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Original Article

Nootropic herbal formulations for the treatment of Alzheimer's disease: *In vivo* pharmacological assay and molecular docking studies

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ABSTRACT

Background and Aims: The main aim of the study was to enhance the cognitive function of the brain by nootropic herbal formulations in animal models. Polyphyto herbal formulations were known to enhance the cognition and memory function by several pathways such as anti-oxidative, anti-inflammatory, and cell signaling pathways. In this study, six formulations were prepared by mixing specified plant parts and were coded as NHF1, NHF2, NHF3, NHF4, NHF5, and NHF6.
Methods: The potency of the formulations was assessed by *In vivo* (photo actometer, rod walking test, pole climbing test, and Ellman's acetylcholinesterase test) studies.
Results: NHF1 and NHF5 exhibited greater activity than the standard drug donepezil *in vivo* (Ellman's acetylcholinesterase test) analysis. NHF1 and NHF5 formulations containing plant parts were further investigated against several published literatures for the identification of chemical constituents and those chemical constituents were subjected to molecular docking and *in silico* ADME prediction studies to figure out the possible compounds responsible for the cholinesterase inhibition activity.
Conclusion: In conclusion, the computational studies also reveal that presence of chemical constituents such as sarsasapogenin (13.13 nM), racemosol (16.26 nM), and beta-sitosterol (30.47 nM) having binding energy (-10.75 kcal/mol), (-10.63 kcal/mol), (-10.25 kcal/mol), might be directly responsible for the nootropic activity.

Keywords: Herbal, nootropic, acetylcholinesterase, Alzheimer, autodock 4.2.6, sarsasapogenin, SwissADME

INTRODUCTION

Alzheimer's disease is a progressive neuronal damage that leads to shrinkage of the brain, which is characterized by the presence of plaques of amyloid beta and tangles of tau protein (Waldemar et al., 2007). It is the most common cause of dementia accounting for 60 - 80% in elder people. Alzheimer's disease has no therapeutic treatment, however, certain medications are available for symptomatic relief and improvement of cognition. In fact, the prescribed medications have serious side effects as well as pharmacokinetic limitations (De la Monte, 2012; Dos Santos Pisoni et al., 2010)

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Natural compounds are known to be one of the best sources for treating most of the clinical problems and they continue to inspire as the best alternatives (Musthaba et al., 2010). Many pathological conditions related to the central nervous system, in one way or the other causes the loss of memory. Alzheimer's and dementia are major known conditions for loss of memory (Aggleton, J. P., Pralus, A., Nelson, A. J., & Hornberger, M., 2016; McKhann et al., 2001). However, the nootropic herbal formulations can be used to enhance the cognition and improve memory function without producing any side effects (Shibnath, K., Madhav, N. V. S., & Sarkar, C. N., 2016). Nootropic herbs mainly enhance memory function by certain ways either by increasing blood circulation to the brain, which further improves brain activity, or showing anti-oxidative and anti-inflammatory activity, which results in the prevention of neurodegeneration. Some other herbs such as *Bacopa monnieri* have been found to act by inhibiting the acetylcholinesterase inhibition pathway to enhance memory (Murray, A. P., Faraoni, M. B., Castro, M. J., Alza, N. P., & Cavallaro, V., 2013).

Acetylcholinesterase is an enzyme which is involved in many physiological conditions in the central nervous system. Its main function is to convert acetylcholine into thiocholine and acetate, which results in a decrease of acetylcholine levels in the presynaptic region of neurons. This gradual decrease in acetylcholine levels leads to many pathological conditions such as Alzheimer's disease and dementia (Da Silva Goncalves, Franca, & Vital de Oliveira, 2016). There are many herbal phytochemicals which are known to inhibit the acetylcholinesterase without any side effects (Rashed, Cardoso Sucupira, Moita Neto, & Feitosa, 2013). Recently, many drugs successfully completed clinical trial investigation (Ghribia, Ghouilaa, Omrib, Besbesb, & Janneta, 2014).

