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Synthesis of Ginsenoside Nanoparticles and Insilico Docking of Ginsenosides to SOD1 and TARDBP Targets in Amyotrophic Lateral Sclerosis (ALS)

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Abstract : Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that attacks the motor neurons of the brain and spinal cord of healthy adults. The disease progresses rapidly and is fatal, leaving patients paralyzed and unable to breathe. The pathological determinants of disease progression remain poorly understood. Currently, there is no known cure or effective treatment available for ALS. But with the advances in technology, there has been enormous increase in the volume of the genetic data produced and - thus stored for analysis and interpretation. The present study is focused on one such usage of the data extracted from ALS Online Database (The study is based on the *insilico* approach for selecting appropriate targets and screening suitable ginsenoside, a herbal drug molecules against targets of ALS. The patient's data and their respective targets were obtained from ALS database. The selection of the targets, SOD1 and TARDBP is based on the reports and the frequency as well as the types of mutations occurring in the population under study.The structure of the targets was modeled using SWISSMODEL. About 50 ginsenoside and their derivative obtained from Pubchem compound database were screened and docked against the targets. *Insilico* results showed that in comparison to ginsenoside alone, combinations of two give better e- values with respect to SOD1. For preliminary characterization, the phytosome complex was subjected to microscopic and spectroscopic analysis. The phytosome complex was observed under the microscope at 100X magnification while LPC and ginsenoside Rg1 did not show any complex structures when observed individually. The OD of phytosome was 1.7 times greater than LPC revealing the formation of complex between LPC and ginsenoside Rg1.Thus, *insilico* results were used to minimize the number of ginesenosides to be used for phytosome complex synthesis.

Introduction

Amyotrophic **l**ateral **s**clerosis (ALS) or "Lou Gehrig's Disease," is a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord[1]. The progressive degeneration of the motor neurons in ALS leads to their death and thus loss in the ability of the brain to initiate and control muscle

movement[2]. The pathological determinants of disease progression remain poorly understood. Currently, there is no known cure or effective treatment available for ALS.

Phytocompounds are secondary metabolites of plant origin know to .possess features such as hepatoprotective, antioxidant, immunomodulation, anticancerous activity apart from preventing or delaying the degeneration of neurons[3].With several of these applications, the efficient delivery of the bioactive constituent of phytocompound at the site of target has been a challenge[4]. These compounds are absorbed poorly either due to their size or poor miscibility with the lipid molecules making bioavailability a serious concern[5,6]. The curcumin exhibit poor water solubility and thus is rapidly eliminated from the body. To improve the bioavailability of curcumin in the body, the curcumin-phytosome loaded chitosan microspheres were used as drug delivery system[7].

To enhance their bioavailability, delivery of the phytocompounds in the form of novel complexes called phytosome could be an alternate. Phytosomes of many plant compounds obtained from extracts of *Ginko bilboa, Silybum marianum, Cucurmin,* have been successfully developed[8,9].

Ginsenosides are a class of bioactive triterpenoid saponins extracted from *Panax Ginseng* roots and are well known in the treatment of Alzheimer's disease, amyotrophic lateral sclerosis, Huntingtons diseases, etc.[10,11]Studies on mutations in a key enzyme of ALS, SOD1 transgenic mice showed that ginseng powder from *Panax quinuefolium* delayed the onset of symptoms and prolonged the survival of the mice[12].

With the advent of new technologies and *insilico* tools available, selection of appropriate targets and drugs as well as studies on their interactions have been possible. This further reduces the effort and cost of wetlab research and findings. The present study describes an approach towards insilico selection of appropriate targets (SOD1 & TARDBP) and ginsenosides for ALS.The data obtained from *insilico* studies have been considered as basis for the wetlab research and phosphatidyl choline-ginsenoside complexes have been synthesized. Further, these complexes were subjected to spectroscopic and microscopic analysis for preliminary characterization.

