Combined Addiction and Neurobiological Targets: An In Silico Analysis of Areca Nut and Areca Nut with Tobacco Biomolecules

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Background: The addictive potential of areca nut (AN) and tobacco is well-documented, but their combined neurobiological effects in AN-containing tobacco products (ANTP) remain obscure. This study employed a three-stage in silico approach to investigate the potential targets and pathways associated with the addictive properties of AN alone and in ANTP. **Materials and Methods:** Bioactive molecules were retrieved for AN and tobacco, followed by target prediction and pathway enrichment analysis. The identified biomolecules were categorized into AN and ANTP groups. **Results:** A total of 195 bioactive molecules were identified (38 to AN, 157 to tobacco). Absorption, distribution, metabolism, and excretion (ADME) details were retrieved. Predicted bioactivity (gene/protein interaction probability ≥80%) was analysed, revealing 13 shared targets between AN and tobacco, 12 exclusive to AN, and 33 exclusive to tobacco. AN and ANTP influenced 21 and 27 pathways (FDR ≤ 0.05), respectively, with distinct footprints. Notably, GABAergic and cholinergic synapses, nicotine addiction, calcium signaling, and morphine addiction pathways were differentially enriched between AN and ANTP. **Discussion:** This study highlights the distinct and synergistic neurobiological effects of AN and tobacco in ANTP. The identified differences in target genes and pathways underscore the need for tailored interventions and cessation strategies for users of AN and ANTP products. Further research is warranted to validate these findings, explore interplay between diverse addiction factors, and develop effective prevention and treatment programs.

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INTRODUCTION

Tobacco and Areca nut (Areca catechu, AN) are the first and fourth most widely consumed psychoactive substances respectively across the globe. AN use is prevalent in South and Southeast Asia. Areca or betel nut have a rich history, deeply intertwined with the cultural and religious practices of these regions and still holds a vital place in socio-cultural practices (1). Tobacco has roots from Mayan-Aztec civilizations and spread globally (2). In India, the smokeless forms of tobacco are often AN-tobacco containing Products (ANTP) and 224 million Indians use these AN and ANTP.(1,3,4) However, the widespread use of both AN and ANTP is not without significant health repercussions, including oral cancer and cardiovascular disease (1,5). The first step towards preventing these diseases and conditions is the cessation of use of AN and ANTP in any form (1).

With chewing of AN, the active component arecoline, is absorbed, transported to brain, where it triggers a series of several complex neural pathways. In the brain, arecoline binds to M5 muscarinic acetylcholine receptors on Gamma-aminobutyric acid (GABA) terminals on dopamine neurons in the ventral tegmental area (VTA), facilitating the release of dopamine (5–10). On the other hand, when tobacco in any form is consumed, its principal component nicotine, is absorbed, transported and initiates a series of complex neural pathways. In the brain, nicotine binds to nicotinic acetylcholine receptors on GABA terminals on dopamine neurons in the VTA, triggering the release of dopamine (11). This increase in dopamine concentration in the VTA and other projection areas are through mechanisms carried out by the mesocorticolimbic system, which includes the VTA, nucleus accumbens and prefrontal cortex (PFC). This system is considered a principal pathway of drug reward (11).

The addictive potential of AN and ANTP has been the subject of numerous studies (1,3,5–10,12). While there are established protocols for tobacco cessation, protocols for cessation of AN and ANTP are limited (1,13). This is particularly alarming considering the synergistic effects of these substances when consumed together. Animal studies have indicated that the few constituents (probably from AN) in ANTP such as *Gutka* can have antagonist effect to tobacco, limiting the pathway(s) of nicotinic stimulation of dopamine by preferential interaction(s) (9).

Due to lack of understanding, the cessation protocols for AN products are limited (13,14). The neurobiology of arecoline, a major constituent of AN, has been reported, but the neuro-molecular biology of the whole AN and ANTP extract still remains unexplored. The neuro-molecular biology of nicotine is well-studied, the combined, cumulative end-effect of total smoked and smokeless tobacco content, largely remain under-investigated (11,15–19). Moreover, the role of lime (commonly used alkaline substance along with chewable versions of tobacco), that increases the absorption of nicotine, is not well-explored (16).

Interestingly, there is a significant difference in the chemical composition of smoked and smokeless forms of tobacco. In vitro studies have shown that these 2 different forms trigger distinctly different gene pathways in blood, buccal and immune cells (16,18,19). This is a critical area of research, especially considering that the intention and attempts to quit are more common in smokers than in smokeless tobacco users (20). These inherent variations can possibly have a cascading effect on cessation treatment too.

Understanding this complex molecular mechanism is crucial for the development of effective prevention and treatment strategies. The recent advances in computational tools and biological databases provide unprecedented opportunities to delve deeper into this complex issue.

This study aims to leverage bioinformatics tools to systematically investigate the potential targets and pathways associated with the addictive properties of AN and ANTP. Through a comprehensive analysis of their bioactive compounds, predicted target interactions, and enriched biological pathways, we aim to elucidate the molecular underpinnings of their addictive potential. This may pave the way for targeted interventions to combat this prevalent public health concern, thereby contributing to the global efforts in the fight against addiction.

MATERIAL AND METHODS

The study was done in three stages – the first was to collect the bioactive compound and process the same. The next was to assess their bioavailability and target (gene/protein) prediction. Using the target, pathway enrichment analysis was carried out.