The nootropic herbal formulations (NHFs) used in this study are composed of various herbal plant parts mixed in different ratios to achieve the desired effect (Kulkarni, Girish, & Kumar, 2012). The current study was concentrated on investigating the safety and efficacy of nootropic herbal formulations to enhance cognition, as well as to identify the natural compounds responsible for the acetylcholinesterase inhibition activity that are present in the NHF1 and NHF5 formulations through a computational study. In the study, different combinations of herbal formulations made from herbal plant parts such as *Aloe vera, Areca catechu, Asparagus racemosus, Avena sativa, Bacopa monnieri, Curcuma longa, Cinnamomum zeylanicum, Convolvulus pluricaulis, Glycine max, Hibiscus rosasinensis, Juglans regia, Lactuca sativa, Mentha piperita, Phyllanthus emblica, Piper nigrum, Ribes nigrum, Terminalia arjuna, Vigna mungo, Zingiber officinale* were incoporated. Specific plant parts from the mentioned plants were combined with a specific quantity to achieve the desired formulation, and all the six formulations were named as NHF1, NHF2, NHF3, NHF4, NHF5, and NHF6.

These prepared formulations were evaluated by *In vivo* (photo actometer, rod walking test, pole climbing test, and Ellman's acetylcholinesterase test) studies. The best formulations containing plant parts were further analyzed against several published literature studies for the identification of chemical constituents. Those selected chemical constituents were subjected to molecular docking and ADME prediction studies to figure out the possible compounds responsible for the cholinesterase inhibition activity.

In fact, the chemical constituents (Sarsasapogenin (Kashyap, Muthusamy, Niranjan, Trikha, & Kumar, 2020; Sy et al., 2016), Recemosol (Sivanandam, 2007), beta-sitosterol (Ayaz et al., 2017; Zeng et al., 2019)) which were found to be active in this study were also evaluated separately in several other studies stating their potential for treating symptomatic relief in Alzheimer's models. In other studies, the active molecules (Sarsasapogenin (Wang et al., 2018; Yang et al., 2018), recemosol, beta-sitosterol) were also modified synthetically to improve the activity, and achieved greater results in treating the Alzheimer's related symptoms in mice models. Therefore, this study provides evidence that these safer and pharmacologically active nootropic formulations can be an alternative drug therapy for symptomatic relief, and prevent clinical patients from progressing to the Alzheimer's disease.

MATERIALS AND METHODS

Plant materials and preparation of formulation

A variety of plant parts, as shown in Table 1, were collected from the local source, and were identified and authenticated

Table 1. Different plants and its parts used in this study.

S.No	Scientific Name	Family	Plant parts
1	*Aloe vera*	Xanthorrhoeaceae	Leaves
2	*Areca catechu*	Arecaceae	Fruit
3	*Asparagus racemosus*	Lilliaceae	Roots
4	*Avena sativa*	Poaceae	Fruit
5	*Curcuma longa*	Zingiberaceae	Rhizome
6	*Cinnamomum zeylanicum*	Lauraceae	Bark
7	*Convolvulus pluricaulis*	Convolvulaceae	Herbs
8	*Glycine max*	Fabaceae	Seed
9	*Hibiscus rosa sinensis*	Malvaceae	Flower
10	*Juglans regia*	Juglandaceae	Fruit
11	*Vigna mungo*	Fabaceae	Seed
12	*Mentha piperita*	Labiatae	Leaves
13	*Phyllanthus emblica*	Euphorbiaceae	Fruit
14	*Piper nigrum*	Piperaceae	Seed
15	*Ribes nigrum*	Grossulariaceae	fruit
16	*Zingiber officinale*	Zingiberaceae	Rhizome
17	*Bacopa monnieri*	Plantaginaceae	Herbs
18	*Terminalia arjuna*	Combretaceae	Bark
19	*Lactuca sativa*	Asteraceae	Leaves

by Sri Venkateshwara University, Thirupathi. The plant parts were kept for air dry under a shady place and grounded, and then passed over #100 sieves to prepare the specified formulations, as shown in Table 2.

Table 2. The composition of nootropic herbal formulations.