Experimental

Insilico studies

The ALS database has been used for analyses and identification of target (http://alsod.iop.kcl.ac.uk). The protein targets identified in the present study are SOD1 (Ala4val) and TARDBP (Ala382Thr).The structures of the identified targets were modeled via homology modeling using SWISS-MODEL workspace (**Fig.1a & b**).The isomorphs of ginsenoside were obtained in smiles format from the molecular database (molecules.gnu-darwin.org).The tool openbabel (version 2.3) was used to convert smiles format of compounds to PDB.The protein targets SOD1 and TARDBP with the ligand structures in PDB format were uploaded in the Hex server and docked using the using Hex tool (version 6.2). All the 50 Ginsenosides were docked individually to each of the targets SOD1 and TARDBP. Once the docking is complete, a docked structure indicating the corresponding e-values displayed.

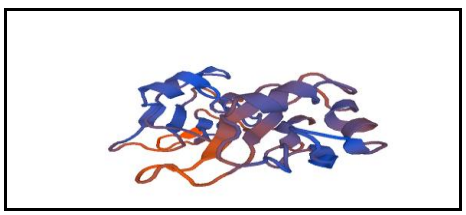


Fig.1 a: TARDBP structure obtained via homology modeling using SWISS MODEL workspace.

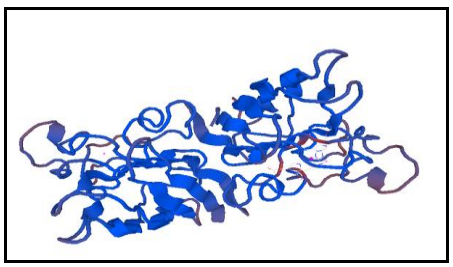


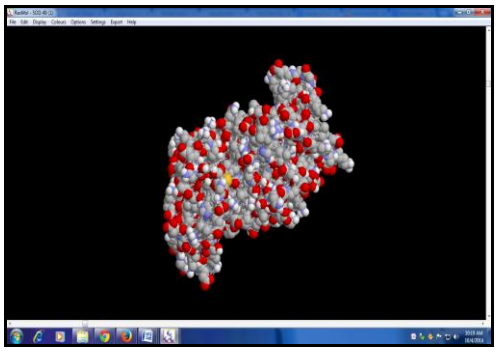
Fig.1 b:SOD1 structure obtained via homology modeling using SWISS MODEL workspace.

Synthesis of phytosomes

The *"salting out"* method was employed in the present study to develop ginsenoside phytosomes complex. Based on the insilico results, ginsenoside Rg1 compound was complexed with L-α phosphatidylcholine (LPC).The ginsenoside Rg1 was procured from Natural Remedies Pvt. Ltd, Bangalore and L-α phosphatidylcholine from Sigma chemicals. All reagents used in the study were analytical grade purity.

The equimolar weights (0.01M) of ginsenoside Rg1 and LPC in aprotic solvent were used for the experiment. Ginsenoside Rg1 was first suspended in methylene chloride followed by inclusion of LPC. The mixture was then incubated at room temperature for 2 hours to allow the formation of complexes. Then n-hexane was then added to precipitate the complexes and stored at -20˚c until further characterization.

Results and discussion



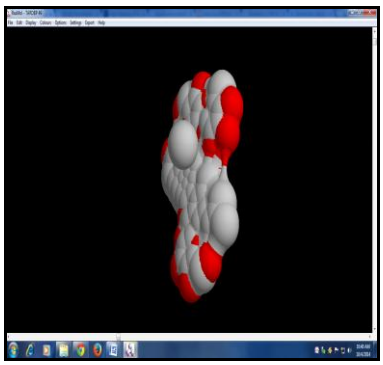


Fig. 2a : SOD1 docked with Ginsenoside Rd mixture (Rg1.Re & Rg1)
Fig. 2b:TARDBP docked with Ginsenoside Rd mixture w/Re

The Hex has been used as a tool for docking in the present study. Hex has been successfully used and applied for docking compounds obtained from *Pogstemon herba* against COX-1 inhibitor[13]. Docking and detailed molecular dynamics of interactions of Pla protein isolated from biological warfare agent *Yersinia pestis* with the mammalian plasminogen system has been analyzed[14]. Insilico results showed that in comparison to ginsenoside alone, combinations of two give better e- values with respect to SOD1 (Rg1.re & Rg1 with GA1)(**Fig. 2 & Table1**).The Rg1 alone has evalues,-155.33 for SOD1 and -78 for TARDBP which is close to the evalues of the combined ginsenoside.The modified natural compound has been proven a better drug than commercial drugs in case Antimalarial Drugs for Malaria[15].The mutations in SOD1 and TARDBP has been known to be leading cause for ALS and docking with Rg1 known to reduce the e- values. A clear dockingresults showed that glycoprotein and mutant protein of antibiotic resistance strain of HIV can be treated with the ligand 1TAM[16].

