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RESEARCH ARTICLE

In Silico Studies Revealed Multiple Neurological Targets for the Antidepressant Molecule Ursolic Acid

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Abstract: ***Background*:** Ursolic acid, a bioactive pentacyclic triterpenoid had been evaluated for its interaction with the neurological targets associated with antidepressant drugs. Current study was to mechanistically analyze the probable site of action for ursolic acid on the target proteins.

***Methods*:** Ursolic acid has been docked with monoamine oxidase isoforms: MAO-A and MAO-B, LeuT (homologue of SERT, NET, DAT) and Human C-terminal CAP1 using GRIP docking methodology.

***Results*:** Results revealed its non-selective antidepressant action with strong binding affinity towards LeuT and MAO-A proteins, which was found to be comparable with the reference ligands like chlorgyline, clomipramine, sertraline and deprenyl/selegiline.

***Conclusion*:** Significant binding affinity of ursolic acid was seen with MAO-A, which indicated its potential role in other neurological disorders, for example, Alzheimer's disease and Parkinson disease besides depression.

Keywords: Ursolic acid, MAO-A inhibitor, MAO-B inhibitor, adenylyl cylase inhibitor, LeuT inhibitor, docking studies.

1. INTRODUCTION

Ursolic acid, UA (Fig. **1**), an isolate of *Rosmarinus officinalis*, is a widely reported phytoconstituent [1-21]. The antidepressant attribute of this pentacyclic triterpenoid, UA had been evaluated by Machado and co-workers wherein it was found to exert its effect *via* monoaminergic and dopaminergic systems [1, 22]. These studies were performed by using *in vitro* and *in vivo* assay techniques where *in vivo* methodologies adopted for antidepressant evaluation was the tail suspension test (TST) and the forced swim test (FST). UA has a strong potential to reduce the immobility time in the TST and FST at effective concentration of 0.01 mg/kg, p.o. and 10 mg/kg, p.o. respectively. The anti-depressant like effect by UA (0.1 mg/kg, p.o.) in the TST test was similar to the effect produced by standard bupropion administered at a dose 100 folds higher *i.e.* 10 mg/kg, p.o. Hence, UA is at par when compared with standard bupropion [1, 22].

Significant inhibition of MAO-A and MAO-B, can effortlessly up-regulate monoamines like dopamine, epinephrine, nor-epinephrine, tyramine and other monoamine analogs [23]. Human C-terminal CAP1 is reported to be depressogenic with the key role in the modulation of actin G-F conversion [24]. Leucine transporter (LeuT) is a homolog of Serotonin Transporter (SERT), Norepinephrine transporter (NET) and Dopamine transporter (DAT) and shares 20-25% sequence identity and 40-45% structural similarity with these human neurotransmitter transporters [25]. Docking with these proteins can help us to analyze the molecular interactions in the mechanism of action of UA as antidepressant and can give us the comparative results with standard and co-crystallized drugs deprenyl, chlorgyline, sertraline and clomipramine.

To the best of our knowledge, the exact mechanism of action of UA as an antidepressant is still not explored well. Hence, the aim of the current study has been to dock UA with the above mentioned proteins and analyze its site of action on the neurological targets.

2. MATERIALS & METHODS

2.1. Proteins

LeuT in complex with sertraline (3GWU), crystal structure analysis of LeuT complexed with L-leucine, sodium, and clomipramine (2Q6H), human monoamine oxidase A in complex with chlorgyline, crystal form A (2BXR), human monoamine oxidase B in complex with deprenyl (2BYB) and crystal structure of the Human C-terminal CAP1 (1K8F) were taken from RSCB-PDB [26-30]. Protein structures taken from the RCSB were cleaned and energy minimized using the tools available in Vlife MDS 4.6.

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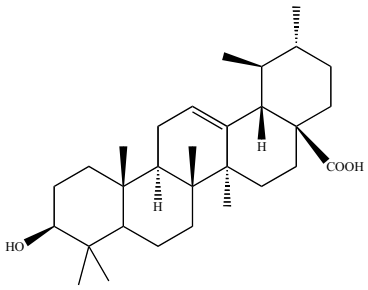

Fig. (1). Structure of pentacyclic triterpenoid, Ursolic Acid.