Formulation	Crude Powder	Quantity (g)
NHF1	*Cinnamon zeylanicum*	1.5
	Vigna mungo	1.5
	Avena sativa	2
	Asparagus racemosus	2
	Areca catechu	3
NHF2	*Mentha piperita*	2.5
	Ribes nigrum	2.5
	Aloe vera	2.5
	Glycine max	2.5
	Piper nigrum	2
NHF3	*Convolvulus pluricaulis*	2.5
	Zingiber officinalis	2.5
	Vigna mungo	3
	Avena sativa	2
	Asparagus racemosus	2.5
NHF4	*Lactuca sativa*	2.5
	Hibiscus rosasinensis	3
	Zingiber officinalis	0.5
	Convolvulus pluricaulis	1.5
	Curcuma longa	1
NHF5	*Phyllanthus emblica*	2
	Mentha piperita	2
	Hibiscus rosasinensis	3
	Terminalia arjuna	1
	Asparagus racemosus	1
NHF6	*Hibiscus rosasinensis*	2.5
	Convolvulus pluricaulis	2.5
	Bacopa monieri	3

NHF: Nootropic herbal formulation

Experimental animals

Albino Wistar rats belonging to the adult age group of male sex and weighing about (180±20 g) were procured and kept in polypropylene cages in a laboratory under ambient temperature with a regular day/night cycle. All the animals were randomized into two per cage and acclimatized for one week in the animal facility under standard conditions following OECD guidelines. A standard pellet diet and water were given *ad libitum,* and all the experiments were conducted in the day time (9.30 AM to 5.00 PM). The study protocol was approved by the Institutional ethical committee (1015/C/06/CPCSE9).

Phytochemical analysis

All the formulations which were selected for the activity were subjected to phytochemical analysis. Phytochemicals were extracted using 10 mL methanol and dried to achieve residue. To the obtained residue, dilute HCl was added, shaken well and filtered, the obtained filtrate used for analysis of alkaloids using the Dragendorff's, Mayer's, Hager's and Wagner's tests, for glycosides, the Legal's, Liebermann's, Foam, Haemolytic, and Borntrager's tests, for flavonoids, the Shinoda test, and for Steroids, the Liebermann's tests were performed (Odebiyi, & Sofowora, 1978; Trease, & Evans, 1996).

Acute and sub-chronic toxicity studies

To assess the acute (24 hours) and sub-chronic (14 days) toxicity of a nootropic herbal formulation, a single dose was given orally in pellet form to the randomized male Wistar rats, which were procured and kept in standard conditions following OECD guidelines. All NHF1, NHF2, NHF3, NHF4, NHF5 and NHF6 formulations were administered orally at a dose of 300, 1000 and 2000 mg/kg to a group of rats, which were fasted for 6 hours. All the animals were allowed free access to food and water under standard conditions (Chinedu, Arome, & Ameh, 2013). Six animals were observed for abnormal behavior and percentage of the mortality rate for a period of 14 days. The control group was treated with normal saline and the test group was treated with standard drug donepezil. After the observation period, blood was collected from all the animals for hematological observations. All the six formulations NHF1, NHF2, NHF3, NHF4, NHF5, and NHF6 were observed to be safe for administration.

Experimental methods

Actophotometer

The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on a photoelectric cell which is in circuit with a counter. When the beam of the light falling on the photocell was cut off by the animal, a count was recorded and displayed digitally. The actophotometer contains a circular or square arena in which the animal moves. The animals were tested for the activity before and after the administration of the formulation (Reddy, & Kulkarni, 1998).

Rod walking test

The ability of the rat to balance on a stationary, horizontal rod and walk on it to come in one end of the rod measures cognitive study and learning activity. Animals were placed in the center of a rod measuring 100 cm long, 20 mm in diameter and positioned 50 cm above the table surface, latency to transfer to its one end was recorded. All the rats were tested three times to observe the holding time or transfer latency on different groups (Dunham, & Miya, 1957).