Table 1a-1d-:The evalues obtained from docking of SOD1 & TARDBP with 50 isomorphs of ginsenosides using HEX tool.

Table :1a

SL NO	ID	COMPOUND NAME	E- VALUES	
			TARGET GENE: SOD 1	TARGET GENE: TARDBP
1)	CID 73149	Ginsenoside Re	- 149.10	-78.54
2)	CID 9898279	Ginsenoside Rb 1	- 149.10	-78.72
3)	CID 73598	Ginsenoside Rb 2	- 150.39	-77.44
4)	CID 9918693	Ginsenoside Rg 3	-147.10	- 74.78
5)	CID 11815492	Ginsenoside Ro	-158.09	-77.50
6)	CID 6917976	Ginsenoside Rb 2	-160.19	-77.44
7)	CID 441923	Ginsenoside Rg1	- 155.33	-78.32
8)	CID 119037	Ginsenoside Rh 2	- 144.33	-77.70
9)	CID 9918692	Ginsenoside F2	-160.19	-77.44
10)	CID 432524	Ginsenoside Rb 1	-160.88	-74.11
11)	CID 441922	Ginsenoside Rg	-155.33	-78.32

Table: 1b

12)	CID 100018	Ginsenoside Re	-160.19	-77.44
13)	CID 12855920	Ginsenoside Rh 1	-151.34	-77.66
14)	CID 441921	Ginsenoside Re	-158.78	-78.54
15)	CID 6441009	Ginsenoside Rg 2	-155.54	-78.30
16)	CID 162741	Ginsenoside F1	-144.60	-77.66
17)	CID 6439048	Ginsenoside Rh 3	-153.34	-77.99
18)	CID 3085260	Ginsenoside Rh 1	-151.34	-77.66
19)	CID 432449	Ginsenoside Re	-149.10	-78.54
20)	CID 130009	Ginsenoside La	-144.60	-77.89
21)	CID 12901617	Ginsenoside Rg 3	-155.54	-78.30
22)	CID 5458674	Ginsenoside Rb 2	-160.19	-77.44
23)	CID 12855917	Ginsenoside Rh 1	-151.34	-77.66
24)	CID 2159998	Ginsenoside Rh 4	-151.69	-77.70
25)	CID 46887678	Ginsenoside F3	-154.51	-77.89

Table: 1c

26)	CID 12855889	Ginsenoside Rc	-160.19	-77.44
27)	CID 14681290	Ginsenoside Rh 2	-151.69	-77.70
28)	CID 432450	Ginsenoside Rb 2	-160.19	-77.44
29)	CID 404133	Ginsenoside Rh 2	-151.69	-77.70
30)	CID 44593678	Ginsenoside Rh 2	-155.93	-78.18
31)	CID 45479443	Ginsenoside Rg 3	-155.54	-78.30
32)	CID 44204122	Ginsenoside Rg 3	-155.54	-78.03
33)	CID 77906406	Ginsenoside Rh 3	-151.69	-77.44
34)	CID 45479218	Ginsenoside Rh 2	-151.69	-78.22
35)	CID 25203503	Ginsenoside Rb 1	-151.69	-78.22
36)	CID 71317070	Ginsenoside Rb 1	-160.88	-78.66
37)	CID 45358174	Ginsenoside Rh 1	-151.34	-77.66
38)	CID 328775	Ginsenoside Rg 1 mixed with G A1	-170.94	-24.19
39)	CID	Ginsenoside	-155.70	-78.34