For the bioactive compound retrieval and processing, bioactive compounds associated with areca nut (*Areca catechu*) and tobacco (*Nicotiana rustica & Nicotiana tabacum*) were retrieved from the latest version of Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT-2.0). This ensures access to

Table 1. Pathway enrichment analysis of areca nut group

Pathway	KEGG PATHWAY Number	Genes	Number of Genes	Total Pathway Genes	Fold Enrichment	Enrichment FDR
PPAR signaling pathway	hsa03320	PPARD FABP3 PPARG FABP2 FABP1 FABP5 FABP4 PPARA	8	75	101.3156	3.36e-13
Nicotine addiction	hsa05033	GABRA3 GABRB2 GABRA2	3	40	71.2375	8.39e-05
Nitrogen metabolism	hsa00910	CA2	1	17	55.87255	0.043117
Cholinergic synapse	hsa04725	SLC5A7 CHRM3 CHRM1 CHRM4 CHRM2 CHRM5	6	113	50.43363	2.88e-08
Fat digestion and absorption	hsa04975	FABP2 FABP1	2	42	45.23016	0.004133
GABAergic synapse	hsa04727	GABRA3 GABRB2 GABRA2 SLC6A1	4	89	42.68914	2.77e-05
Taste transduction	hsa04742	GABRA3 CHRM3 GABRA2	3	85	33.52353	0.000598
Morphine addiction	hsa05032	GABRA3 GABRB2 GABRA2	3	91	31.31319	0.000598
Retrograde endocannabinoid signaling	hsa04723	GABRA3 FAAH GABRB2 GABRA2	4	148	25.67117	0.000119
Gastric acid secretion	hsa04971	CA2 CHRM3	2	76	24.99561	0.011326
Insulin secretion	hsa04911	FFAR1 CHRM3	2	86	22.08915	0.013401
Reg. of actin cytoskeleton	hsa04810	CHRM3 CHRM1 CHRM4 CHRM2 CHRM5	5	217	21.88556	2.79e-05
Neuroactive ligand-receptor interaction	hsa04080	GABRA3 CHRM3 GABRB2 GABRA2 CHRM1 CHRM4 CHRM2 CHRM5	8	350	21.71048	2.88e-08
Pancreatic secretion	hsa04972	CA2 CHRM3	2	101	18.80858	0.017116
Calcium signaling pathway	hsa04020	CHRM3 CHRM1 CHRM2 CHRM5	4	240	15.83056	0.000598
Alcoholic liver disease	hsa04936	FABP1 PPARA	2	141	13.47281	0.028771
CAMP signaling pathway	hsa04024	CHRM1 CHRM2 PPARA	3	219	13.01142	0.006482
Non-alcoholic fatty liver disease	hsa04932	PPARG PPARA	2	155	12.25591	0.032565
Alzheimer disease	hsa05010	CHRM3 CHRM1 CHRM5	3	383	7.439948	0.023363
Pathways of neurodegeneration	hsa05022	CHRM3 CHRM1 CHRM5	3	475	5.998947	0.035284
Pathways in cancer	hsa05200	PPARD PPARG TERT	3	530	5.376415	0.043117

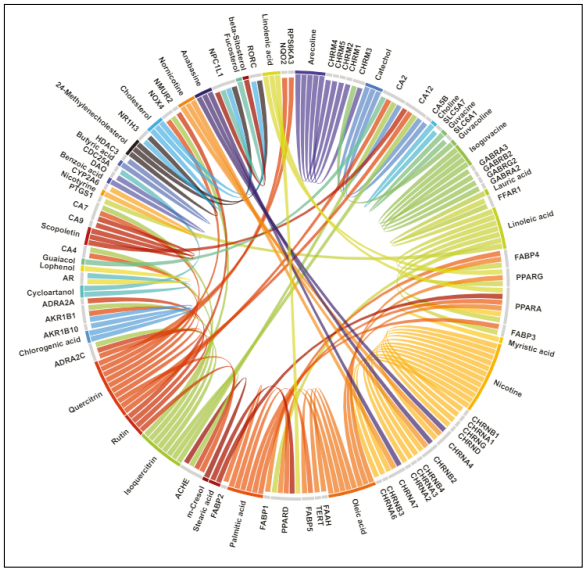
a comprehensive and updated database of relevant biomolecules (21). Compound names were manually extracted from IMPPAT 2.0, ensuring accuracy and comprehensiveness. Phytochemical names were converted to their Simplified Molecular Input Line Entry System (SMILES) format at https://cactus.nci.nih.gov/chemical/structure for easier computational analysis. This step standardizes the representation of each compound for further processing.

For bioavailability assessment and target prediction, Absorption, Distribution, Metabolism and Excretion(ADME) studies were done. For each SMILES string collected at the last step, SWISS ADME (http://www.swissadme.ch/index.php) was used to predict key bioavailability characteristics including lipophilicity, LogP, solubility, and various ADME parameters (22). This provides insights into the potential absorption, distribution, metabolism, and excretion of each compound, aiding in understanding their biological significance. Subsequently, each SMILES string was used as input in the web tool of Swiss Target Prediction

