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Arecoline Biological Activity and Biotransformation: A review

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ABSTRACT

Arecoline is a psychoactive alkaloid containing a reduced pyridine nucleus, isolated from *Areca catechu* L. (Arecaceae) with different biological activities on cardiovascular, digestive, nervous, and endocrine systems. Arecoline is the main toxic component of *A. catechu* responsible for oral carcinoma. It possesses a variety of pharmacological activities; it exhibits anticancer activity. It was reported that arecoline causes cytotoxicity through apoptosis in human endothelial cells. Moreover, the death of human leukemia K562 cells was induced by arecoline. Biotransformation is a structural modification of compounds such as amino acids, toxins, and drugs by enzymatic chemical reactions within living organisms. According to enzymatic sources, biotransformation is classified into three major types; microbial, plant cell culture, and animal cell culture transformation. It was suggested that microorganisms could be employed as a model of mammalian metabolism. Studying both mammalian and microbial transformation of arecoline is of interest by which we can reduce its toxicity, increase its bioavailability, and produce more active metabolites.

Keywords: Arecoline, Microbial Transformation, Biological Activity, Biotransformation, Cytotoxicity

INTRODUCTION

Biotransformation is defined as a biochemical reaction that converts xenobiotics with the aid of a plant cell, an animal cell, a microorganism, or an isolated enzyme into metabolites. It is mainly an enzyme-catalyzed reaction.[1] The main goal of biotransformation is the conversion of poorly excreted lipophilic molecules into more easily excreted hydrophilic metabolites.[2] Biotransformation results in the conversion of an active molecule to more active metabolites (bioactivation) or less active metabolites (inactivation or detoxification). It also causes the activation of inactive substances such as pro-drugs.[3]

In nature, there are many natural products such as flavonoids, alkaloids, anthraquinones, tannins, carbohydrates, lipids, etc. Among these natural products, alkaloids are considered an important class with various pharmacological activities.[4] Alkaloids are produced by complex biosynthetic pathways from many sources such as marines, bacteria, and plants, or by synthetic reactions. They possess wide pharmacological effects.[5, 6] Alkaloids chemistry is among the most exciting and significant areas of bioorganic chemistry. The most common alkaloids classification is based on their distribution according to the principal structure and principal C-N skeleton such as pyridine, piperidine, pyrrolidine, indole, quinoline, quinazoline, isoquinoline, etc. Another classification is according to the biogenetic precursors they are derived from amino acids such as ornithine, lysine, tyrosine, tryptophan, etc.[7]

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Biotransformation of alkaloids continued to cover a wide range of substrates and enzymes. The identification of novel biosynthetic and catabolic pathways for alkaloids helps to provide new biocatalysts for chemical synthesis.[3]

Arecoline is a psychoactive alkaloid isolated from areca nuts. In addition to arecoline, areca nuts contain the alkaloids guvacoline, arecaidine, and guvacine.[8] Arecoline has a variety of effects on the central nervous system such as alertness, stimulation, euphoria, and anxiolytic action due to its partial agonist activity on the nicotinic and muscarinic acetylcholine receptors (mAChR).[9]

1. Physical properties

Arecoline is an oily base, volatile with steam, and miscible with many solvents such as ether, alcohols, dimethyl sulfoxide (DMSO), dimethyl formamide, and water.[10]

2. Biosynthesis of arecoline

Arecoline is expected to be biosynthesized from nicotinic acid as a precursor (Figure 1). The biosynthesis of nicotinic acid starts from the amino acid tryptophan through an oxidative cleavage reaction of the indole system to give *N*-formyl kynurenine. The latter compound loses its formyl group to generate L.kynurenine which by ring hydroxylation produces 3-hydroxy kynurenine that is converted to 3-hydroxy anthranillic acid. Ring-opening by oxidation followed by ring closure results in the formation of nicotinic acid from 3-hydroxyanthranilic acid. Arecoline is formed from nicotinic acid by esterification, *N*-methylation, and partial reduction of the ring.[11, 12]

3. Biological activity and mechanism of action of arecoline

Arecoline is the main active component in the areca nuts responsible for the central nervous system effects. Arecoline is a partial agonist on the nicotinic receptors as well as mAChR such as M_1, M_2, M_3, and M_4 receptors, which is believed to be the primary cause of its parasympathomimetic effects.[12-15]

Arecoline is a cyclic bioisostere of acetylcholine with a tertiary amino group. It binds to muscarinic receptors as it is structurally related to acetylcholine and muscarine.[16]

3.1. Anticancer activities

The cytotoxic activity of arecoline is mediated through its action on muscarinic receptors. Muscarinic receptors are expressed in numerous primary and metastatic tumors such as prostate, ovary, colon, and lung cancers.[17-19] In some of these tumors such as small-cell lung carcinoma, acetylcholine is possibly synthesized by the tumor cells. Therefore, acetylcholine could affect tumor cell proliferation and migration via an autocrine pathway mediated by nicotinic and/or muscarinic receptors.[20] It was reported that M3-mAChRs antagonists inhibit the growth of small-cell lung carcinoma.[21, 22]

Moreover, muscarinic agonists can produce cytotoxic activity in a dose-dependent manner. For example, arecaidine as M2-mAChR agonist inhibited cell proliferation in human glioblastoma cancer.[23] It was also reported that cholinergic receptors M3-mAChR have a significant role in the proliferation, differentiation, and apoptosis of leukemia cells. For example, the death of human leukemia K562 cells was induced by arecoline, which was associated with the up-modulation of tumor necrosis factor receptor-2 surface expression. The effect of arecoline was mediated by its muscarinic activity.[24]