Pole climbing test

The pole climbing study was performed by incorporating a Cook's pole climbing apparatus; this experiment was utilized to understand the learning and its retention in response to stimuli applied by the instrument. The apparatus contains a chamber (25x25x25 cm) which was made of a stainless steel grid floor for the experimental area. In the center, a pole hangs, measuring 2.5 cm in diameter, which helps the rat to avoid shock by climbing it. Initially, a rat was placed in the experimental chamber and allowed to habituate the area for 45 sec. A simultaneous

conditioned stimulus and unconditional stimulus, i.e., buzzer signal and electric shock respectively were applied for 45 sec. The animal avoids the shock by climbing the pole after an alert from the conditional stimuli associated learning. Each rat was subjected on the first day and 24 hours later to 05 trails maximum. The transfer latency and escape latency were noted during the study period (Cook, & Weidley, 1957; Soman, Mengi, & Kasture, 2004).

In vivo acetylcholinesterase estimation

collection of brain samples

All the animals were euthanized by cervical decapitation 90 min after the last dose on the 15th day. The brain was removed carefully using forceps, weighed and homogenized in a glass homogenizer containing sterile normal saline. The supernatant which was obtained after centrifugation (Remi, Hyderabad, India) at 3000 rpm for 10 min was used for the analysis of cholinesterase activity using 3 replicas (Thomsen, Kewitz, & Pleul, 1988).

Ellman acetylcholinesterase activity

In vivo acetylcholinesterase activity was measured using a modified Ellman's method. About 0.5 mL of the supernatant which was obtained from the result of centrifugation was pipetted out into an 8 mL of freshly prepared DTNB solution (10 mg DTNB in 100 mL of Sorenson phosphate buffer) having pH 8.0. The above solution was divided into two equal parts and 2 drops of eserine solution were added to only one part. Then, 1 mL of substrate solution (75 mg of acetylcholine iodide per 50 mL of distilled water) was added to both tubes and incubated for 10 min at 30°C. The eserine containing solution was used for zeroing the colorimeter (Insif electronics, Hyderabad, India). The resulting yellow color was due to the reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of the substrate. After the instrument (Shimadzu, Hyderabad, India) was calibrated, the absorbance change per minute of the sample was read at 420 nm (Ellman, Courtney, Andres, & Featherstone, 1961).

Computational methods

Based on the *in vivo* pharmacological evaluation, it was found that NHF1 and NHF5 had better activity than the standard drug. Therefore, these formulations were considered for further evaluation to figure out activity responsible chemical constituents using computational techniques. The main phytochemicals present in the plants which belong to NHF1 and NHF2 were downloaded from the NCBI-PubChem database in .sdf format.

Protein and ligand preparation

The crystal structure of acetylcholinesterase bearing PDB ID: 4M0E_A was downloaded from the RCSB PDB website (https://www.rcsb.org/). All the cocrystal ligands and water molecules were removed from the protein. Finally, the hydrogen atoms and Gasteiger charges were applied using Autodock tools (ADT) (Morris et al., 2009). All the natural product ligands which were identified and retrieved from NCBI-PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2019) were saved in sdf format. All these files were converted to mol2 format using the OpenBabel software (O'Boyle et al., 2011).

Molecular docking

Molecular docking studies were performed using the Autodock 4.2.6 and ADT tools. The selected protein was refined by deleting the crystal ligands, chain B and crystal water molecules. The downloaded ligands were saved in .pdbqt file format, and grid maps were setup using a grid box with coordinates of X=-11.708, Y=-42.266 Z=21.559 having a number of points of 60 for all the x,y,z dimensions. Finally, the Lamarckian genetic algorithm was incorporated for docking ligands into the binding pocket region (Morris et al., 2009).

ADME properties

ADME properties play a crucial role in predicting the druggable properties of small molecules (Katsila, Spyroulias, Patrinos, & Matsoukas, 2016). The 24 best active natural compounds were selected based on results from the molecular docking studies. All the selected molecules were analyzed for ADME analysis using a SwissADME server (Daina, Michielin, & Zoete, 2017).

