Original Article

Anti-Alzheimer's Activity of Compounds from the Methanolic Extract of *Lawsonia inermis* Seeds: *In vivo* and *in silico* Molecular Docking Studies

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ABSTRACT

Background: Alzheimer's disease (AD) hallmark feature is neurodegeneration due to the accumulation of β-amyloid plaques and the formation of neurofibrillary tangles in the aged brain. The prevalence of AD in humans is doubled for every two decades and is expected to reach 74.7 million worldwide by 2030. Numerous treatment approaches for AD are currently available but success rate is very limited, therefore novel medicines that minimize AD progression are urgently needed. **Methods:** In this study, *in vivo* experiments were performed to test the anti-Alzheimer's property of MELIS on male albino rats under D-galactose induced AD. Estimation of ACh content and AChE activity in cerebral cortex was done in different groups of rats. In addition, in *in silico* analysis, molecular docking of MELIS compounds against AChE was performed in Auto dock vina software tool. **Results:** MELIS exhibited Anti-alzheimer's properties in rats by modulating ACh and AChE. Further, *in silico* molecular docking studies found top 10 MELIS compounds such as Dihydromyricetin, Quercitrin, Zearalenone, Leupeptin, Moricizinesulfone, Lecanoric acid, Sulfamerazine, 3-Deoxyguanosine, N-(3-indolylacetyl)-lisoleucine and Trimethoprim exhibited anti-acetylcholinesterase (AChE) property through showing good binding affinity by forming hydrogen bond interactions with active site amino acids. **Conclusion:** It is conclude from the results that MELIS compounds exhibit anti-Alzheimer property by modulating the ACh content, AChE activity and interacting to AChE active site amino acids. Therefore MELIS could be preferred source of active compounds for isolation and identification of new drugs for the AD treatment.

Key words: Alzheimer's Disease, AChE, ACh, Cholinergic systems, Docking studies and *Lawsonia inermis*.

INTRODUCTION

Alzheimer's Disease (AD) is characterized by forfeiture in behavioural domains such as attention, perceptual and constructive skills, language, memory, orientation, functional skills and problem solving.[1] Consequently, more than 15 million people are suffering globally from AD due to aging and hence medicinally their recovery becomes hard.[2] Till now, AD patients have not been effectively treated but their life span had been increased by using certain drugs.[3] The neuropathological features of the AD are formation of extracellular β amyloid plaques and neurofibrillary intracellular tangles in the brain.[4] A wide range of evidences indicate that the existence of many other pathological symptoms of different neurodegenerative diseases and aging are contributing for increased number of AD patients.[5] Deficiency of a key neurotransmitter, viz. Acetylcholine (ACh), which transmits the signal between the neurons has been linked to AD. Acetylcholine esterase (AChE), associated with the cholinergic transmission plays a vital role in depletion of acetylcholine through rapid hydrolysis. Hence, AChE is considered as a therapeutic target enzyme to develop cholinergic inhibitors to treat AD.[6] Another reason for selecting AChE as target

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is its association with other pathological features of AD such as β-amyloid precursor plaques and neurofibrillary tangles, which collectively induce a series of complex events in progression of AD.[7] Identification of novel inhibitors against AChE was started since a few decades by pharmacological companies and research institutions. The main reason for their failure in producing AChE inhibitors in animal models and cell lines are involvement of a huge expenditure, no reproducible results, time consuming, manpower.[8] To overcome all these problems, a new stream of biology that bioinformatics, which deals with computer applications on biological molecules has been introduced. Structure-based virtual screening is a part of bioinformatics which explores the potential inhibitors from subset of millions of compounds stored in various databases against therapeutic target protein/enzymes of interest computationally within short period of time. [9,10]

As per report of the AD international 2015, neurodegenerative diseases are doubling for every two decades due to aging and dementia, which will recapture 74.7 million by 2030. Treatment for neurodegenerative disorder such as AD is currently very limited, new medications that reduce AD progression are urgently needed. Ethnopharmacological screening of natural plants against diseases are a primary step towards the production of new therapeutic medicines.[11,12] Naturally available sources have been used to isolate FDA approved drugs such as Galantamine and Rivastigmine as anti-Alzheimeric agents for mild to moderate AD treatment.[13]

Selection and screening of inhibitors for any target is the most important aspect in structure- based virtual screening because resulted inhibitors will be used for further studies in animals. In this connection, selection of naturally available compounds of medicinal plant origin are good choice because they do not exert any adverse effects.[14,15] On this basis, in the present study, we selected the compounds from medicinal plant, *Lawsonia inermis,* commonly known as Henna for screening against AChE. Henna has many medicinal properties such as anti-Pyretic, anti-Fertility, anti-Arthritic, anti-Cancer, anti-Tubercular, anti-Diabetic, anti-Inflammatory and anti-Alzheimers.[16]

MATERIALS AND METHODS

Chemicals

All the chemicals used in the present investigation were Analar grade (AR) and Solvents obtained from the following scientific companies: Sigma (USA), Fisher (USA), Merck (India), Himedia (India), TCI (China), Molychem (India) and SRL (India).