2.2. Docking Studies

Vlife MDS 4.6 (Vlife Sciences, India), a robust software having all the necessary simulation modules, was employed for the docking studies. The structure of UA under study has been drawn using Chem Draw Ultra and then exported to VLife MDS 4.6 for its conversion into 3D form by using default conversion procedure. Energy minimization was done using MMFF force field and charge [31]. This is to record here that the structure of reference ligands which were extracted from the target proteins will be used as such without any structure cleaning and will be used primarily for tracking the active site. Accuracy and validation of docking algorithm was done by repeating docking studies with the redrawn structures of reference ligands and compared the dock score of co-crytallized form with the redrawn form. Molecular docking energy evaluations are usually carried out with the help of scoring function like dock score, PLP score, potential of mean force (PMF) score, steric and electrostatic score, *etc*. The PLP function is incorporated by the Vlife MDS software in the GRIP docking method which calculates the ligand - receptor binding affinity in terms of the PLP score. The PLP score is designed to enable flexible docking of ligands to perform a full conformational and positional search within a rigid binding site. UA was docked into the active site of 3GWU, 2Q6H, 2BXR, 2BYB and 1K8F that can be obtained in the co-crystallized form with sertraline, clomipramine, chlorgyline, deprenyl respectively and cavity no. 1 in case of 1K8F. Along with the active sites, UA was also docked with all the cavities present with the target proteins. The parameters fixed for docking simulation were like this; number of placements: 100, rotation angle: 10^{o}, exhaustive method, ligand-wise results: 10, scoring function: dock score. By rotation angle, ligand would be rotated inside the receptor cavity to generate different ligand poses inside the receptor cavity. By placements, the method will check all the 100 possible placements into the active site pocket and will result out best placements out of 100. After docking simulation, the best docked conformer of UA and reference ligand were then checked for their interactions with targeted proteins like hydrogen bonding, hydrophobic, pi-staking/ aromatic, charge and vanderwaal's interactions [32-37].

2.3. Pharmacokinetic & Toxicological Studies

ADMET studies were performed using StarDrop software of Optibrium Ltd and integrated Derek Nexus module of LHASA Ltd [38-40]. UA was subjected for the ADMET profiling.

3. RESULTS & DISCUSSION

Though UA seemed to have potential docking affinity towards all the tested proteins like Human C-terminal CAP1, MAO-A, MAO-B and LeuT, its affinity towards MAO-A was found to be particularly high (Table **1**; -61.95 dock score). Relative affinity of UA for the target proteins were in the following order: MAO-B (2BYB)< Human C-Terminal CAP1s (1K8F)< LeuT (2Q6H)< LeuT (3GWU)< MAO-A (2BXR). Dock score of UA and the reference co-crystallized drugs with co-crystallized active site and all other cavities of target proteins are presented in Table **1**.

