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Atherogenic effect of Arecoline: A computational study

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Abstract:
There are over 600 million people worldwide covering Asian and Oceanic countries including India have the habit of chewing areca nut as masticator in different forms. Arecoline ($C_8H_{13}NO_2$) has been reported as one of the abundant constituents of areca nut. A good number of scientific publications have made Arecoline responsible for oral cancer. Based on observation from clinical situation in North East India, one of the most betel quid chewing region of the country, we suspected a link between consumption of areca nut and Cerebro Vascular Disease like stroke. Therefore, we considered Low Density Lipoprotein (LDL) receptor as target and Arecoline as ligand and studied ligand –target interaction using computational tools. Also we considered High Density Lipoprotein (HDL) receptor as another target to see if Arecoline has any binding potential with it over and above LDL receptor. Docking result indicated that Arecoline and Cholesterol both, have affinity towards extracellular domain of Human LDL receptor but affinity of Arecoline is much higher (-12.3560.) than that of Cholesterol(-0.1810). Docking of Arecoline and 1, 2-Hexyl-1-cyclopentanone thiosemicarbazone (thiosemicarbazone) with Bovine HDL receptor showed that Arecoline also has the potential (Score, -6.2690Kcal/Mol) to block HDL receptor though its potential is less than that (score, -10.0509 Kcal/Mol) of control (thiosemicarbazone). We, therefore, suggest that by inhibiting endocytosis of LDL cholesterol because of blocking LDL receptor function and also by preventing LDL cholesterol uptake by liver from blood because of interference with HDL receptor, Arecoline may contribute to atherosclerosis. The study therefore, indicates a positive correlation between chewing of betel quid and Cerebro Vascular Disease.

Key words: Arecoline, Atherogenic effect, LDL receptor, HDL receptor, Cerebro Vascular Disease, *Insilico*

Background:
There are over 600 million people worldwide covering Asian and Oceanic countries, have the habit of chewing areca nut as masticator in different forms. Chewing the mixture of areca nut and betel leaf is a part of cultural tradition, custom or ritual in these countries which dates back thousands of years. Arecoline ($C_8H_{13}NO_2$) has been reported as one of the abundant constituents of areca nut. A good number of scientific publications have focussed on Arecoline as a responsible agent for oral cancer. Interestingly there are some controversial scientific reports available about the role of Arecoline in relation to oral cancer.

Reports are available that Arecoline inhibits p53, represses DNA repair, and triggers DNA damage response in human epithelial cells **[1].** It is also reported that Arecoline induced disruption of expression and localization of the tight junctional protein ZO-1 is dependent on the HER 2 expression in human endometrial Ishikawa cells **[2].** Adverse effects of Arecoline and nicotine on human periodontal ligament fibroblasts in vitro has also been reported **[3].** In a review article it is suggested that Areca nut should be highly suspected as a human carcinogen but toxicity studies relating to areca nut containing polyphenols and tannins are not conclusive, with both carcinogenic and anti-carcinogenic effects being reported **[4]**. The mutagenicity and

genotoxicity of areca alkaloids has been detected by many short-term assays. However, their genotoxicity to oral fibroblasts and keratinocytes, the target cells of betel quid, has not been identified. It would thus appear that areca nut toxicity is not completely due to its polyphenol, tannin and alkaloid content **[4].** Decreasing interleukin-6 production and induction of apoptosis and cell cycle arrest in human basal cell carcinoma cells by Arecoline has been reported **[5].** Report regarding induction of cell cycle arrest, apoptosis, and cytotoxicity to human endothelial cells by Arecoline is also available **[6].** Contradictory report saying that areca nut extract and Arecoline induced the cell cycle arrest but not apoptosis of cultured oral KB epithelial cells has also been published **[7]**

It is thus seen that over the last two decades scientists have been paying attention to Arecoline only from the point of view of cancer. No significant attention has been focussed on other possible adverse effect of Arecoline. Although there are some reports on possible correlation between Betel Quid chewing and Cardiovascular Disease available **[8, 9],** sufficient evidence for the mechanisms of action of Arecoline specifically enhancing Cerebro Vscular Disease (CVD) are still lacking. It is observed that in North East India – one of the most betel quid chewing areas of the country, prevalence of stroke is increasing at an alarming rate though there is no hard statistics available and stroke patients die or suffer from different degrees of disability because of absence of properly organised stroke services in any of the privately or publicly funded hospitals. Based on observation from clinical situation, we suspected a link between consumption of areca nut and stroke. Therefore, we focused on Low Density Lipoprotein (LDL) receptor discovered only in 1985 as our target. We studied the binding potential of Arecoline with LDL receptor using computational tools because mechanism of endocytosis of LDL cholesterol is LDL receptor dependent process. The hypothesis of the work is that if any molecule can bind with active site of LDL receptor with higher affinity than that of LDL cholesterol, that molecule will have atherogenic activity and atherosclerosis in turn is responsible for the majority of Cerebro Vascular Diseases (CVD) including stroke. Though, High Density Lipoprotein (HDL) cholesterol is a good cholesterol (athero-protective) as it helps in lowering LDL cholesterol, article published in the Jan. 13 issue of the *New England Journal of Medicine* 2011 specifically states that a specific receptor is essentially required for the athero-protective function of HDL as this receptor mediates uptake of cholesterol by liver from HDL.We, therefore, also looked at Arecoline HDL receptor bonding *in silico* to see if Arecoline has any inhibitory role on HDL receptor over and above its action on LDL receptor. For this experiment, thiosemicarbazone a commercially available known HDL receptor inhibitor was considered as control.