Statistical analysis

All the experiments were done in triplicate, and all the data were shown as mean ± SD. The data were analyzed using the Graphpad Prism 5 program trial version. Statistical differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison test. Mean values were considered statistically significant when $p<0.001$.

RESULTS

Toxicity studies

All the NHF1, NHF2, NHF3, NHF4, NHF5, and NHF6 formulations were found to be safe and no mortality was seen in both acute and sub chronic toxicity studies, even at the high dose escalation of 2000 mg/kg. All the animals were observed to be normal in the consumption of food, behavior and physical activity during and at the end of the observation period of 14 and 28 days for acute and sub chronic toxicities, respectively. After the observation period, blood was collected from the tail for hematological analysis. Hematological results showed (Table 3) no significant variation in hemoglobin, platelet count and total WBC.

Pharmacological screening

Locomotor activity test

The test groups which were treated with NHF2 and NHF4 nootropic herbal formulations revealed a statically significant reduction in escape latency compared to the control and standard, indicating greater activity. The results were shown in a bar diagram with a statistical significance value in Figure 1.

Pole climbing apparatus

The test groups which were treated with NHF2 and NHF6 nootropic herbal formulations revealed a statically significant reduction in escape latency compared to the control, indicating greater activity. However, NHF5 shows significant compared with the standard. The results were shown in a bar diagram with statistical significance value in Figure 2.

Table 3. Group mean – hematology reports of animals treated with nootropic herbal formulations.

Formulations	Dose	Mean ± SD	Hb (g %)	Platelets ($x10^5$/c)	Total WBC ($x10^3$/cmm)
Control		Mean	11.33%	1.9	7.5
		± SD	0.00412	0.126	0.54
NHF1	Medium	Mean	11%	2.5	8.9
		± SD	0.00358	0.113	0.34
	High	Mean	11.14%	2.93	10.37
		± SD	0.00431	0.103	0.489
NHF2	Medium	Mean	11.19%	1.8	6.5
		± SD	0.00398	0.25	0.34
	High	Mean	11.24%	1.96	7.06
		± SD	0.00427	0.103	0.15
NHF3	Medium	Mean	11.22%	0.9	1.2
		± SD	0.00336	0.0816	0.26
	High	Mean	11.00%	0.358	1.95
		± SD	0.00701	0.0917	0.08
NHF4	Medium	Mean	11%	1.5	8.4
		± SD	0.00228	0.2	0.98
	High	Mean	10.95%	1.9	9.333
		± SD	0.00055	0.126	0.816
NHF5	Medium	Mean	11.26%	1.6	8
		± SD	0.00521	0.34	0.86
	High	Mean	11%	1.9	8.83
		± SD	0.00854	0.126	1.16
NHF6	Medium	Mean	11.20%	1.6	6.8
		± SD	0.002	0.14	0.87
	High	Mean	11.05%	1.4	6.56
		± SD	0.00089	0.12679	0.69

Values are mean of triplicate determination (n=3) ± standard deviation

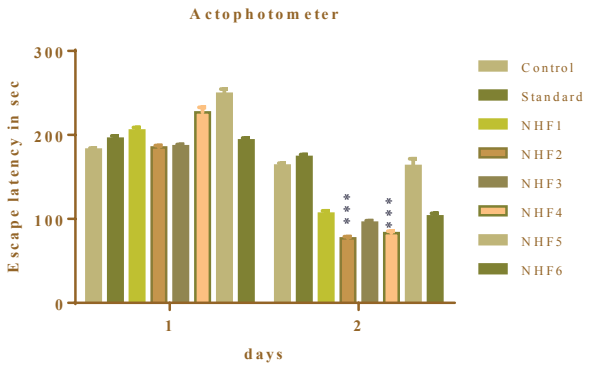

Figure 1. Bar graph of escape latency of rat in sec using actophotometer ***=p<0.001 standard (donepezil) vs. NHF2 and NHF4.