Table: 1d

	6439252	Rg 4		
40)	CID 5458669	Ginsenoside A 1	-155.33	-78.32
41)	CID 44584745	Ginsenoside R 10	-155.33	-78.07
42)	CID 10699455	Ginsenoside Rh 5	-152.96	-78.07
43)	CID 6450175	Ginsenoside Rg 5	-155.70	-78.34
44)	CID 177397	Ginsenoside Rs 3	-148.00	-77.82
45)	CID 44202120	Ginsenoside Rb 2 & c	-150.39	-77.44
46)	CID 10629247	Ginsenoside R 25	-155.58	-78.48
47)	CID 46887679	20 (R) – Ginsenoside Rg 2	-155.84	-78.30
48)	CID 12912322	20(R) – Ginsenoside Rg 2	-155.54	-78.30
49)	CID 5458671	Ginsenoside Rg mixed with Re	-172.49	-80.78
50)	CID 5458670	Ginsenoside Rg 1 mixed with A1	-155.33	-78.32

Keeping the complexities of interactions of phytocompounds, in the present study Rg1 alone was used for forming phytosome complex with LPC. The microscopic observation and spectrophotometric analysis of the complexes have been carried out as a part of preliminary characterizationof phytosomes. The complex formed was observed under the microscope at 100X magnification **(Fig. 3)**. The LPC and ginsenoside Rg1 did not show any complex structures under microscope when viewed individually. The phytosome complex and LPC in 5 ml of 1M NaOH were analyzed at 266nm in UV-Vis spectrophotometer and the OD values were tabulated (**Table2**).The OD of phytosome was 1.7 times greater than LPC revealing the formation of complex between LPC and ginsenoside Rg1.Various characterization studies have been done on phytosomes. The spectrophotometric analysis of the phytosomes for its drug content has been employed[17]. Phytosomes have been earlier used as efficient tool to deliver drugs and known to increase bioavailability of the compounds [18,19,20]. Also, the luteolin-loaded phytosomes have been known to sensitize human breast carcinoma cells to doxorubicin[21].

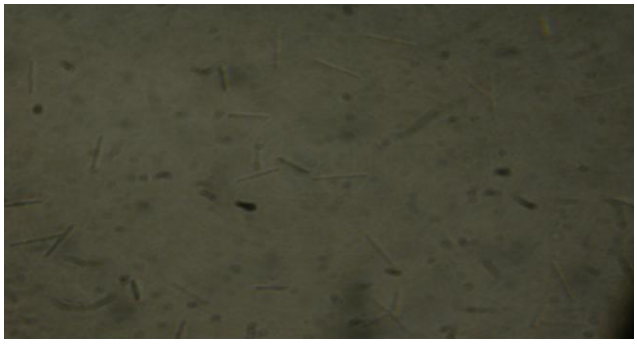


Fig.3: The LPC-Ginesenoside complex as viewed under 100x magnification.

Table.2. OD values of the complexes against a blank solution of LPC

COMPOUND	**OPTICAL DENSITY (266nm)**
Phytosome complex	**0.764**
L-α phosphatidylcholine	**0.49**

In conclusion, the ginsenoside and their derivative obtained from Pubchem compound database were docked against the targets SOD1 and TARDBP.The *insilico* studies revealed that the combination of ginsenosides give better e- values for SOD1 target. The phytosome complex was observed under microscope and the optical density of phytosome complex synthesized by LPC entrapping the ginsenoside Rg1 was 1.7 times greater than either LPC ginsenoside Rg1.It is also proposed that usage of nanoencapsulated ginsenoside with specific receptors for the target cells would increase the efficacy of ginsenoside by increasing its bioavailability.