Table 2. Pathway enrichment analysis of areca nut and tobacco group

Pathway	KEGG PATHWAY Number	Genes	Number of Genes	Total Pathway Genes	Fold Enrichment	Enrichment FDR
Nitrogen metabolism	00910	CA2 CA9 CA4 CA7	4	17	95.78151	1.34e-06
Caffeine metabolism	00232	CYP2A6	1	5	81.41429	0.043028
PPAR signaling pathway	03320	NR1H3 PPARD FABP3 PPARG FABP2 FABP1 FABP5 FABP4 PPARA	9	75	48.84857	5.40e-12
Cholinergic synapse	04725	CHRNA3 ACHE CHRNA4 SLC5A7 CHRNB4 CHRM3 CHRNA6 CHRNB2 CHRM1 CHRM4 CHRM2 CHRM5	12	113	43.22882	2.47e-15
Nicotine addiction	05033	GABRA3 GABRB2 GABRA2 CHRNB2	4	40	40.70714	4.10e-05
Proximal tubule bicarbonate reclamation	04964	CA2 CA4	2	23	35.39752	0.009201
Folate biosynthesis	00790	AKR1B1 AKR1B10	2	26	31.31319	0.010881
Fat digestion and absorption	04975	NPC1L1 FABP2 FABP1	3	42	29.07653	0.001657
Galactose metabolism	00052	AKR1B1 AKR1B10	2	31	26.26267	0.013629
Fructose and mannose metabolism	00051	AKR1B1 AKR1B10	2	33	24.671	0.013803
Neuroactive ligand-receptor interaction	04080	GABRA3 CHRNA3 CHRNA4 CHRNB4 CHRNA2 NMUR2 CHRM3 CHRND CHRNA1 GABRB2 CHRNB3 CHRNA6 ADRA2A GABRA2 CHRNB2 CHRM1 CHRM4 CHRM2 ADRA2C CHRM5 CHRNG	21	350	24.42429	3.25e-22
Pentose and glucuronate interconversions	00040	AKR1B1 AKR1B10	2	34	23.94538	0.013911
GABAergic synapse	04727	GABRA3 GABRB2 GABRA2 SLC6A1	4	89	18.29535	0.000858
Chemical carcinogenesis	05207	CHRNA3 CHRNA4 CHRNB4 CHRNB2 CDC25A AR RPS6KA3 PPARA	8	197	16.53082	5.91e-07
Reg. of lipolysis in adipocytes	04923	PTGS1 FABP4	2	56	14.53827	0.032079
Taste transduction	04742	GABRA3 CHRM3 GABRA2	3	85	14.36723	0.008819
Glycerolipid metabolism	00561	AKR1B1 AKR1B10	2	60	13.56905	0.035113
Morphine addiction	05032	GABRA3 GABRB2 GABRA2	3	91	13.41994	0.009201
Insulin resistance	04931	NR1H3 RPS6KA3 PPARA	3	108	11.30754	0.013137
Retrograde endocannabinoid signaling	04723	GABRA3 FAAH GABRB2 GABRA2	4	148	11.00193	0.004242
Gastric acid secretion	04971	CA2 CHRM3	2	76	10.71241	0.049015
Reg. of actin cytoskeleton	04810	CHRM3 CHRM1 CHRM4 CHRM2 CHRM5	5	217	9.379526	0.001878
Alcoholic liver disease	04936	NOX4 FABP1 PPARA	3	141	8.661094	0.021178
Non-alcoholic fatty liver disease	04932	NR1H3 PPARG PPARA	3	155	7.878802	0.026266
Calcium signaling pathway	04020	CHRM3 CHRM1 CHRM2 CHRM5	4	240	6.784524	0.013803
Alzheimer disease	05010	NOX4 CHRM3 CHRM1 CHRM5	4	383	4.251399	0.049015
Metabolic pathways	01100	CA12 AKR1B1 PTGS1 CA2 CA9 DAO CA4 CA7 CA5B AKR1B10 CYP2A6	11	1527	2.932407	0.008523

Figure 1. Image depicting relationship of phytobiomolecule with genes



(http://www.swisstargetprediction.ch/) to identify potential macromolecular targets with high binding affinity.(22) This step predicts likely protein interactions, shedding light on the mechanisms of action for each compound. Swiss Target Prediction offers predictions based on 2D and 3D similarity with a vast library of known ligands (23). To increase precision, only gene/protein targets in Homo sapiens with a predicted bioactivity interaction probability of ≥80% were considered for further analysis. This stringent filtering helps identify the most probable and relevant targets for investigation.

The final step was pathway enrichment analysis. For this identified biomolecules were categorized into two groups: Group – A (areca alone) and B (with Areca and tobacco together). This segregation allows for separate analysis of effects specific to each combination. ShinyGO version 0.77 (http://bioinformatics.sdstate.edu/go/) was employed for gene enrichment analysis.(24) Gene lists for group-A and B were input to ShinyGO to identify enriched human Gene Ontology (GO) terms. This step reveals broader functional categories associated with the gene sets. ShinyGO facilitates the generation of hierarchical clustering trees with enriched GO terms. Specifying an aspect ratio of 4 enhances readability and clarity of the visualization. Focusing on addiction/dependence related pathways, KEGG pathway analysis was performed with a false discovery (FDR) rate cut-off of ≤0.05 and top 30 pathways selection. This step identifies specific metabolic and signalling pathways potentially involved in addiction biology associated with AN and ANTP consumption. The difference in key addiction related Kyoto Encyclopaedia of Genes and Genomes(KEGG) pathways in both groups are highlighted.