Preparation of the plant extract

Seeds of the plant, *L. inermis*, duly confirmed by a taxonomist Dr. Madhava Chetty, have been collected in Sri Venkateswara University campus, Tirupati and its surrounding areas. Initially, they were cleaned with tap water followed by distilled water. Seeds, dried under shade was powdered with scientific grinder, soaked in methanol (100%), kept in dark for 3 days with occasional stirring using glass rod. After 3 days, the seed compounds dissolved in methanol were filtered orderly with muslin cloth, non-absorbent cotton and finally with what man No. 1 filter paper. The filtrate was further concentrated using rotary evaporator and the methanolic extract so obtained was preserved in refrigerator for further usage in various experiments.

In vivo studies

Induction of Alzheimer's Disease

The anti-AChE activity of MELIS was tested in three months old wistar strain male albino rats (160 ± 20) purchased from Sri venkateswara enterprises, Bangalore. For acclimatization, they were housed in cages in an animal house by maintaining 25-28°C temperature, 50-55% humidity and regular light/ dark condition for ten days. The experimental rats were fed with standard pellet diet and water *ad libitum*. The general health status of experimental rats during acclimatization was monitored every day. All the protocols followed in this study were approved by the ethical committee of Sri Venkateswara University (No.09 (i)/a/ CPCSEA/ IAEC/SVU/ZOOL/KY/ Dt. 08-07-2012).

Grouping of animals

Treatment : 60 days

Group I : (Normal control-NC): Rats received normal saline through oral gavage

Group II : (Disease control-DC): Rats received D-galactose (120 mg/kg) through intraperitoneal injection

Group III : (Plant control-PC) -Rats received only MELIS 150 mg/kg through oral gavage

Group IV : (Plant treatment-PT)- AD rats which received MELIS through oral gavage

Tissue isolation

For biochemical estimations, all rats of six groups were sacrificed on the 60th day of treatment by cervical dislocation. The brain was isolated immediately and placed on a chilled glass plate. The selected brain region viz. cerebral cortex was separated by following standard anatomical protocol.[17] Further, it was frozen in liquid nitrogen (-180°C) and then stored at -40°C until further

use. At the time of biochemical analysis, the cerebral cortex tissue was thawed.

Acetyl choline content

ACh content in the cerebral cortex was estimated by the method of Metcalf (1951).[18] Cerebral cortex was weighed accurately by using the scientific balance and transferred into test tubes, was placed in a boiling water bath for 5 min to terminate the AChE activity and also to release the bounded ACh. The tissue was homogenized in 1ml of distilled water. 1ml of alkaline hydroxylamine hydrochloride and 1ml of 50 % hydrochloric acid solutions were added to the obtained homogenate. The contents were mixed thoroughly and centrifuged at 1000 rpm/min. Supernatant was taken and 0.5 ml of 0.37 M ferric chloride was added. The resulted brown color was read in UV-Vis spectrophotometer at 540nm against a blank (all contents where tissue homogenate was replaced with distilled water). The ACh content was expressed as μ moles of ACh/gm wet weight of tissue.

AChE Activity

AChE activity was estimated by the Ellmans method (1961).[19] 2% (w/v) homogenate of cerebral cortex was prepared in 0.25 M ice cold sucrose solution. To start the reaction, 100 μl of homogenate was added to the reaction mixture containing 3 ml of phosphate buffer (pH 8.0), 20 μM of substrate (0.075 M) and 100 μM of dithiobistrinitrobenzene (DTNB, 0.01M). The contents were incubated at 37°C for 15 min. The resulted colour was read at 412 nm in a spectrophotometer against a reagent blank containing 3.0 ml of phosphate buffer (pH 8.0), 20 μM of substrate (0.075 M) and 100 μM of dithiobistrinitrobenzene (DTNB, 0.01 M).The enzyme activity was expressed as μM of ACh hydrolyzed/mg protein/hour.

Statistical analysis

All the experiments in triplicate were performed and the findings were expressed in mean ± SD. One-way ANOVA has been used to test the significance of differences between experimental groups followed by multiple range tests by Tukey and Dunnet. SPSS.20v was used to conduct statistical analysis. The data at $p<0.05$ was regarded as a significant difference.

In silico studies

Selection of therapeutic target protein

Experimentally elucidated X-ray diffraction of AChE with resolution 2.697 Å was downloaded from protein data bank (PDB). The PDB id for this structure is 5FPQ.[20] This PDB structure was directly uploaded into protein preparation wizard of SPDB viewer to get optimized A chain along with energy minimization. All hetero atoms and water molecules present in the protein chain were deleted using Argus lab software. Further, partial charges were added to both the hetero groups and hydrogen atoms.

Molecular docking studies

3D coordinate of AChE was imported into auto dock vina software and converted it into pdbqt, a vina compatible format. All compounds, identified in MELIS by using HR-LC/Q-TOF/MS (see supplementary material Figure 1 and Table 1) were uploaded into auto dock vina wizard in a 2D format. All 2D co-ordinates, subjected to energy minimization were then converted into pdbqt format. Compounds and protein were simulated for docking according to genetic algorithm by setting up eight poses for each compound in the active site of protein. Among 8 poses, the pose which contains least energy with good binding affinity was selected for further analysis.