References

1. Lewis P. Rowland, M.D. and Neil A. Shneider, Amyotrophic lateral sclerosis, N. Engl. J Med., 2001, 344,1688-1700.
2. Sabatelli M., Madia F., Conte A., Luigetti M., Zollino M., Mancuso I., Lo Monaco M., Lippi G. and Tonali P., Natural history of young-adult amyotrophic lateral sclerosis, *Neurology, 2008,* 12, 876–81.
3. Nilesh Jain, Brahma P. Gupta, Navneet Thakur, Ruchi Jain, JitendraBanweer, Deepak Kumar Jain, Surendra Jain, Phytosome: A Novel Drug Delivery System for Herbal Medicine, International Journal of Pharmaceutical Sciences and Drug Research, 2010, 4, 224-228.
4. Kidd P.M., Bioavailability and activity of phytosome complexes from botanical polyphenols: the silymarin, curcumin, green tea, and grape seed extracts, Altern. Med. Rev., 2009,14,226-46.
5. Surendra Tripathy, Dilip K. Patel, LipikaBaro, Suresh K. Nair, A review on phytosomes, their characterization, advancement & potential for transdermal application, Journal of Drug Delivery & Therapeutics, 2013, 3,147-152.
6. Allais G., D'Andrea G., Maggio M., Benedetto C., The efficacy of ginkgolide B in the acute treatment of migraine aura: an open preliminary trial**,**Neurol Sci., 2013,34 ,161-3.
7. Zhang J., Tang Q., Xu X., Li N., Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery, Int. J. Pharm., 2013,1,168-74.
8. Patel Amit, Tanwar Y.S., Suman Rakesh, Patel Poojan, Phytosome: Phytolipid Drug Dilivery System for Improving Bioavailability of Herbal Drug, JPSBR, 2013 ,3, 51-57.
9. Angelico R., Ceglie A., Sacco P., Colafemmina G., Ripoli M., Mangia A., Phyto-liposomes as nanoshuttles for water-insoluble silybin-phospholipid complex,Int. J. Pharm., 2014, 25;471(1-2):173-81.
10. Mehari Endale, EunJuIm, Joo Young Lee, Sung Dae Kim, TaddesseYayeh, Yong-Bum Song, Yi-SeongKwak, Chaekyun Kim, Seung-Hyung Kim, Seong-Soo Roh, Jae Youl Cho, andManHee Rhee,Korean Red Ginseng Saponin Fraction Rich in Ginsenoside-Rb1, Rc and Rb2 Attenuates the Severity of Mouse Collagen-Induced Arthritis, Mediators of Inflammation ,2014, 14 pages.
11. VivekJatwa, Praveen Khirwadkar, Kamlesh Dashora , Indian traditional memory enhancing herbs and their medicinal benefits,Indian Journal of Research in Pharmacy and Biotechnology ,2014,2,1030-1037.
12. Ik-Hyun Cho,Effects of *Panax ginseng* in Neurodegenerative Diseases, J. Ginseng Res.,2012, 36, 342–353.
13. Raharjo S.J., FatchiyahF.,Virtual screening of compounds from Thepatchouli oil of Pogostemonherba for COX-inhibition,Bioinformation, 2013,9, 321-4.
14. Ruback E., Lobo L.A., França T.C., PascuttiP.G.,Structural analysis of Pla protein from the biological warfare agent *Yersinia pestis*: docking and molecular dynamics of interactions with the mammalian plasminogen system, Biomol. Struct. Dyn.,2013,31,477-84.
15. Guruprasad N. M., Harish B. M, Jalali S.K., Puttaraju H., Insilico modeling of *Wolbachia*and its potentials incombating mosquito borne diseases Chikungunya and Dengue, International Journal of Mosquito Research, 2014, 3, 61-68.
16. RaghunathSatpathy, GyanRanjanSatpathy, PrangyaRanjan Rout,Molecular docking study of HIV NS3 proteaseinhibitors on dengue virus,Biotechnol. Bioinf. Bioeng., 2011, 2,179-182.
17. May S. Freag, Yosra S.R. Elnaggar, Ossama Y. Abdallah, Lyophilized phytosomalnanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation, International Journal of Nanomedicine, 2013,8, 2385–2397.
18. Mukesh S. Sikarwar, Shalini Sharma, Amit K. Jain, and S. D. Parial,Preparation, Characterization and Evaluation of Marsupsin–Phospholipid Complex, PharmSciTech., 2008, 9, 129–137.
19. Thurapathi Pandu Raju, MettuSrikanth Reddy, VeerareddyPrabhakar Reddy, Phytosomes, A Novel Phyto-phospholipid Carrier For Herbal Drug Delivery, IRJP,2011, 6, 28-33.
20. Bombardelli E., Phytosome: new cosmetic delivery system,BollChim Farm., 1991,11,431-8.
21. Sabzichi M., Hamishehkar H., Ramezani F., Sharifi S., Tabasinezhad M., Pirouzpanah M., Ghanbari P., Samadi N., Luteolin-loaded phytosomes sensitize human breast carcinoma MDA-MB 231 cells to doxorubicin by suppressing Nrf2 mediated signaling,Asian Pac J Cancer Prev., 2014,13,5311-6.

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