RESULTS

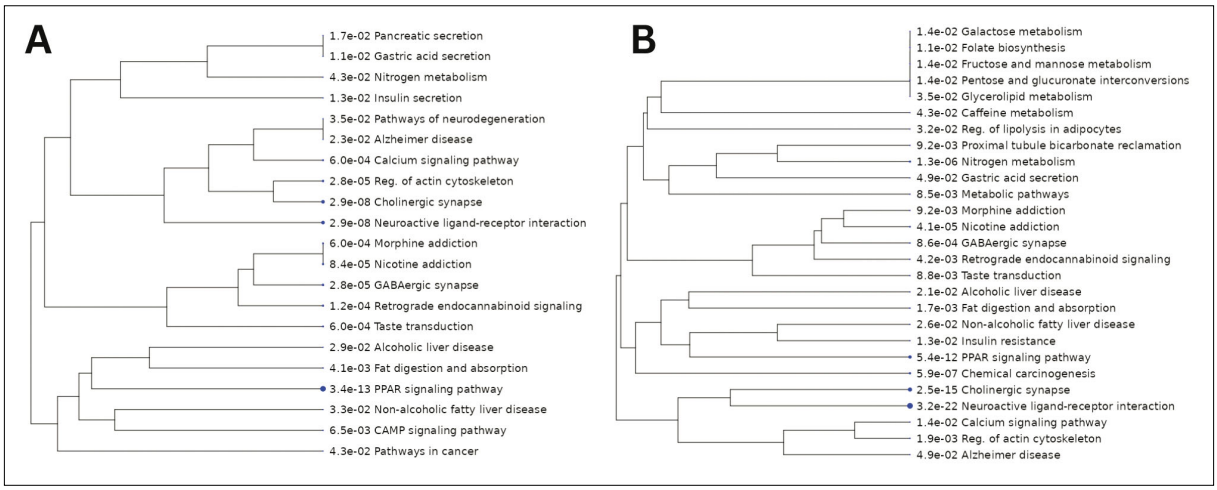
From the IMPPAT 2.0, there were 38 and 157 bioactive molecules retrieved for areca nut and tobacco leaf/stem products respectively. From these, 36(94.74%) and 141(89.81%) SMILES for these biomolecules were retrieved. The ADME details of all these products are given separately, as supplemental file-1 and 2. Swiss target prediction revealed that, of these biomolecules, there were 25 and 46 gene/protein for AN and tobacco respectively, which served as target site of action with a probability of ≥80%. The gene-biomolecule interaction are depicted in Figure 1.

Of these, 13 genes were observed to be associated with both areca and tobacco biomolecules. They are – CA2, CA12, FFAR1, FABP4, PPARG, PPARA, FABP3, FAAH, TERT, FABP5, PPARD, FABP1 and FABP2. Twelve genes (CHRM4, CHRM5, CHRM2, CHRM1, CHRM3, CA5B, SLC5A7, SLC6A1, GABRA3, GABRB2, GABRG4, GABRA2) were exclusive to AN while 33 genes (CHRNB1, CHRNA1, CHRNG, CHRND, ACHE, ADRA2C, AKR1B10, AKR1B1, ADRA2A, AR, CA4, CA9, CA7, PTGS1, CYP2A6, DAO, CDC25A, HDAC3, NR1H3, NOX4, NMUR2, CHRNA4, CHRNA7, CHRNA3, CHRNA2, CHRNB2, CHRNB4, CHRNB3, CHRNA6, NPC1L1, RORC, NQO2 and RPS6KA3) were exclusive to tobacco.

The Figure 2. shows the hierarchical clustering trees with enriched GO terms while Figure 3. demonstrates the network. Table 1., 2. shows the enrichment analysis of group-A and B genes. Pathway analysis with high FDR and adjusted p-values revealed difference between the group-A and B set of genes. There were 21 and 27 pathways with FDR≤0.05 for group-A and B genes respectively.

The role of particular A and B group genes in KEGG pathways of GABAergic synapse, cholinergic synapse, nicotine addiction, calcium channel signalling pathway and morphine addiction were highlighted (Supplemental files:3-8). Certain genes in group-A exclusively worked in the GABAergic synapse (GABRA3, GABRB2, GABRA2, SLC6A1) as well as in morphine addiction (GABRA3, GABRB2, GABRA2). By virtue of AN related genes presence in group-B, these two pathways were also involved.

Figure 2. The hierarchical clustering trees with enriched Gene ontology terms. A. Areca nut B. Areca nut with tobacco



The cholinergic pathway in group-A was associated with SLC5A7, CHRM3, CHRM1, CHRM4, CHRM2, CHRM5 while in group-B, it involved more genes namely - CHRNA3, ACHE, CHRNA4, SLC5A7, CHRNB4, CHRM3, CHRNA6, CHRNB2, CHRM1, CHRM4, CHRM2 and CHRM5. The nicotine addiction pathway (supplement file-3) in group-A involved GABRA3, GABRB2 and GABRA2 while the same in group-B was associated additionally with CHRNB2. Similarly, the retrograde endocannabinoid signalling pathway (supplement file-4) in group-A and B involved GABRA3, FAAH, GABRB2 and GABRA2 genes. In the calcium channel signalling pathway (supplement file-5), group-A influences G protein-coupled receptors (GPCR) internalization and acts through CHRM3, CHRM1, CHRM2 and CHRM5 while for the group-B it involves CHRNA7(ROC-Receptor operated channel) besides the CHRM3, CHRM1, CHRM2 and CHRM5.