In case of Human C-Terminal CAP1s, there was no co-crystallized ligand and even there was only one cavity. In cavity 1, UA had vanderwaal's interactions with His417A, Glu436A, Asn438A, Pro452A, Glu455A, Ile473A, Ile347D, Gly365D, Lys366D, Asp383D, Asp384D and Gly403D while displaying hydrophobic interactions with Glu436A, Pro452A, Glu455A, Ile473A, Gly475A, Leu347D, Gly365D, Lys366D, Asp383D, Asp384D and Gly403D amino acid residues of 1K8F. In case of LeuT (2Q6H), UA had vanderwaal's interactions with Leu29A, Arg30A, Val33A, Gln34A, Gly318A, Ala319A, Phe320A, Asn321A, Lys398A, Asp401A and Asp404A while hydrophobic interactions were recorded with Leu29A, Arg30A, Val33A, Ile111A, Gly318A, Ala319A, Phe320A, Leu322A, Leu400A, Asp401A and Phe405A amino acid residues of 2Q6H. Along with this, carbonyl oxygen of UA was having charge interaction with Arg30A at bond distance of 4.533 angstrom (Fig. **2**). Co-crystallized structure of clomipramine was also docked in 2Q6H. Clomipramine had vanderwaal's interactions with Leu25A, Leu29A, Arg30A, Val33A, Gln34A, Tyr107A, Tyr108A, Ile111A, Phe253A, Ala319A, Phe320A, Lys398A, Leu400A, Asp401A and Asp404A while having hydrophobic interactions with Leu29A, Arg30A, Gln34A, Ile111A, Ala319A, Leu400A and Asp404A of 2Q6H. Along with this, nitrogen of azepine ring of clomipramine showed hydrogen bonding with Arg30A at bond distance of 2.019 angstrom while the nitrogen of tertiary amine group of side chain had charge interaction with Asp401A at bond distance of 3.101 angstrom (Fig. **3**). Since UA showed highest affinity with Cavity 8, so protein ligand interactions in Cavity 8 were also studied. UA had vanderwaal's interactions with Leu173A, Met176A, Phe177A, Val180A, Leu380A, Trp381A, Ala383A, Ala384A and Phe387A while having hydrophobic interactions with Leu173A, Met176A, Phe177A, Val180A, Leu380A, Trp381A, Ala383A, Ala384A and Phe387A residues of Cavity 8 of 2Q6H. Unlike the site which was tracked by co-crystallized ligand, UA did not have hydrogen bonding and charge interactions with amino acid residues of cavity 8 of 2Q6H. In case of LeuT (3GWU), UA showed vanderwaal's interactions with Arg30A, Val33A, Gln34A, Ala319A, Leu400A, Asp401A, Glu402A, Asp404A, Phe405A and Ile475A while having hydrophobic interactions with Arg30A, Val33A, Gln34A, Ala319A, Leu400A, Asp401A, Glu402A, Asp404A, Phe405A, Ile475A, Thr479A residues of 3GWU. Along with this, carbonyl oxygen of UA had charge interaction with Arg30A at a bond distance of 2.698 angstrom (Fig. **4**). Sertraline, which was the co-crystallized ligand in 3GWU, was also evaluated for its interaction

Table 1. Docking studies of ursolic acid and the reference ligands with various target proteins, a – PLP score.

Proteins under Docking Study	Dock Score [a]										
	Ursolic Acid										Reference Ligand in Co-crystallized Site
	Ref. Ligand Tracked Active Site	Cavities									
		1	2	3	4	5	6	7	8	9	
Leucine Transporter LeuT complexed with L-leucine, sodium and Clomipramine (2Q6H)- Ref. Ligand Clomipramine	-50.95	-51.45	-30.96	-48.57	-51.37	-49.61	-23.66	-48.28	-57.93	-37.27	-84.52
Human MAO-A in complex with Chlorgyline crystal form A(2BXR)- Ref. Ligand Chlorgyline	-61.95	-50.47	105.94	-49.99	NA	NA	NA	NA	NA	NA	-62.97
Human C-terminal CAP1s(1K8F)	NA	-54.72	NA	NA	NA	NA	NA	NA	NA	NA	NA
Leucine Transporter LeuT in complex with Sertraline (3GWU)- Ref. Ligand Sertraline	-45.17	-57.77	-36.81	-21.82	-41.19	-48.83	-59.66	-48.25	NA	NA	-62.41
Human MAO-B in complex with Deprenyl (2BYB)- Ref. Ligand Deprenyl	-31.09	8.02	447.99	-38.58	-48.09	-46.62	-27.86	NA	NA	NA	-67.99

NA: Not Available.