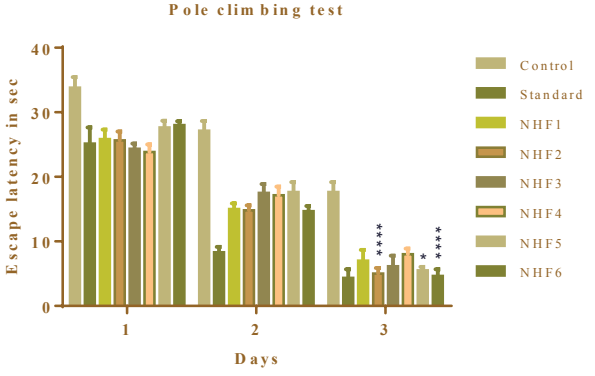

Figure 2. Bar graph of escape latency of rat in sec using cook's pole climbing apparatus ***=p<0.001 Standard (donopezil) vs. NHF5.

Rod walking test

The test groups which were treated with nootropic herbal formulations revealed a statically significant reduction in escape latency compared to the control, indicating greater activity. The results were shown in a bar diagram with statistical significance value in Figure 3.

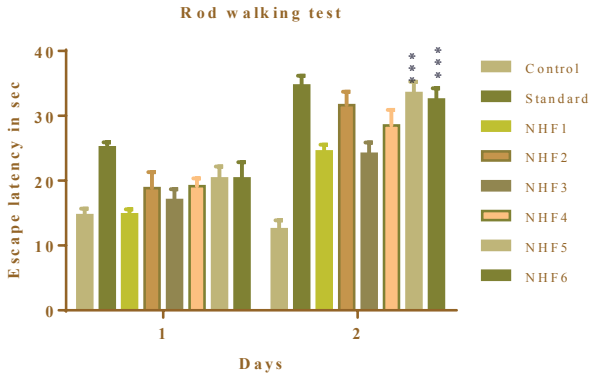

Figure 3. Bar graph of escape latency of rat in seconds using cook's pole climbing apparatus ***=p<0.001 Standard (donepezil) vs. NHF5.

In vivo acetylcholinterase estimation

In vivo acetylcholinesterase activity was measured using a modified Ellman's method. The yellow color observed was due to the reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of the substrate. After the instrument was calibrated, the absorbance change per minute of the sample was read at 420 nm. Acetylcholine is converted to thiocholine and acetate by the action of the acetylcholinesterase enzyme. The thiocholine, which was broken from acetylcholine, reacts with dithiobisnitrobenzoate and produces a yellow color. More yellow color represents less inhibition, whereas, less yellow color represents more inhibition of the acetylcholinesterase activity. The absorbance of NHF5 and NHF1 was found to be lower than that of the standard drug donepezil, which directly indicates that the NHF5 and NHF1 herbal formulations were more active than the standard drug. The values were represented in a bar diagram represented in Figure 4.

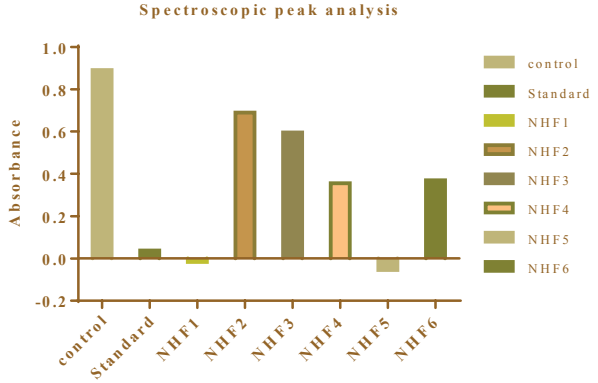

Figure 4. Graph shows spectroscopic absorbance peak analysis of control, standard and nootropic herbal formulations at 420 nm.