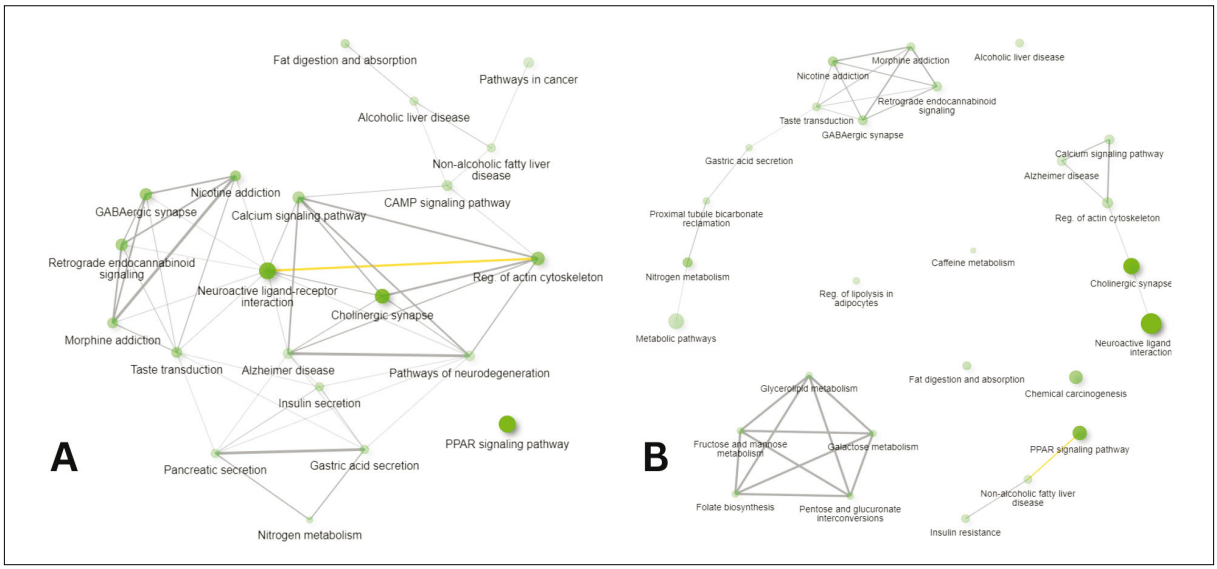
DISCUSSION

The neurobiological effects and dynamics of tobacco, as nicotine, are widely studied while the AN and ANTP are not as widely studied (16-18,25). This is one of the several reason for the non-evolution of AN and ANTP specific cessation and dependence management protocol (1,13,14). The present study was undertaken to find the similarities and differences in potential targets and pathways associated with the addictive properties of AN and ANTP through an in-silico approach.

From the results of the present study, it is observed that like any other substances of abuse, the reward pathway is at the central to the AN and tobacco use. The dopamine signalling pathway is at the core (26). Gene/protein including PPAR and FABPs regulate dopamine transport and metabolism, playing a vital role in the addiction of AN and ANTP. Both group of genes/proteins converge in this core action, activating these reward pathways through distinct mechanisms. The target is usually the dopamine neurons in the VTA and substantia nigra in the midbrain. AN stimulates dopamine release via nicotinic receptors and potentially through its own alkaloids, involving CHRM4, CHRM5 (nicotinic receptors), PPARG, FABP4, FABP3 that facilitate dopamine transport, metabolism and reward. Tobacco acts primarily through nicotine, directly binding to nicotine receptors and acting through CHRNB1, CHRNA2, CHRNA4 (nicotinic receptors) and DAT (dopamine reuptake transporter) ultimately triggering dopamine release. The signalling molecules would include possibly the dopamine, acetylcholine and anandamide (endocannabinoid). Tobacco and AN activates nicotinic receptors, leading to dopamine release. Individually, AN activates opioid receptors, enhancing dopamine reward while tobacco directly stimulates DAT, causing sustained dopamine levels. Concomitant use, as in ANTP, may lead to synergistic effects, significantly amplifying dopamine signalling or preferential docking, as reported by some studies. It is widely agreed that the dopamine-mesolimbic motivation-reward-reinforcement cycle a hall mark feature in addiction (5,6,8-11,14,18,26).

After this stage, the pathways between AN and ANTP diverges and branches out to additional players. AN activates opioid signalling and probably

Figure 3. The network relationship of the pathways identified in figure-2 A. Areca nut B. Areca nut with tobacco



influence the glutamatergic pathway, further enhancing reward and potentially mediating its unique psychoactive effects. As a part of the opioid signalling pathway, AN triggered genes/proteins could possibly liaise with a μ opioid receptor OPRM1 and FAAH (endocannabinoid degradation enzyme) (5,6,12,14,27). Resultantly, β-endorphin (endogenous opioid) and anandamide (endocannabinoid) could be triggered enhancing reward and pleasure. Increased FAAH activity breaks down anandamide, potentially contributing to dependence (28). Tobacco, on the other hand, has no action on opioid signalling and mediates through stress and inflammatory pathways, possibly contributing to its mood-altering and withdrawal symptoms (11).

Tobacco and AN connect again at neuroadaptation and tolerance pathways (12,25). This could be the basis of both AN and tobacco, leading to tolerance and dependence over time. In ANTP, the combined activation of multiple reward pathways leads to significantly higher dopamine levels and stronger reward signals. Additionally, ANTP may accelerate neuroadaptation and tolerance due to the activation of both stress and reward pathways.