Prediction of molecular properties

Docking studies conducted on the top 10 compounds of MELIS have been subjected to Lipinski rule of 5 predictions such as molecular weight, cLogP, hydrogen bond donor count, hydrogen bond acceptor count using molinspiration.[21] Also, the adverse effects of compounds such as mutagenicity, tumarogenicity, reproductive toxicity and irritant were measured with OSIRIS server.[22]

RESULTS

In AD-induced experimental rats, the ACh content in the cerebral cortex region of the brain was significantly decreased ($p<0.05$) when compared with normal control rats (Figure 1). However, AD rats treated with MELIS for 60 days resulted in increment of ACh content. There were no significant changes observed in MELIS alone supplemented rats. On the contrary, the AChE activity was significantly ($p<0.05$) increased in AD rats, whereas its activity was significantly decreased in AD rats treated with MELIS. No changes were observed in the AChE activity of both MELIS control rats and normal control rats.

Molecular docking studies explored the binding mode of MELIS compounds with active site amino acids of AChE, as follows: Table 1 represents the docking outcomes such as binding affinity, hydrogen bond interaction, bond distances and bond angles of top

Table 1: Docking results of best 10 compounds of MELIS (PC ID: Pubchem ID).

S. No.	Compound Name and Pubchem ID	Hydrogen bonding	BondDistance (Å)	Bond Angle (°)	Binding energy (k cal/mole)
1	Dihydromyricetin PC ID161557	Asp_{74} N-H-------------H_{20}-O_{19} Trp_{86} C-O--------------H_{22}-O_{21} Asn_{87} CG-OD_{1}--------H_{20}-O_{19} His_{447} C-O------------H_{28}-O_{27}	2.2 2.1 2.4 1.9	123.3 120.3 168.0 124.8	-10.4
2	Quercitrin PC ID 5280459	Trp_{86} CA-C-O---------H_{20}-O_{19} Trp_{86} CA-C-O---------H_{22}-O_{21} Gly_{121} CA-N-H--------O_{31}-C_{27} Gly_{122} CA-N-H--------O_{31}-C_{27} Glu_{202} CG-CD-OE_{1}---H_{34}-O_{33} Ser_{203}CB-OG-HG-----O_{31}-C_{27} Phe_{295} CA-N-H-------H_{38}-O_{37}	2.2 2.2 2.3 2.1 2.6 2.1 2.1	120.3 93.5 138.6 124.4 125.1 131.0 118.9	-10.0
3	Zearalenone PC ID 5281576	Trp_{86} CA-C-O----------H_{24}-O_{23}	2.2	102.0	-9.5
4	Leupeptin PC ID 72429	Trp_{86}CA-C-O-----------H_{0}-N_{28} Trp_{86}CA-C-O ----------H_{33}-N_{30} Gly_{121}-C-N-H----------O_{4}-C_{3} Gly_{122}C-N-H------------O_{4}-C_{3} Ser_{203} CB-OG-HG-----O_{4}-C_{3} Tyr_{337}-CE_{2}-CZ-O------H_{23}-N_{22}	2.0 2.3 2.7 2.5 2.6 1.9	110.0 136.8 121.2 104.6 135.1 121.6	-9.1
5	Moricizinesulfone PC ID 3083448	Asp_{87}-CB-CG-OD_{1}----N_{20}-C_{19} Gly_{121}-C-N-H-----------O_{2}-S_{0} Gly_{122}-C-N-H-----------O_{2}-S_{0} Ser_{203}-CB-OG-GH-----H_{1}-S_{0} His_{447}-CD_{2}-NE_{2}-HD_{1}--O_{1}-S_{0}	3.5 2.3 2.8 2.1 2.5	134.2 105.5 109.5 140.0 113.9	-9.0
6	Lecanoric acid PC ID 99613	Glu_{202}-CG-CD-OE_{2}----H_{11}-O_{10}	1.8	106.9	-9.0
7	Sulfamerazine PC ID 5325	Tyr_{72} CA-C-O----------H_{21}-N_{20} Asn_{87}-CB-C-OD_{1}------H_{22}-N_{20} $Tyr_{337}$$CE_{2}$-CZ-O-------$H_{1}$-$N_{5}$	2.1 1.9 1.9	147.3 145.7 103.4	-8.9
8	3-Deoxyguanosine PC ID 165138	Tyr_{72}-CA-C-O ----------H_{1}-N_{6} Trp_{86}-CA-C-O-----------H_{15}-N_{7} Trp_{86}-CA-C-O-----------H_{13}-N_{4} Tyr_{124}-CE_{2}-CZ-O-------H_{1}-N_{5}	2.4 2.3 2.6 2.3	135.5 123.3 109.4 138.3	-8.9
9	N-(3-Indolylacetyl)- Lisoleucine PC ID 644226	Tyr_{337}-CE_{1}-CZ-O-------H_{3}-N_{1}	2.3	109.5	-8.8
10	Trimethoprim PC ID 5578	Gln_{71} CG-CD-OE_{1}--------H_{9}-N_{4} Tyr_{72}-CAC-O--------------H_{10}-N_{4} Tyr_{72}-CAC-O--------------H_{0}-N_{2} Trp_{86}-CA-C-O-------------H_{1}-N_{3} Tyr_{124}-CE_{2}-CZ-O---------H_{24}-N_{24}	2.6 2.0 2.3 2.1 2.1	136.8 109.4 124.7 119.1 97.4	-8.4
11	Galantamine (Standard) PC ID 9651	Glu_{201}-CD-OE_{1}-----------O_{18}-C_{11} Ser_{203} OG-HG-------------O_{20}-C_{21} Tyr_{337}-CZ-OH------------N_{1}-C_{9} His_{447}-NE_{2}-HD_{1}----------O_{20}-C_{21}	2.6 2.2 2.9 2.7	145.1 124.9 139.3 124.4	-10.0

ten compounds of MELIS with AChE protein. The docking interaction visualizations of all compounds with AChE were represented in Figure 2.

