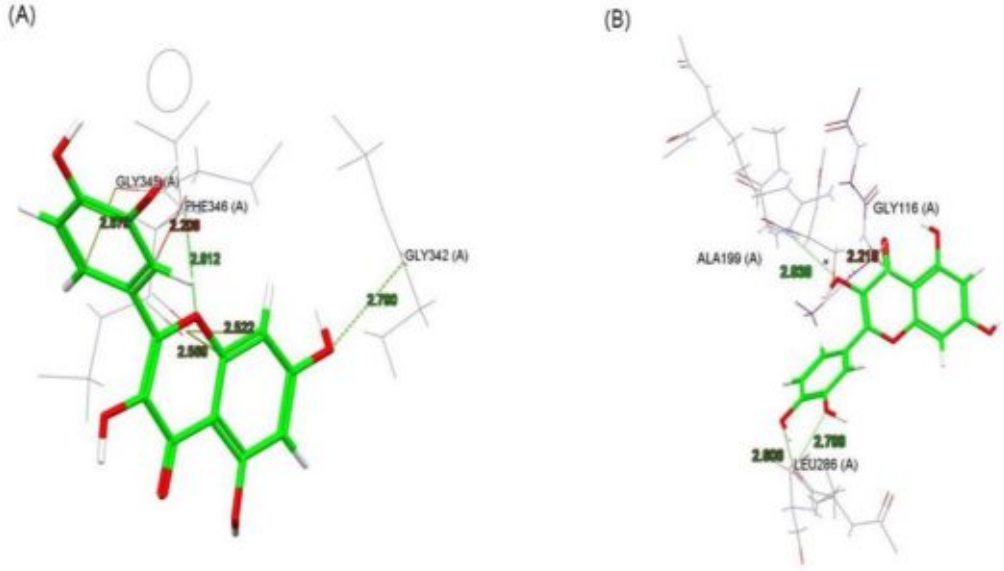

Figure 3: Molecular Docking Interactions of Quercetine with (A) Acetyl Choline Esterase (PDB ID: 2X8B) (B) Butyl Choline Esterase (PDB ID: 2WIL)

This outcome indicated that the leaf extract of *capparis sepiaria* might be active against the neuroinflammation as most of the phytoconstituents showed inhibition of the AChE. Similarly, in case of BChE, donepezil showed highest docking score compared to the other marketed drugs. In this docking study quercetine showed highest docking score compared to the donepezil.

Interestingly docking study against both AChE & BChE revealed that the quercetine is one of the important phytoconstituent of *Capparis Sepiaria* which might be playing an important role against the AChE and BChE and showing the activity of *capparis sepiaria* against the neuroinflammation.

CONCLUSION

Neuroinflammation is the major problem associated with the Alzheimer's disease where older people are most affected. Available drugs for the treatment of neuroinflammation having some side effects & toxic effects on the different organs. In addition to this these drugs were found to be in ineffective for the long-term treatment of the neuroinflmmation. Hence in the present research phytoconstituents of *Capparis sepiaria* leaf were explored against the molecular targets of neuroinflammation where viz. α-amyrine, β-amyrine, and quercetin were found to be most potent and safe at molecular level for the treatment of Neuroinflammation. Hence our study concludes that the leaf extract of *Capparis sepiaria* would be effective against the neuroinflammation as it comprises α-amyrine, β-amyrine, and quercetin. Additionally, our study also suggests that components like α-amyrine, β-amyrine, and quercetin could be used separately for the new drug development as active pharmaceutical ingredient.

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