Carbonic Anhydrase (CA) activation is associated with enhanced memory. In the brain, CAs play a pivotal role in several physiological functions, including the regulation of pH levels in neurons and the extracellular space through the regulation of ionic gradients of bicarbonate ions. Specifically, CAs contribute to the availability of protons and bicarbonate ions, which are necessary for the transmission of neuronal signalling. This, in turn, it influences the function of proton-sensitive membrane proteins, regulating the kinetics and the concentration of pH transition into intra- and extracellular compartments. These proteins include GABA agonist receptors (GABAARs), N-methyl-D-aspartate receptors (NDMA), and ionic channels (29). In the hippocampus of rats, it has been demonstrated that the excitation mediated by GABA receptors depends on HCO_3^- concentration, which is regulated by the cytosolic activity of CAs. Information processing and memory storage require synchronized neuronal activity, commonly known as hippocampal theta rhythm. This rhythm is associated with the GABAergic postsynaptic depolarization into pyramidal cells in the hippocampus, with an inverted potential from Cl^- to HCO_3^-, and this process is regulated by CAs. Importantly, this CA modulation and theta rhythm specifically affect learning, but not other sensory or locomotor behaviours. CA activators may increase the efficacy of temporal activity of cholinergic and GABAergic inputs, transforming the hyperpolarizing GABAergic postsynaptic potential from inhibitory to excitatory. This may be via the reduced intracellular concentrations of HCO_3^-, favouring its outflow through the channel receptor GABAA. Thus, the regulation of ionic gradients has several effects on post-synaptic depolarization, with benefits of increasing memory and learning. As the GABAergic postsynaptic potential becomes excitatory, it potentiates the signal transfer (29).

In light of these facts, exposure to group-B (ANTP), could lead to alterations in the CAs as opposed to lesser changes in group-A(AN). These subtle changes could influence cognition, memory formation, and consolidation. Present results indicate that with ANTP gene/protein of CA2, CA12, CA5B, CA4, CA9 and CA7 are modulated. The variation between AN and ANTP are marked in terms of CA family expression. AN and ANTP could potentially modulate CA activity, thereby influencing cognitive functions. Further research is needed to fully understand these effects, particularly the impact of AN and ANTP on higher executive function, memory formation and consolidation.

The changes in calcium channel signalling also can bring in changes in permeability of the cell as well as passing of neural signals across synapses. Many drugs of abuse are known to employ this pathway to cause their effect (30). The changes in these signalling pathway can have ramification in the addiction biology. In the presence of tobacco, receptor operated channels (ROC) are involved, allowing movement of CHRNA7 that could further alter the intracellular Ca^{2+} ion movement, influencing the signalling pathway (31).

The nicotine addiction pathway (Supplemental file-3) has emerged more interesting. With use of tobacco, all nicotinic acetylcholine receptors are triggered increasing the synaptic transfers in the VTA. The GABAergic and glutamatergic triggers are suppressed in tobacco users, while AN in the ANTP would trigger the GABA and glutamatergic receptors also, increasing the increased excitation of the dopaminergic neuron releasing more dopamine in the nucleus accumbens contributing to the "high". Also, these signals alter the Cl^{-}, Ca^{2+} and Na^{+} ion flow, which further influences the "high". Superimposed on this is the changes in calcium channel signalling pathway that could further alter the flow of ions in the involved cells.

As outlined, the addiction biology between AN and ANTP probably emanates from differences in nicotine addiction (Supplemental file-3), calcium channel signalling pathway (Supplemental file-5), cholinergic synapse (Supplemental file-6), GABAergic synapse (Supplemental file-7), and morphine addiction pathways (Supplemental file-8). Other physical factors of the AN and ANTP such as moisture content, pH of the salivary bolus, shape & texture of particles besides volume of saliva, the protonated and unprotonated forms of tobacco could influence the neuro-biological variations between AN and ANTP at molecular level.(3)

The strength of the study is that it aims to leverage bioinformatics tools to systematically investigate the potential targets and pathways associated with the addictive properties of AN and ANTP. It is a crucial and under-researched area that may have ramification on public health and policies. The study employed a three-stage approach encompassing biomolecule retrieval, target prediction, and pathway enrichment analysis, using established and reliable tools like IMPPAT, SwissADME, Swiss Target Prediction, and ShinyGO. The performance of these tools has been peer-reviewed and accepted by the scientific community. The study had employed stringent filtering criteria and appropriate statistical tests to draw meaningful conclusions. The study identified potential differences in the addiction biology of AN and ANTP, highlighting the role of distinct pathways and the synergistic effects of combined use.

While this study provides valuable insights into potential AN and ANTP mechanisms, further in vitro and in vivo studies are needed to validate the findings. The study relies on existing databases for biomolecules and targets, which may not be exhaustive and could potentially miss other relevant, important bioactive entities. The complex interplay between biological pathways and their role in addiction may not be fully captured by this analysis. Furthermore, the study exclusively addresses the neurobiology of addiction but does not consider the psychological and social factors that contribute to behaviour and dependence.

CONCLUSION

This study provides valuable insights into the potential molecular mechanisms underlying the addictive properties of AN and ANTP. The identified differences in neurobiological pathways and the synergistic effects of combined use highlight the need for targeted interventions and tailored cessation strategies for users of these products. Further research is necessary to validate these findings, explore the interplay of diverse factors influencing addiction, and develop effective prevention and treatment programs.