It has been predicted that the Dihydromyricetin compound exhibited good binding affinity (-10.4 kcal/mole) among all docked compounds in the active site of AChE. The complex, Dihydromyricetin and AChE showed that the ligand molecule interacted with His_{447}, Trp_{86}, Asn_{87} and Asp_{74} through hydrogen bond interaction. Another atom H_{20} of Dihydromyricetin was formed hydrogen bond with hydrogen atom of His_{447} amino acid. It was also noted that H_{22} atom of compound formed another hydrogen bond interaction with Oxygen atom of $Trp_{86.}$ OD_1 atom of Asn_{87} was formed hydrogen bond with H_{20} atom of compound. HIS_{447} showed hydrogen bond interaction with H_{28} atom of compound. The bond angles and distances of the above said interaction were represented in Table 2.

After Dihydromyricetin, Quercitrin was shown to have -10.0kcal/mole binding affinity to AChE 's active site amino acids and occupied second place. Two atoms H_{20} and H_{22} of Quercitrin were interacted with H of Trp_{86}. Two amino acids such as Gly_{121} and Gly_{122}were interacted with O_{31} atom of compound by hydrogen bond. H atom of compound got interacted with OE_1 of Glu_{202}. O_{31} atom of compound acted as hydrogen bond donor and it formed bond with H of of Ser_{203}. Finally, H atom of Phe_{295} interacted with H_{38} of compound. Similarly, Zearalenone compound exhibited binding affinity score -9.5 kcal/mole with single hydrogen bond interaction. This interaction formed in between O atom of Trp_{86} and H_{24} of compound. Next to this compound, Leupeptin exhibited binding affinity -9.1 kcal/mole with the active site of the AChE through six hydrogen bond interactions. They include O atom of Trp_{86} formed two hydrogen bond interactions with the H_0 and H_{33}. O_4 of Leupeptin formed two hydrogen bond interactions with the H atoms of Gly_{121} and Gly_{122} of protein. Besides, gama H of Ser_{203} amino acid interacted with O_4 of compound. Finally, O atom of Tyr_{337} exhibited hydrogen bond interaction with H_{23} atom of compound.

Another top fifth compound, Moricizinesulfone was showed binding affinity -9.0 kcal/mole. Compound was unveiled five hydrogen bond interactions with active site amino acids of protein. OD_1 of Asp_{87} amino acid made a hydrogen bond interaction with N_{20} atom of compound. Also amino acids Gly_{121} and Gly_{122} interacted with same atom that oxygen of compound. Next, gama hydrogen of Ser_{203} showed interaction with

Table 2: Prediction of lipinski rule of five and adverse effects of compounds of MELIS (MW: Molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; Mut: mutagenicity; Tum: tumorigenesis; Rep Tox: reproductive toxicity; Irri: irritant)

S.No.	Compound	MW (g/mol)	cLogP	HBA	HBD	Mut	Tum	Rep Tox	Irri
1	Dihydromyricetin PC ID161557	320.25	0.6122	8	6	None	None	None	None
2	Quercitrin PC ID 5280459	448.379	0.5798	11	7	None	None	None	None
3	Zearalenone PC ID 5281576	318.368	3.888	5	2	None	None	None	None
4	Leupeptin PC ID 72429	426.560	-0.4337	10	5	None	None	None	None
5	Moricizinesulfone PC ID 3083448	459.522	2.3847	9	1	None	None	High	None
6	Lecanoric acid PC ID 99613	318.280	2.2259	7	4	None	None	None	None
7	Sulfamerazine PMD ID 5325	264.308	0.4856	6	2	None	None	High	None
8	3-Deoxyguanosine PC ID 165138	267.244	-1.9543	9	4	None	None	None	None
9	N-(3-Indolylacetyl)-Lisoleucine PC ID 644226	288.346	1.7523	5	3	None	None	None	None
10	Trimethoprim PC ID 5578	290.322	1.0686	7	2	None	None	None	None
11	Galantamine (Standard) PC ID 9651	287.358	1.1901	4	1	None	None	None	None

H_1 of compound. HD_1 of His_{447} amino acid formed hydrogen bond interaction with O_1 of compound.