with its binding site. In case of Sertraline, vanderwaal's interactions were observed with Leu25A, Leu29A, Arg30A, Tyr107A, Tyr108A, Ile111A, Phe253A, Ala319A, Phe320A, Leu400A, Asp401A, Asp404A and Thr409A while hydrophobic interactions were seen with Arg30A, Ile111A, Ala319A, Phe405A and Thr409A residues of 3GWU. In addition to this, nitrogen of the secondary amine group of Sertraline had hydrogen bonding with Asp404A at bond distance of 2.34 angstrom (Fig. **5**). But UA had highest affinity with cavity 6 of 3GWU, so protein ligand interactions in cavity 6 were also studied. UA showed vanderwaal's interactions with Met176A, Phe177A, Val180A, Leu380A, Trp381A, Ala383A, Ala384A and Phe387A while having hydrophobic interactions with Leu173A, Met176A, Phe177A, Val180A, Leu380A, Trp381A, Ala383A, Ala384A and Phe387A residues of cavity 6 of 3GWU. In case of 2BXR, UA had the highest binding affinity with the binding site which was tracked by the co-crystallized ligand Chlorgyline. UA dispayed vanderwaal's interactions with Tyr69A, Val93A, Leu97A, Arg109A, Ile180A, Asn181A, Ile207A, Phe208A, Ser209A, Val210A, Glu216A, Cys323A, Ile325A, Ile335A, Leu337A, Phe352A, Tyr407A and Tyr444A while hydrophobic interactions were noted with Tyr69A, Val93A, Leu97A, Ile180A, Asn181A, Ile207A, Phe208A, Ser209A, Val210A, Glu216A, Cys323A, Ile325A, Ile335A and Leu337A residues of 2BXR. Chlorgyline had vanderwaal's interactions with Tyr69A, Ile180A, Glu216A, Cys323A, Ile335A, Leu337A, Met350A, Phe352A, Tyr407A and Tyr444A while showing hydrophobic interactions with Tyr69A, Ile180A, Glu216A, Ile335A, Leu337A and Tyr407A residues of 2BXR. In case of 2BYB, UA had vanderwaal's interactions with Gly57A, Gly58A, Ser59A, Tyr60A, Phe168A, Leu171A, Cys172A, Ile198A, Ile199A, Gln206A, Lys296A, Tyr326A, Phe343A, Tyr398A and Tyr435A while having hydrophobic interactions with Gly58A, Ser59A, Tyr60A, Leu171A, Cys172A, Ile198A, Ile199A, Gln206A, Lys296A and Tyr398A residues. Deprenyl, which was co-crystallized in 2BYB, was also evaluated for its interaction with its binding site. Deprenyl showed vanderwaal's interactions with Phe168A, Leu171A, Cys172A, Ile199A, Gln206A, Tyr326A, Phe343A, Tyr398A and Tyr435A while it's hydrophobic interactions were seen with residues: Leu171A, Ile199A and Gln206A of 2BYB. Besides this, UA had highest affinity towards cavity 4 of MAO-B, hence protein - ligand interactions in Cavity 4 were also studied. UA had vanderwaal's interactions with Gln163A, Thr166A, Leu167A, Asn170A, Asp318A, Gly319A, Glu320A, Ala325A, His347A and Lys348A while having hydrophobic interactions with Gln163A, Thr166A, Leu167A, Gly319A, Glu320A, Ala325A and Lys348A residues of cavity 4 of 2BYB.

Various parameters, which were included in the ADMET studies, are LogS, logS @ pH7.4, LogP, LogD, 2C9 pKi, hERG pIC50, BBB log ([brain]:[blood]), BBB category, HIA category, P-gp category, 2D6 affinity category, PPB90 category as pharmacokinetic features and Mitochondrial dysfunction, Thyroid toxicity, Photoallergenicity, Skin sensitization, Occupational asthma, Respiratory sensitization, Developmental toxicity, Teratogenicity, Bradycardia, Adrenal gland toxicity, Blood in urine, Mutagenicity *in vitro*, Mutagenicity *in vivo*, Photomutagenicity *in vitro*, alpha-2-mu-Globulin nephropathy, Anaphylaxis, Bladder urothelial hyperplasia, Cardiotoxicity, Cerebral oedema, Chloracne,

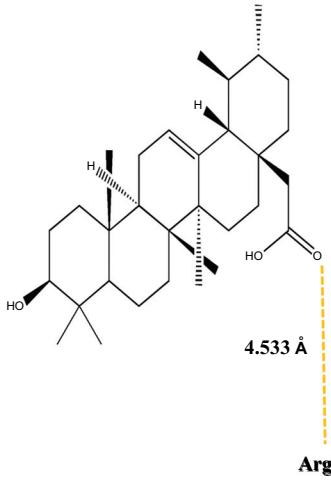

Fig. (2). Charge Interactions of Ursolic Acid with 2Q6H. Dotted line (yellow) represents charge interaction (View online version for colour graphics).