SUPPLEMENTAL FILES

– Use the link to access https://figshare.com/s/848009d22549252b14e8

- Supplemental file-1: ADME characteristics of biomolecules associated with areca nut only
- Supplemental file-2: ADME characteristics of biomolecules associated with areca nut and tobacco products
- Supplemental file-3: KEGG Pathway of nicotine addiction. Red colour – gene/protein influenced by areca nut; Red color with blue circle - gene/protein influenced by areca nut product with tobacco
- Supplemental file-4: KEGG Pathway of Retrograde endocannabinoid signalling pathway. Red colour – gene/protein influenced by areca nut; Red colour with blue circle - gene/protein influenced by areca nut product with tobacco
- Supplemental file-5: KEGG Pathway of Calcium channel signalling pathway. Red colour – gene/protein influenced by areca nut; Red colour with blue circle - gene/protein influenced by areca nut product with tobacco
- Supplemental file-6: KEGG Pathway of cholinergic synapse. Red colour – gene/protein influenced by areca nut; Red colour with blue circle - gene/protein influenced by areca nut product with tobacco
- Supplemental file-7: KEGG Pathway of GABAergic synapse. Red colour – gene/protein influenced by areca nut; Red colour with blue circle - gene/protein influenced by areca nut product with tobacco
- Supplemental file-8: KEGG Pathway of morphine addiction. Red colour – gene/protein influenced by areca nut; Red colour with blue circle - gene/protein influenced by areca nut product with tobacco

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REFERENCES

1. Thavarajah R, Ranganathan K, Joshua E, Rao U. Areca nut use disorder: A dynamic model map. Indian J Dent Res. 2019;30(4):612.
2. Carod-Artal FJ. Hallucinogenic drugs in pre-Columbian Mesoamerican cultures. Neurología (English Edition). 2015 Jan;30(1):42–9.
3. Sharma P, Cheah NP, Kaur J, Sathiya Kumar S, Rao V, Morsed FA, et al. Physical and chemical characterization of smokeless tobacco products in India. Sci Rep. 2023 Jun 1;13(1):8901.
4. Singh PK, Yadav A, Singh L, Mazumdar S, Sinha DN, Straif K, et al. Areca nut consumption with and without tobacco among the adult population: a nationally representative study from India. BMJ Open. 2021 Jun;11(6):e043987.
5. Garg K, Kumar A, Kizhakkethil V, Kumar P, Singh S. Overlap in oncogenic and pro-inflammatory pathways associated with areca nut and nicotine exposure. Cancer Pathogenesis and Therapy. 2023 Sep;S2949713223000538.
6. Sariah A, Guo S, Zuo J, Pu W, Liu H, Rolls ET, et al. Acute and Chronic Effects of Betel Quid Chewing on Brain Functional Connectivity. Front Psychiatry. 2020 Mar 17;11:198.
7. Li M, Pang X, Guo Z, Yang Y, Gu Z, Zhang L. Integrated metabolomics and network pharmacology to reveal the mechanism of areca nut addiction. Addiction Biology. 2023 Dec;28(12):e13352.
8. Papke RL, Horenstein NA, Stokes C. Nicotinic Activity of Arecoline, the Psychoactive Element of "Betel Nuts", Suggests a Basis for Habitual Use and Anti-Inflammatory Activity. Simon SA, editor. PLoS ONE. 2015 Oct 21;10(10):e0140907.
9. O'Neill B, Lauterstein D, Patel JC, Zelikoff JT, Rice ME. Striatal Dopamine Release Regulation by the Cholinergic Properties of the Smokeless Tobacco, Gutkha. ACS Chem Neurosci. 2015 Jun 17;6(6):832–7.
10. Yang Y, Huang H, Cui Z, Chu J, Du G. UPLC–MS/MS and Network Pharmacology-Based Analysis of Bioactive Anti-Depression Compounds in Betel Nut. DDDT. 2021 Nov;Volume 15:4827–36.
11. Gogineni HP, Sachs DPL, Brunzell DH. Neurobiology and Mechanisms of Nicotine Addiction. In: Eakin MN, Kathuria H, editors. Tobacco Dependence [Internet]. Cham: Springer International Publishing; 2023 [cited 2024 Jan 11]. p. 45–74. (Respiratory Medicine). Available from: https://link.springer.com/10.1007/978-3-031-24914-3_3
12. Jensen KP, DeVito EE, Yip S, Carroll KM, Sofuoglu M. The Cholinergic System as a Treatment Target for Opioid Use Disorder. CNS Drugs. 2018 Nov;32(11):981–96.
13. Sumithrarachchi S, Jayasinghe R. Pharmaceutical Agents for Areca Nut Cessation. In: Warnakulasuriya S, Ranganathan K, editors. Oral Submucous Fibrosis [Internet]. Cham: Springer International Publishing; 2023 [cited 2023 Nov 13]. p. 349–55. (Textbooks in Contemporary Dentistry). Available from: https://link.springer.com/10.1007/978-3-031-12855-4_21
14. Sumithrarachchi SR, Jayasinghe R, Warnakulasuriya S. Betel Quid Addiction: A Review of Its Addiction Mechanisms and Pharmacological Management as an Emerging Modality for Habit Cessation. Substance Use & Misuse. 2021 Nov 10;56(13):2017–25.
15. Le Foll B, Piper ME, Fowler CD, Tonstad S, Bierut L, Lu L, et al. Tobacco and nicotine use. Nat Rev Dis Primers. 2022 Mar 24;8(1):19.