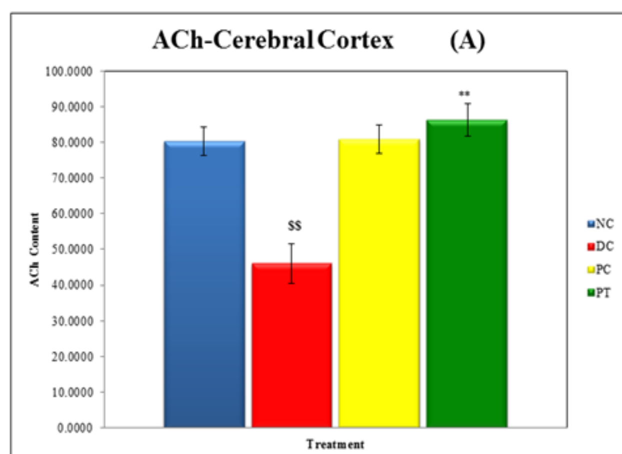
With regard to Lecanoric acid, it exhibited binding affinity -9.0 kcal/mole with single hydrogen bond interaction. Amino acid Glu_{202} only formed the hydrogen bond interaction with H_{11}atom of compound. Next in the order was Sulfamerazine, which showed the binding affinity -8.9kcal/mole. Tyr_{72} amino acid interacted with H_{21} atom of compound by giving hydrogen bond donor in the form of O atom. OD_1 of Asn_{87} interacted with H_{22} of compound whereas O atom of Tyr_{337} showed bonding with H_1 of compound. Further, 3-Deoxyguanosine also exhibited binding affinity -8.9 kcal/mole with active site of AChE protein. Tyr_{72} amino acid interacted with H_1 of compound. Oxygen of Trp_{86} formed 2 hydrogen bonds with H_{13} and H_{15} of compound. Fourth hydrogen bond formed in between oxygen atom of Tyr_{124} and H_1 of 3-Deoxyguanosine.

Following the above, the compound, N-(3-Indolylacetyl)-Lisoleucine exhibited -8.8 kcal/mole binding affinity by forming single hydrogen bond interaction. Tyr_{337} was involved in hydrogen bond interaction with H_3 atom of compound. Finally, Trimethoprim compound also showed -8.4 kcal/mole binding affinity score which was least among all above mentioned compounds. It was interacted to 4 amino acids with 5 hydrogen bonding interactions which include OE_1 atom of Gln_{71}formed hydrogen bonding with H_9 of compound. Oxygen atom of Tyr_{72} formed two hydrogen bond interactions with H_0 and H_{10} atoms of compound. H_1 of compound interacted with oxygen atom of Trp_{86}. At end, H_{24} atom of Trimethoprim exhibited bonding with Tyr_{124} amino acid.

Molecular property prediction of MELIS compounds revealed that all compounds were qualified Lipinski rule of five. Further, these compounds also not showed any adverse effects except the two viz. Sulfamerazine and Moricizinesulfone which exhibited reproductive toxicity.

DISCUSSION

In the present study, we have screened the anti-Alzheimer's properties of the compounds from MELIS against D-galactose induced AD. The observations revealed that MELIS has significantly increased the ACh content in the cerebral cortex region of rat



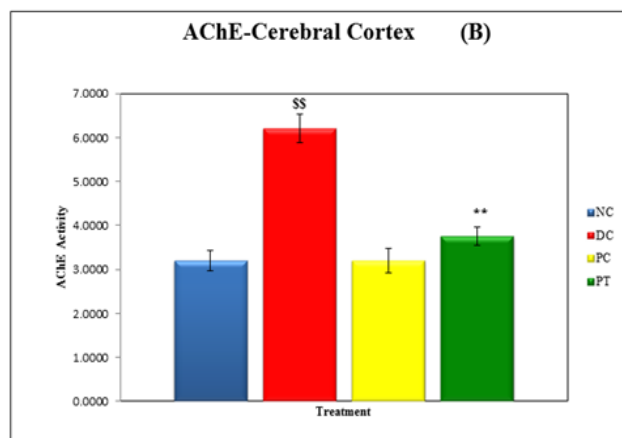


Figure 1: Effect of MELIS on ACh content (A) and AChE activity (B) in cerebral cortex region of AD –induced rats Brain.

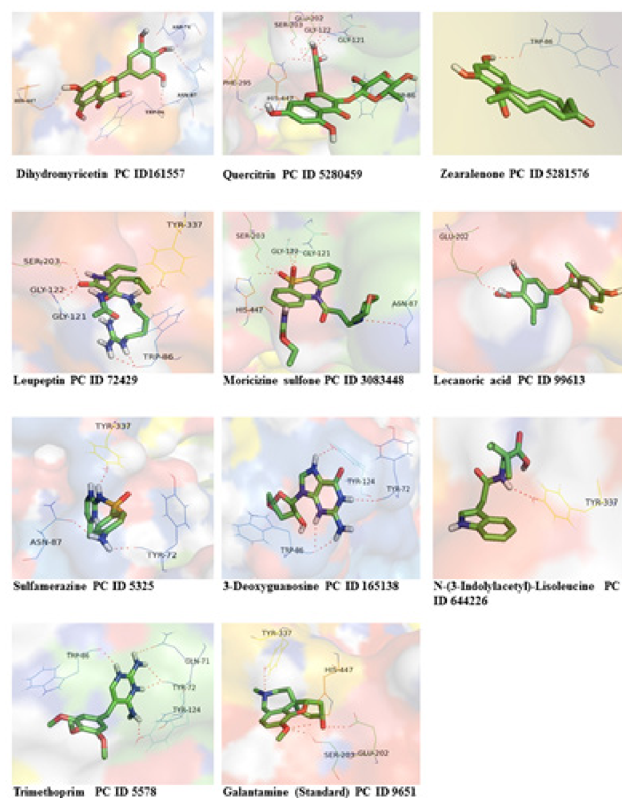


Figure 2: Molecular interactions of MELIS in active site of AChE where thick sticks denote compounds of MELIS whereas thin sticks denote interacted amino acids of AChE.

brain under AD-induced conditions. Additionally, MELIS also decreased the activity levels of AChE in AD rats. AChE is a vital enzyme involved in rapid hydrolysis of neurotransmitter ACh in the central and peripheral nervous system. Increased AChE activity reduces the content of ACh which causes cognitive deficits.[23] Recent findings have revealed that AChE is also involved in non-cholinergic mechanism intricate in neurodegeneration. Amyloid β peptide aggregation has been accelerated by increasing AChE activity which results in neurodegeneration.[24] In view of these observations, it is therefore inferred that, MELIS prevented neurodegeneration and amyloid beta plaques formation in cerebral cortex by inhibiting AChE activity. Further, persistent amount of ACh was also observed in MELIS-treated AD rats due to inhibition of AChE, which improves the neuronal transmission.