Computational results

Molecular docking analysis

In this molecular docking study, about 39 natural products were selected from the plant parts which belong to the NHF1 and NHF5 formulations through a thorough search of the literature reports. The target protein acetylcholinesterase was retrieved from a protein data bank (PDB) having PDB ID: 4M0E_A, which contains 542 amino acids and x-ray diffraction resolution of 2.0 Å. The most active residues in the binding area interacting with the ligands were "Tyr 341, Ser 293, Glu 292, Phe 295, Ser203, Arg 296, Glu 202, Ser 125, Tyr 124, Asp 74, and Trp 286". Among 39 docked phytochemicals, 3 molecules such as Sarsasapogenin (-10.75 kcal/mol), racemosol (-10.63 kcal/mol), and beta-sitosterol (-10.25 kcal/mol) showed the best binding energies. The superimposition of the 3 best active phytochemicals were inserted into the active site of the protein and represented in Figure 5.

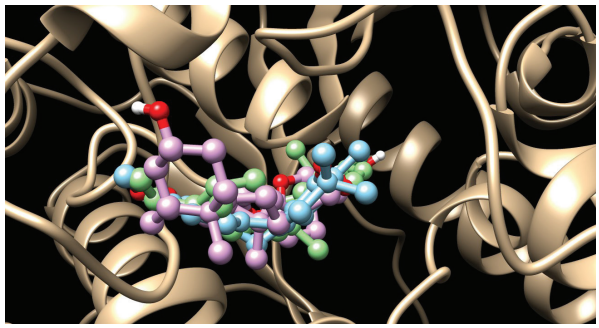

Figure 5. Superimposition of the best 3 ligands from the docking studies were superimposed in binding region of the 4M0E protein.

ADME analysis

In this study, about 24 best active molecules out of 39 compounds from molecular docking studies were analyzed for ADME parameters by the SwissADME online tool to understand the drug likeness nature of nootropic herbal chemicals. In this study, physicochemical, lipophilicity, water solubility, pharmacokinetics and drug likeness parameters were analyzed, which were shown in the Table 4.

DISCUSSION

The nootropic herbal formulations which were prepared were firstly tested for phytochemical analysis for ensuring the presence of the most important class of phyto-constituents such as alkaloids, glycosides and flavonoids, and the phytochemical tests show the presence of those phytochemicals. Secondly, all the nootropic herbal formulations were evaluated for acute and sub-chronic toxicity studies with the dose escalation of 2000 mg/kg body weight of the animal. Hematological reports suggest that all the formulations were safe for administration, and no mortality was observed during the study.

In vivo pharmacological studies such as locomotor activity, pole climbing study, and rod walking studies were performed using actophotometer, rod walking apparatus, and cook's pole

Table 4. SwissADME properties of best active phytochemicals.

S.No	Compound ID	MW	HB-A	HB-D	TPSA	iLOGP	GI-absorption	BBB -permeation	CYP2D6 inhibition	CYP3A4 inhibition	Lipinski violation	Bioavailability Score	Brenk alerts	SA
1	14632996	218.33	1	0	17.07	3.11	High	Yes	No	No	0	0.55	1	4.53
2	5281157	299.28	5	4	106.86	1.19	High	No	No	No	0	0.56	2	2.36
3	10087955	329.3	6	4	116.09	1.78	High	No	No	No	0	0.56	2	2.57
4	11723200	315.28	6	5	127.09	0.71	High	No	No	No	0	0.56	3	2.47
5	222284	414.71	1	1	20.23	5.07	Low	No	No	No	1	0.55	1	6.30
6	196216	218.33	1	0	17.07	3.14	High	Yes	No	No	0	0.55	1	4.17
7	9064	290.27	6	5	110.38	1.33	High	No	No	No	0	0.55	1	3.5
8	420422	305.37	5	0	48	3.41	High	Yes	Yes	No	0	0.55	0	4.25
9	156777	354.35	6	3	96.22	2.63	High	No	Yes	Yes	0	0.55	0	4.08
10	585939	258.27	4	3	69.92	1.7	High	Yes	Yes	No	0	0.55	0	3.05
11	442770	340.37	5	3	86.99	1.98	High	No	Yes	Yes	0	0.55	1	3.79
12	5281855	302.19	8	4	141.34	0.79	High	No	No	No	0	0.55	3	3.17
13	72276	290.27	6	5	110.38	1.47	High	No	No	No	0	0.55	1	3.5
14	5280961	270.24	5	3	90.9	1.91	High	No	Yes	Yes	0	0.55	0	2.87
15	5280520	270.24	5	3	90.9	1.36	High	No	Yes	Yes	0	0.55	0	2.89
16	5282074	286.24	6	4	111.13	1.48	High	No	Yes	Yes	0	0.55	0	2.95
17	5280863	286.24	6	4	111.13	1.7	High	No	Yes	Yes	0	0.55	0	3.14
18	119269	356.37	6	4	107.22	2.24	High	No	Yes	Yes	0	0.55	1	3.85
19	71629	306.27	7	6	130.61	1.19	High	No	No	No	1	0.55	1	3.76
20	5089889	576.5	12	9	209.76	1.8	Low	No	No	Yes	3	0.17	1	5.85
21	5280343	302.24	7	5	131.36	1.63	High	No	Yes	Yes	0	0.55	1	3.23
22	624971	340.41	4	2	58.92	3.23	High	Yes	Yes	Yes	0	0.55	0	4.04
23	92095	416.64	3	1	38.69	4.54	High	Yes	No	No	1	0.55	0	6.88
24	5282230	327.33	5	2	84.86	2.68	High	No	No	No	0	0.56	1	2.57