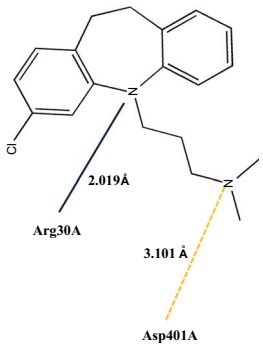

Fig. (3). Hydrogen Bonding and Charge Interactions of clomipramine with 2Q6H. Continuous line (blue) represents hydrogen bonding while dotted line (yellow) represents charge interaction (View online version for colour graphics).

Cholinesterase inhibition, Cumulative effect on white cell count and immunology, Cyanide-type effects, High acute toxicity, Methaemoglobinaemia, Nephrotoxicity, Neurotoxicity, Oestrogenicity, Peroxisome proliferation, Phospholipidosis, Phototoxicity, Kidney disorders, Bone marrow toxicity, Splenotoxicity, Irritation (of the gastrointestinal tract), Irritation (of the respiratory tract), Irritation (of the skin), Lachrymation, HERG channel inhibition *in vitro*, Hepatotoxicity, Non specific genotoxicity *in vitro*, Non specific genotoxicity *in vivo*, Photo-induced non-specific genotoxicity *in vitro*, Photo-induced non-specific genotoxicity *in vivo*, Chromosome damage *in vitro*, Chromosome damage *in vivo*, Photo-induced chromosome damage *in vitro*, Carcinogenicity, Photocarcinogenicity, Pulmonary toxicity, Uncoupler of oxidative phosphorylation, Irritation (of the eye), Testicular toxicity, Ocular toxicity, Kidney function-related toxicity, Bladder disorders and Urolithiasis as toxicological features [38-40]. Results are presented in Table **2**. The data clearly represented the specificity of structural features of UA as choice for treatment of CNS disorders. The reason is that its significant lipophilicity will allow it to cross the blood brain barrier which is actually the major hurdle while targeting the CNS disorders. hERG pIC50 value of UA (4.505) was compared with that of reference ligands taken in the current study *i.e.* chlorgyline (5.438), sertraline (5.364), clompiramine (6.203) and deprenyl (5.195). It was noted that the UA had lowest hERG pIC50 value, rendering it to be a more appropriate molecule. Further, toxicological profiling also predicted UA as safe molecule with no indication of toxicity against 57 toxicological endpoints.

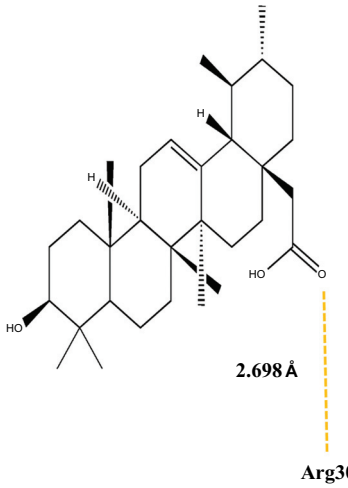

Fig. (4). Charge Interaction of Ursolic Acid with 3GWU. Dotted line (yellow) represents charge interaction (View online version for colour graphics).

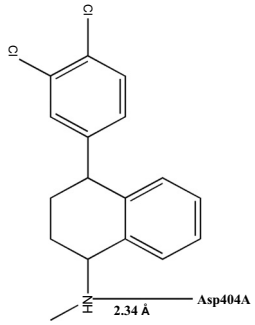

Fig. (5). Hydrogen bonding of sertraline with 3GWU. Continuous line (blue) represents hydrogen bonding (View online version for colour graphics).