One of biological streams such as bioinformatics helps scientists to identify new potential drugs against therapeutic targets of diseases with low-cost, effective and least man power. In the present study, we performed molecular docking studies against AChE protein with MELIS compounds. The structural elucidation of AChE revealed that it has 20 Å deep active site gorge.[25] The catalytic site present at the gorge's base having amino acids His_{447}, Glu_{334} and Ser_{203} called catalytic triad. Along with that, a second site is also present at peripheral region extending beyond Tyr_{337} at the catalytic/peripheral site edge to the entrance of the gorge with numerous aromatic side chains. Kinetic and thermodynamic studies have demonstrated that inhibitors can interfere with either or both of the two AChE binding sites.[26] In the present study, Quercitrin, Leupeptin and Moricizinesulfone have formed hydrogen bond interaction with Ser_{203} of AChE. It is very clear that these compounds would inhibit AChE activity through this hydrogen bond interaction with $Ser_{203,}$ one of the traid. Interestingly, a potential AChE inhibitor Galantamine was also bound to the same active site through four hydrogen bond interactions with Glu_{201}, Ser_{203}, Tyr_{337} and His_{447} amino acids.[27] Similarly, MELIS compounds such as Leupeptin, Sulfamerazine, N-(3-Indolylacetyl)-Lisoleucine were formed hydrogen bond interaction with Tyr_{337} as like Galantamine. Additionally, Dihydromyricetin and Moricizinesulfone were showed hydrogen bond interaction with His_{447} as Galantamine. An interesting aspect to note here is that though Dihydromyricetin and Galantamine bound in the same active site with hydrogen bond interactions with same amino acids, binding affinity rank of Dihydromyricetin (-10.4 kcal/mole) occupied much greater position than Galantamine (-10.0 kcal/mole). Therefore, Dihydromyricetin could be considered as a good AChE inhibitor than Galantamine. This derives a strong support from a recent study wherein Dihydromyricetin was reported as anti-Alzheimer's agent in AD induced rats.[28] Another study revealed that (-)-Huperzine A, also an AChE inhibitor shows its inhibitory activity by forming hydrogen bond interaction with Tyr_{133} and Tyr_{337}, also hydrophobic interaction with Trp_{86} amino acid.[27] In our docking results, interestingly, MELIS compounds viz. Dihydromyricetin, Leupeptin, 3-Deoxyguanosine and N-(3-Indolylacetyl)-lisoleucine have formed hydrogen bond interaction with Trp_{86} similar to (-)-Huperzine A. This observation further lend support to the AChE inhibitory activity of MELIS compounds. Along with these important hydrogen bond interactions, many compounds of henna have formed hydrogen bond interaction with other active site amino acids of AChE which increase the inhibitory potentiality of these compounds thus clearly stating that MELIS contained compounds which act as AChE inhibitors by binding to the active site amino acids.

In the current results, all compounds screened were fulfilled the lipinski rule of five except Moricizinesulfone and Sulfamerazine, hence these compounds might be supplemented through non-oral manner. Lipinski *et al.* (1997)[29] predicted the chemical descriptors for FDA approved oral drugs and finally they found that they contained molecular weight <500, hydrogen bond donors <5, hydrogen bond acceptor <10 and octanol water partition coefficient (clog P) <5. Non-oral drugs,violating the lipinski rule of five indicate that drugs which follow this rule can be used through orally.[30,31] Any drug which is prescribed for the treatment, they should not contain adverse effects like mutagenicity, tumarogenicity, reproductive effect and irritant. In our study, most of the compounds not exhibited any adverse effects except Sulfamerazine and Moricizinesulfone with reproductive toxicity. Here are clear evidences in the present investigation that the best ranked compounds might be the reason for the AChE inhibition in D-gal induced AD in *in vivo.*

CONCLUSION

Based on the findings of this study, it is conclude that MELIS exhibits anti-alzheimer activity in *in vivo* by modulating the content of ACh and AChE activity. *In silico* molecular docking studies revealed that among 63 compounds of MELIS, Dihydromyricetin, Quercitrin, Zearalenone, Leupeptin, Moricizinesulfone, Lecanoric acid, Sulfamerazine, 3-Deoxyguanosine, N-(3-Indolylacetyl)-Lisoleucine and Trimethoprim acted as

AChE inhibitors by showing good binding affinity with active site amino acids of AChE. It is therefore evidence that possible regulation of AChE by MELIS in both *in vivo* and *in silico* could be the choice to take MELIS as the key source of isolation for new and innovative drug compounds.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

ABBREVIATIONS

MELIS: Methanolic Extract of *Lawsonia inermis*; **AD:** Alzheimer's Disease; **AChE:** Acetylcholinesterase; **ACh:** Acetylcholine; **DTNB:** Dithiobistrinitrobenzene; **PDB:** Protein Data Bank; **HR-LC/Q-TOF/MS:** High Resolution-Liquid Chromatography/Quadrupole Time-of-Flight/ Mass Spectrometer; **FDA:** Food and Drug Administration.