MW: Molecular weight, HB-A: Hydrogen Bond Acceptor, HB-D: Hydrogen Bond Donor, TPSA: Topological Polar Surface Area, S.A: Synthetic accessibility

climbing apparatus respectively. In the actophotometer test, the NHF2 and NHF4 formulations were found to be more active than the standard drug. In the cook's pole climbing test, NHF5 was found better than the standard. Similarly, NHF5 was found to be active in the rod walking test.

In vivo acetylcholinesterase activity was analyzed using a modified Ellman's method in which the color intensity was measured as absorbance against the enzyme activity. The more yellow color indicates the more enzyme activity, whereas a less yellow color indicates the less enzyme activity which is due to more inhibition of the acetylcholinesterase enzyme by the inhibitors. The NHF5 and NHF1 were found to be more active than the standard drug donepezil.

Finally, docking studies performed on the acetylcholinesterase revealed that all the docked molecules have a good binding affinity. However, about 7 molecules have a range of -9.00 kcal/mol to -10.75 kcal/mol binding energy and good interaction with the protein residues. Among which 3 molecules shows the most active. Hence, it is believed that the presence of these phytochemicals might be directly responsible for the acetylcholinesterase activity. The ADME parameters predicted from the SwissADME server also supported that all the most active 3 molecules have good drug-like properties.

CONCLUSION

The nootropic herbal formulations which were used in this study showed significant results in behavioral and physiological activities. Specifically, the nootropic herbal formulations NHF1 composed of *Cinnamon zeylanicum, Vigna mungo, Avena sativa, Asparagus racemosus, Areca catechu* and NHF5 composed of *Zingiber officinalis, Convolvulus pluricaulis, Curcuma*

longa, Phyllanthus emblica, Mentha piperita, Hibiscus rosa sinensis showed greater impact in elevation of neuronal acetylcholine in the brain via significant demotion of acetylcholinesterase activity and it is clearly evident in the *In vivo* acetylcholinesterase study. Further study conducted to evaluate the chemical constituents responsible for the activity was predicted using molecular docking studies which suggest that Sarsasapogenin (-10.75 kcal/mol), racemosol (-10.63 kcal/mol), and beta-sitosterol (-10.25 kcal/mol) have the best binding energy and greater interactions with the acetylcholinesterase enzyme. The ADME parameters predicted from the SwissADME server further support that all the best active compounds are proven to be druggable molecules and can permeate through the blood brain barrier (BBB). These shreds of evidence suggest that the neuroprotective and acetylcholinesterase inhibition nature of these formulations maybe due to the presence of the chemical constituents sarsasapogenin, racemosol, and beta-sitosterol. Therefore, these formulations might clinically help patients of dementia and Alzheimer's in recovery by symptomatic relief, and improvement of cognition.

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