Upon analysis of the results contained in Tables **1** and **2**, it had been observed that UA had significant binding affinity towards the target proteins when compared with the reference drugs but displayed comparatively weaker forms of interactions except in case of LeuT (2Q6H and 3GWU). Existence of charge interactions in case of LeuT will somehow support the hypothesis laid down by Machado and co-workers [1, 22]. Only in case of MAO-A, UA had shown much higher binding affinity than the reference ligand chlorgyline for the binding site which was tracked by this co-crystallized ligand itself. MAO-A preferentially catalyzes the oxidative deamination of dopamine, norepinephrine and serotonin. The MAO-A inhibitors were shown to be effective in treating Parkinson's disease and possibly Alzheimer's disease (AD), with concomitant extension of life span [41-43]. Thus, on the basis of the experiments performed by Machado and coworkers [1, 22] and our *in silico* studies, it emerged that UA might prove to be a useful drug in the

Table 2. ADME and toxicological profiling of ursolic acid.

ID	Ursolic Acid	ID	Ursolic Acid	ID	Ursolic Acid
logS	0.7184	Mutagenicity *in vitro*	Inactive	Irritation (of the gastrointestinal tract)	No report
logS @ pH7.4	0.7127	Mutagenicity *in vivo*	No report	Irritation (of the respiratory tract)	No report
logP	5.452	Photomutagenicity *in vitro*	No report	Irritation (of the skin)	No report
logD	2.675	alpha-2-mu-Globulin nephropathy	No report	Lachrymation	No report
2C9 pKi	5.089	Anaphylaxis	No report	HERG channel inhibition *in vitro*	No report
hERG pIC50	4.505	Bladder urothelial hyperplasia	No report	Hepatotoxicity	No report
BBB log([brain]:[blood])	-0.8101	Cardiotoxicity	No report	Non specific genotoxicity *in vitro*	No report
BBB category	+	Cerebral oedema	No report	Non specific genotoxicity *in vivo*	No report
HIA category	+	Chloracne	No report	Photo-induced non-specific genotoxicity *in vitro*	No report
P-gp category	No	Cholinesterase inhibition	No report	Photo-induced non-specific genotoxicity *in vivo*	No report
2D6 affinity category	Very High	Cumulative effect on white cell count and immunology	No report	Chromosome damage *in vitro*	No report
PPB90 category	High	Cyanide-type effects	No report	Chromosome damage *in vivo*	No report
Mitochondrial dysfunction	No report	High acute toxicity	No report	Photo-induced chromosome damage *in vitro*	No report
Thyroid toxicity	No report	Methaemoglobinaemia	No report	Carcinogenicity	No report
Photoallergenicity	No report	Nephrotoxicity	No report	Photocarcinogenicity	No report
Skin sensitisation	Equivocal	Neurotoxicity	No report	Pulmonary toxicity	No report
Occupational asthma	No report	Oestrogenicity	No report	Uncoupler of oxidative phosphorylation	No report
Respiratory sensitisation	No report	Peroxisome proliferation	No report	Irritation (of the eye)	No report
Developmental toxicity	No report	Phospholipidosis	No report	Testicular toxicity	No report
Teratogenicity	No report	Phototoxicity	No report	Ocular toxicity	No report
Bradycardia	No report	Kidney disorders	No report	Kidney function-related toxicity	No report
Adrenal gland toxicity	No report	Bone marrow toxicity	No report	Bladder disorders	No report
Blood in urine	No report	Splenotoxicity	No report	Urolithiasis	No report

treatment of Parkinson and Alzeihmer's disease, which needs to be explored further in much depth.

CONCLUSION

To mechanistically analyze the active site of UA to prove its therapeutic potential as an antidepressant, five proteins covering MAO-A, MAO-B, LeuT & Human C-terminal CAP1 receptors were used along with cocrystallized reference ligands like sertraline, deprenyl, chlorgyline and clomipramine. Results are surprisingly amazing especially for MAO-A, where UA docked significantly with MAO-A, with a dock score of -61.95. Docking results affirmed the role of UA as antidepressant but at the same time raised a strong possibility for UA to be anti-Alzeihmer's and anti-Parkinson agent. Thus, there is a need to assess the potential of ursolic acid for both the neurological disorders in detail.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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