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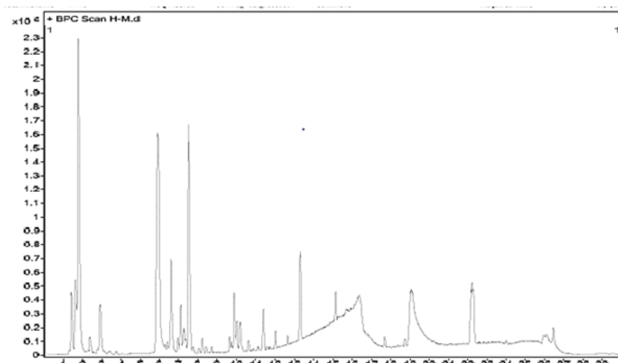
Supplementary Table 1: Phytochemical constituents were detected in MELIS by HR-LC/Q-TOF/MS.

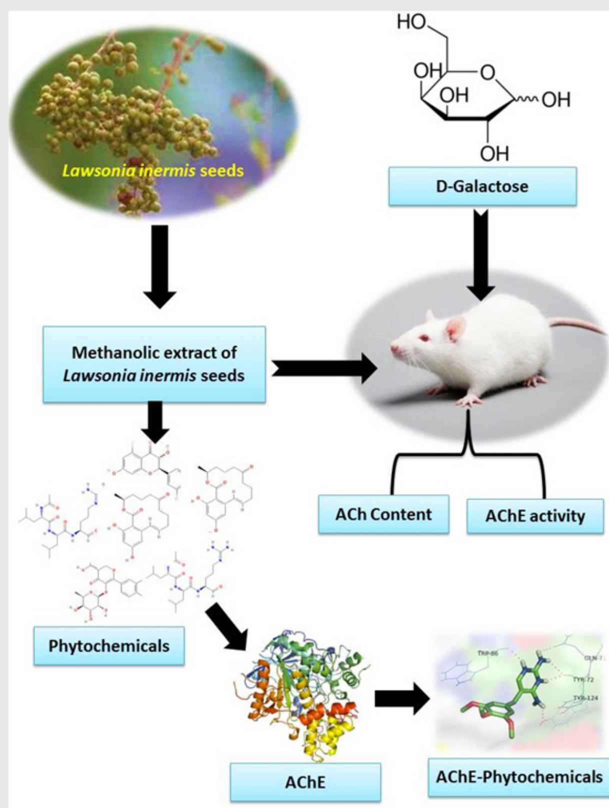
S.No	Retention Time (RT)	Mass	Name	Molecular formula
1	1.433	174.1127	D-Arginine	$C_6H_{14}N_4O_2$
2	1.761	224.188	1,3-Dicyclohexylurea	$C_{13}H_{24}N_2O$
3	1.801	155.0591	Ethosuximide M5	$C_7H_9NO_3$
4	1.854	342.118	Maltose	$C_{12}H_{22}O_{11}$
5	1.868	162.114	Carnitine	$C_7H_{16}NO_3$
6	2.399	290.1364	Trimethoprim	$C_{14}H_{18}N_4O_3$
7	2.4	225.0857	Acyclovir	$C_8H_{11}N_5O_3$
8	2.426	267.0968	3-Deoxyguanosine	$C_{10}H_{13}N_5O_4$
9	2.927	147.0542	O-Acetylserine	$C_5H_9NO_4$
10	5.92	148.0744	Pantoic acid	$C_6H_{12}O_4$
11	5.922	332.1106	Pro Asn Cys	$C_{12}H_{20}N_4O_5S$
12	5.923	327.1544	Ala His Thr	$C_{13}H_{21}N_5O_5$
13	5.923	288.1457	N-(3-Indolylacetyl)-Lisoleucine	$C_{16}H_{20}N_2O_3$
14	6.299	459.1433	Moricizine sulfone	$C_{22}H_{25}N_3O_6S$
15	6.421	346.1255	Aucubin	$C_{15}H_{22}O_9$
16	6.485	401.1604	Tyr Gly Tyr	$C_{20}H_{23}N_3O_6$
17	6.608	401.1622	Meropenem Metabolite (2HPyrrole-2-acetic acid, 5-carboxy-4-[[(3S,5S)-5-[(dimethylamino) carbonyl]-3-	$C_{17}H_{27}N_3O_6S$
18	6.94	306.133	Trimethoprim 1-N-oxide	$C_{14}H_{18}N_4O_4$
19	6.962	418.1866	Asp Val Trp	$C_{20}H_{26}N_4O_6$
20	7.103	184.0383	Everninic Acid	$C_8H_8O_5$
21	7.133	387.2491	Lys Gln Leu	$C_{17}H_{33}N_5O_5$
22	7.237	286.1095	His Met	$C_{11}H_{18}N_4O_3S$
23	7.241	156.0431	Iretol	C7 H8 O4
24	7.262	398.1266	Cys Tyr Asn	$C_{16}H_{22}N_4O_6S$
25	7.321	449.2861	Gentamicin C1a	$C_{19}H_{39}N_5O_7$
26	7.368	168.043	Vanillic acid	$C_8H_8O_4$
27	7.479	475.3021	Netilmicin	$C_{21}H_{41}N_5O_7$
28	7.522	188.0695	Ethyl Oxalacetate	$C_8H_{12}O_5$
29	7.535	324.2093	9a-Fluoro-Bhydroxyandrosterone	$C_{19}H_{29}FO_3$
30	7.62	538.3471	GPCho(16:0/2:0[U])	$C_{26}H_{53}NO_8P$
31	7.744	564.3627	GPCho(18:1(9Z)/2:0[U])	$C_{28}H_{55}NO_8P$
32	8.226	320.0546	Dihydromyricetin	$C_{15}H_{12}O_8$
33	8.435	342.2321	Val Lys Pro	$C_{16}H_{30}N_4O_4$
34	8.711	448.1028	Quercitrin	$C_{21}H_{20}O_{11}$
35	9.639	273.2681	C16 Sphinganine	$C_{16}H_{35}NO_2$
36	9.736	287.2838	C17 Sphinganine	$C_{17}H_{37}NO_2$
37	10.186	264.0678	Sulfamerazine	$C_{11}H_{12}N_4O_2S$
38	11.127	318.075	Lecanoric acid	$C_{16}H_{14}O_7$
39	11.375	180.0428	Acetylsalicylic acid (aspirin)	$C_9H_8O_4$
40	11.398	567.2931	Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$
41	11.398	568.2773	Leukotriene F4	$C_{28}H_{44}N_2O_8S$
42	11.574	284.333	Cetrimonium	$C_{19}H_{42}N$
43	11.663	190.0272	3-(trifluoromethyl)-Benzoic acid	$C_8H_5F_3O_2$