Methodology:

The 3D structure of the extracellular domain of human LDL receptor (PDB ID: 1N7D) was obtained from RCSB Protein Data Bank (PDB). The structure was downloaded in PDB (Text) format. The active site of the receptor was predicted using Q-Site finder **[10].** The amino acids forming the first probable active site were recorded. The structure of Arecoline and Cholesterol were obtained from NCBI PubChem database in SDF format. To study the binding efficacy of Arecoline and Cholesterol to the human LDL receptor, docking experiment was carried out using BiosolveIT FlexX 1.3.0, **[11].** The PDB structure of human LDL receptor was loaded in the BioSolveIT FlexX interface. The bound "12-Tungstophosphate" was considered as cofactors of the protein. Analyzing the Q-Site Finder result, CYS 337 was considered as the central active site amino acid. Active site was defined as an area of 10Å radius surrounding CYS 337. Separate docking experiments were done for Arecoline and Cholesterol molecule with human LDL receptor, taking the same active site and same docking parameters.

As structure of human HDL receptor is not available in PDB and sequence for human HDL receptor could not be obtained, taking into account the c DNA sequence of bovine HDL receptor **[12],** and the sequence obtained from Uni-Prot database (Accession No. O18824), 3D structure of bovine HDL receptor was predicted by homology modelling using Bhageerath-H tool **[13].** Because BLAST against PDB database could not show significant alignment. So, the structure was predicted with a hybrid approach of homology modelling as well as *ab initio* prediction. Active site of the predicted bovine HDL receptor was determined again using Q-Site finder **[10].** Arecoline and thiosemicarbazone were docked against the predicted structure again using BiosolveIT FlexX 1.3.0 **[11].**

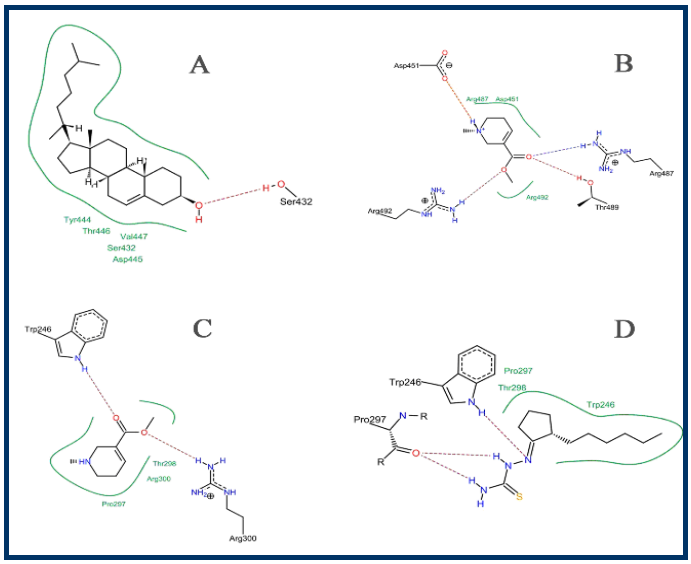

Figure 1: Docking mode of Different Ligands and Receptors. **(A)** Cholesterol bound to Extracellular domain of Human LDL Receptor; **(B)** Arecoline bound to Extracellular domain of Human LDL Receptor; **(C)** Arecoline bound to Bovine HDL Receptor; **(D)** Thiosemicarbazone bound to Bovine HDL Receptor (The red dotted line represents H-bonding and Green curves represents weak interactions)

Discussion:

The extracellular domain of human LDL receptor consists of 699 amino acid residues and 38 β-strands. The structure (PDB ID: 1N7D) was determined using X-Ray Diffraction technique at 3.7Å resolution at a pH of 5.3 **[14].** Analyzing the PDB file with Q-site finder, it was observed the most probable active site consists of CYS 337, GLN 338, ASP 339, PRO 340, THR 342, CYS 343, SER 344, GLN 345, LEU 346, CYS 347 and VAL 348. Docking result showed that both Arecoline and Cholesterol have affinity towards the extracellular domain of human LDL receptor. Cholesterol forms one H-bond with SER 432 and other weak interactions with TYR 444, ASP 445, THR 446, VAL 447 and SER 432. The total docking score for cholesterol-LDL

receptor is -0.1810, whereas, Arecoline has much higher affinity towards the LDL receptor. It forms four H-bonds with the active site amino acids, viz. ASP 451, ARG 487, THR 489 and ARG 492 and other weak interactions with ARG 487, ASP 451 and ARG 492. The total docking score of Arecoline-LDL receptor is -12.3560. It was observed that much of the energy contribution i.e. -16.3511 KCal/mol in the binding of Arecoline and LDL receptor is due to the shape complementarity, whereas, in case of Cholesterol, it is -6.7911 KCal/mol **(Figure 1 A & B), Table 1 (see supplementary material).**

Docking result of Arecoline and thiosemicarbazone with HDL receptor is presented in **(Figure 1 C & D) Table 1 (see supplementary material)** from the table it is clearly observed that Arecoline also has the potential (Score, -6.2690Kcal/Mol) to block HDL receptor though its potential is less than that (score, -10.0509 Kcal/Mol) of control. Thiosemicarbazone forms three Hydrogen bonds with the active site amino acids viz. PRO 297 and TRP 246 and other weak interactions with PRO 297, THR 298 and TRP 246. Whereas, Arecoline forms two Hydrogen bonds with TRP 246 and ARG 300 and other weak interactions with THR 298, ARG 300 and PRO 297.

Although initial report on betel quid as cardiovascular risk factor exists **[8, 9],** our work predicted that Arecoline - the major secondary metabolite of betel quid enhances atherosclerosis by interfering with LDL and HDL receptors. The result of our study showed that Arecoline binds with active site of extracellular domain of human endothelial LDL receptor with higher binding efficacy than that of Chloesterol molecules. This may inhibit initiation of endocytosis by LDL receptor thereby enhancing deposition of cholesterol in the wall of blood vessels. While looking at HDL receptor-Arecoline bonding it is observed that Arecoline has 60% bonding potential with HDL receptor in comparison to control. The work thus suggests two fold activities of Arecoline as risk factor of CVD. Though betel quid chewing habit was correlated with CVD earlier, its exact mode of action was not explained and a number of possibilities have been suggested as follows: (a) Linked to diabetes mellitus and metabolic syndrome which are independent risk factor for atherosclerosis **[15, 16];** (b) Related to elevated level of triglycerides **[17];** (c)Linked to thrombin like enzyme activity and defective fibrinolytic action **[18, 19];** (d) Stimulates platelet aggregation and thromboxane B2 production **[20];** (e) Related to acute myocardial infarction **[9].** Present observation differs from all the explanations mentioned above in the context that it has given a deeper insight into receptor mediated possible molecular mechanism of action of Arecoline in relation to CVD.

Conclusion:

We, therefore, suggest that Arecoline may be responsible for initiating and accelerating the process of atherosclerosis in the population who regularly consume areca nut by the following mechanisms: i) by way of inhibition of endocytosis of LDL cholesterol by interfering with LDL receptor ii) by preventing the uptake of LDL cholesterol by liver by interfering with HDL receptor. This study therefore, hints that there is a positive correlation between chewing betel quid and stroke or other CVD. However, wet lab study either in vitro or in vivo with Arecoline is needed to see the activity in real experimental situation. This suggestion (if confirmed) will have high relevance to the developing nations in the habit of chewing betel nut as the incidence of stroke is rising in these population and there is very little organised health care frame work to cope with this rising tide. Prevention is more cost effective than treating the disease.

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Supplementary material:

Table 1: Hydrogen bond forming components of HDL receptor and LDL receptor with Arecoline, Cholesterol and thiosemicarbazone

H-Bond forming atoms		Bond length (Å)	Bond Energy (KCal/Mol)	Total Docking Score (KCal/Mol)
Ligand	**Receptor**			
1, 2-Hexyl-1-cyclopentanone thiosemicarbazone	HDL Receptor			-10.0509
H37	O-PRO 297	1.93	-3.84	
H38	O-PRO 297	2.09	-3.72	
N12	HE1-TRP 246	1.60	-3.86	
Arecoline	HDL Receptor			
O2	HE1-TRP 346	1.95	-3.70	-6.2690
O1	HH11-ARG 300	1.91	-4.70	
Arecoline	LDL Receptor			
O6	HH21-ARG 492A	1.69	-3.62	
O8	HG1-THR 489A	1.67	-4.19	-12.3560
H25	OD1-ASP 451A	1.82	-8.30	
O8	HH21-ARG 487A	1.89	-0.24	
Cholesterol	LDL Receptor			
O1	HG-SER 432 A	1.96	-4.70	-0.1810