16. Olsson Gisleskog PO, Perez Ruixo JJ, Westin Å, Hansson AC, Soons PA. Nicotine Population Pharmacokinetics in Healthy Smokers After Intravenous, Oral, Buccal and Transdermal Administration. Clin Pharmacokinet. 2021 Apr;60(4):541–61.
17. Schmidt HD, Rupprecht LE, Addy NA. Neurobiological and Neurophysiological Mechanisms Underlying Nicotine Seeking and Smoking Relapse. Complex Psychiatry. 2018;4(4):169–89.
18. Liu G, Arimilli S, Savage E, Prasad GL. Cigarette smoke preparations, not moist snuff, impair expression of genes involved in immune signaling and cytolytic functions. Sci Rep. 2019 Sep 16;9(1):13390.
19. Arimilli S, Madahian B, Chen P, Marano K, Prasad GL. Gene expression profiles associated with cigarette smoking and moist snuff consumption. BMC Genomics. 2017 Dec;18(1):156.
20. Islam MM. Comparison between Smokers and Smokeless Tobacco Users in Their Past Attempts and Intentions to Quit: Analysis of Two Rounds of a National Survey. IJERPH. 2022 Oct 21;19(20):13662.
21. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A. IMPPAT 2.0: An Enhanced and Expanded Phytochemical Atlas of Indian Medicinal Plants. ACS Omega. 2023 Mar 7;8(9):8827–45.
22. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017 Mar 3;7(1):42717.
23. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Research. 2019 Jul 2;47(W1):W357–64.
24. Ge SX, Jung D, Yao R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Valencia A, editor. Bioinformatics. 2020 Apr 15;36(8):2628–9.
25. Christie MJ. Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction. British J Pharmacology. 2008 May;154(2):384–96.
26. Volkow ND, Michaelides M, Baler R. The Neuroscience of Drug Reward and Addiction. Physiological Reviews. 2019 Oct 1;99(4):2115–40.
27. Li M, Pang X, Guo Z, Yang Y, Gu Z, Zhang L. Integrated metabolomics and network pharmacology to reveal the mechanism of areca nut addiction. Addiction Biology. 2023 Dec;28(12):e13352.
28. Parsons LH, Hurd YL. Endocannabinoid signalling in reward and addiction. Nat Rev Neurosci. 2015 Oct;16(10):579–94.
29. Poggetti V, Salerno S, Baglini E, Barresi E, Da Settimo F, Taliani S. Carbonic Anhydrase Activators for Neurodegeneration: An Overview. Molecules. 2022 Apr 14;27(8):2544.
30. Sanchez-Reyes OB, Zilberg G, McCorvy JD, Wacker D. Molecular insights into GPCR mechanisms for drugs of abuse. Journal of Biological Chemistry. 2023 Sep;299(9):105176.
31. Sassano MF, Ghosh A, Tarran R. Tobacco Smoke Constituents Trigger Cytoplasmic Calcium Release. Applied In Vitro Toxicology. 2017 Jun;3(2):193–8.

Kombinált függőség és neurobiológiai támadáspontok: az Areca dió és az Areca dió+dohány biomolekulák in silico elemzése

Háttér: Az areca dió (AN) és a dohány addiktív potenciálja jól dokumentált, de az AN-t tartalmazó dohánytermékekben (ANTP) ezek együttes neurobiológiai hatása kevéssé ismert. Kutatásunk háromlépcsős in silico megközelítést alkalmazott az AN és az ANTP addiktív tulajdonságaihoz kapcsolódó lehetséges célpontok és útvonalak vizsgálatára. **Anyagok és módszerek:** Azonosítottuk az AN és a dohány bioaktív molekuláit, melyet a támadási célpontok predikciója és útvonal enrichment elemzés követett. Az azonosított biomolekulákat AN és ANTP csoportokba soroltuk. **Eredmények:** Összesen 195 bioaktív molekulát azonosítottunk (38 az AN, 157 a dohány esetében). Az abszorpcióval, eloszlással, metabolizmussal és kiválasztással kapcsolatos folyamatok részleteit lekértük. Elemeztük a bejósolt bioaktivitást (gén/fehérje interakció valószínűsége ≥80%), és 13 közös célpontot tártunk fel az AN és a dohány között, 12-t kizárólag az AN, és 33-at kizárólag a dohány esetében. Az AN és az ANTP 21, illetve 27 útvonalat befolyásolt (FDR ≤ 0,05), de eltérő módon. Nevezetesen, a GABAerg és kolinerg szinapszisok, a nikotin-függőség, a kalcium-jelátvitel és a morfium-függőségi útvonalak esetében eltérő feldúsulást tapasztaltunk az AN és az ANTP esetében. **Megbeszélés:** Kutatásunk rávilágít az AN és a dohány különálló és szinergikus neurobiológiai hatásaira az ANTP-ben. A célgénekben és útvonalakban feltárt különbségek rávilágítanak arra, hogy az AN és ANTP termékek felhasználói számára testreszabott beavatkozásokra és leszokási stratégiákra van szükség. További kutatások szükségesek ezeknek az eredményeknek a validálására, a különböző függőségi tényezők közötti kölcsönhatások feltárására, valamint hatékony megelőzési és kezelési programok kidolgozására.

Kulcsszavak: Areca dió, dohány, rágás, viselkedéses addikció, neurobiológia