44	13.308	308.1894	2-Pyrrolidinone, 4-(2-aminoethyl)-1-ethyl-3,3-diphenyl- (AHR 5904)	$C_{20}H_{24}N_{2}O$
45	13.311	329.1633	Sinomenine	$C_{19}H_{23}NO_{4}$
46	14.652	224.1422	Methyl jasmonate	$C_{13}H_{20}O_{3}$
47	15.139	199.1952	Dodecanamide	$C_{12}H_{25}NO$
48	15.826	252.1737	Punctaporin B	$C_{15}H_{24}O_{3}$
49	15.903	594.4254	GPCho(11:0/11:0[U])	$C_{30}H_{61}NO_{8}P$
50	15.982	550.3988	GPCho(O-18:1(9Z)/2:0[S])	$C_{28}H_{57}NO_{7}P$
51	16.114	426.3008	Leupeptin	$C_{20}H_{38}N_{6}O_{4}$
52	16.601	318.1461	Zearalenone	$C_{18}H_{22}O_{5}$
53	17.13	375.9845	Ambroxol	$C_{13}H_{18}Br_{2}N_{2}O$
54	17.695	297.2683	(Z)-N-(2-hydroxyethyl)hexadec-7-enamide	$C_{18}H_{35}NO_{2}$
55	18.391	464.3139	3alpha,6alpha,7alpha,12alpha-Tetrahydroxy-5betacholest-24-en-26-oic acid	$C_{27}H_{44}O_{6}$
56	19.049	504.3461	Madecassic Acid	$C_{30}H_{48}O_{6}$
57	19.091	299.2848	N-(2-hydroxyethyl)palmitamide	$C_{18}H_{37}NO_{2}$
58	19.234	566.383	(5b,12a), 9-anthracenylmethyl ester, 12-hydroxy-Cholan-24-oic acid	$C_{39}H_{50}O_{3}$
59	19.29	460.3197	1alpha,24,25,28-tetrahydroxyvitamin D2 /1alpha,24,25,28-tetrahydroxyergocalciferol	$C_{28}H_{4}4O_{5}$
60	22.233	606.2895	Trandolapril glucuronide	$C_{30}H_{42}N_{2}O_{11}$
61	23.999	430.2718	17-phenyl-trinor-PGF2alphaisopropyl ester	$C_{26}H_{38}O_{5}$
62	26.409	152.1083	(3-Hydroxyphenyl)trimethylamm	$C_{9}H_{14}NO$
63	26.414	166.1236	Edrophonium	$C_{10}H_{16}NO$



Supplementary Figure 1: Chromatogram of MELIS obtained from HR-LC/Q-TOF/MS.

PICTORIAL ABSTRACT



SUMMARY

- Methanol extract of *Lawsonia inermis* (MELIS) exhibited the property of Anti-alzheimer in D-Galactose induced Alzheimer's disease (AD) in albino male rats of wistar strain. Treatment with MELIS increased the ACh content of brain cerebral cortex in AD rats.
- Additionally, the activity of AChE in brain cerebral cortex in AD rats was decreased by MELIS.
- Docking studies of compounds present in MELIS against AChE enzyme revealed good binding interactions which indicate possible MELIS compounds inhibiting AChE.
- It was therefore evidence that possible regulation of AChE by MELIS in both *in vivo* and *in silico* could be the choice to take MELIS as the key source of isolation for new and innovative drug compounds.

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