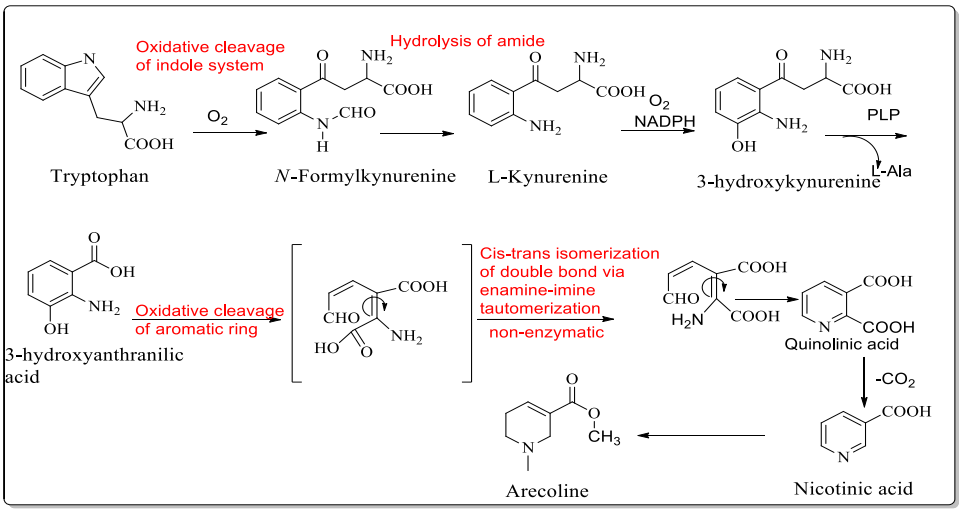

Figure 1. Biosynthesis of arecoline from L-tryptophan

Arecoline, as a partial agonist for muscarinic receptors, can cause cytotoxicity through apoptosis to human endothelial cells EAHY 926 (hybridoma of human endothelium and lung adenocarcinoma A549/8).[25] Arecoline has the ability to prevent tumorigenesis of basal cell carcinoma via lowering the levels of interleukin-6, increasing levels of p53 (a tumor suppressor factor), and finally inducing cell cycle arrest and apoptosis.[26]

3.2. Effects on the nervous system

Arecoline has the ability to reverse memory loss and learning impairment in Alzheimer's dementia due to its effect on muscarinic receptors.[27, 28] Arecoline has the ability to stimulate muscarinic receptor (M receptor) therefore it promotes body excitability and improves the abilities of learning and memory. Crushed areca nuts are frequently combined with lime and covered with piper betel leaves for chewing. It has been suggested that the mentioned processing could quantitatively hydrolyse arecoline into arecaidine. Additionally, it has been found that arecaidine is a powerful inhibitor of the uptake of the central inhibitory transmitter GABA, also known as gamma-aminobutyric acid.[29]

3.3. Effects on the digestive system

Arecoline stimulates smooth muscles of the duodenum and colon via activation of mAChR.[30]

According to the effects of arecoline on the digestive system, previous studies demonstrated that arecoline could improve digestion functions by stimulating muscarinic receptors.[31] In 1999, an interesting experiment conducted on guinea pigs showed that spontaneous contraction of the ileum could be significantly enhanced by arecoline in a dose-dependent manner.[32] Moreover, previous studies also reported that areca nut water extracts (containing 0.06% arecoline) could boost the contractions of gastric smooth muscle and muscle strips of the duodenum, ileum, and colon significantly.[33]

3.4. Anti-parasitic effects

Arecoline is an old anthelmintic that has been used as a taenicide for cats, dogs, and poultry. The mechanism is related to its paralytic effect.[34]

Previous studies demonstrated that arecoline is an active agent against tapeworms, and the mechanism was related to its paralytic effect.[35] Many researches have revealed that arecoline possesses an important synergetic effect against oncomelania when combined with esculentoside and pentachlorophenol sodium, and the potential mechanism was associated with regulation of the smooth muscle contractions.[36-38] Furthermore, arecoline was also proved to be highly effective against cysticercus in vitro and also leads to inhibition of Fasciola hepatica. [39, 40]

3.5. Antimicrobial activity

In 2010, Luo et al. reported that arecoline showed antibacterial activities against *Candida albicans*, *Bacillus anthracis*, and *Bacillus proteus* with MIC values of 0.8, 0.8, and 0.8 mg/mL, respectively.[41]

3.6. Effect on the cardiovascular system

Arecoline exhibits vasodilator and antithrombotic activity due to its effect on mAChRs.[42]

The vasodilator effect of the sub-fraction of areca nut (mainly contains arecoline and condensed tannin) was examined by Goto et al. (1997). The results showed that the sub-fraction caused relaxation of the rat aorta intact endothelium.[43] Ling et al. (2012) reported that arecoline improved ACh-induced vasorelaxation in high fructose-fed rats, and the potential mechanism might be related to the increase of cystathionine- c-lyase expression and stimulation of KATP channels.[44]

Moreover, arecoline was reported to have an antithrombotic effect, and its antithrombotic effect was closely associated with activating the endothelial target for acetylcholine.[45] In 2004, Shan et al. reported that arecoline had antiatherogenic effects, and the mechanism appeared to be thoroughly involved in increasing the plasma level of nitric oxide.[46]

3.7. Antidiabetic effect

Yao et al. (2009) reported that arecoline could improve glucose and lipid metabolism in type 2 diabetic rats.[47]Another research by Hsu et al. (2010) demonstrated that arecoline could inhibit adipogenic differentiation, induced adenylyl cyclase-dependent lipolysis and affected insulin-induced glucose uptake.[48] In addition, it was reported that arecoline could prevent the dysfunction of β cells of the pancreas induced by high fructose.[49-51]

3.8. Recent uses of arecoline derivatives

Muscarinic acetylcholine receptors (mAChRs) are an interesting target for positron emission tomography (PET) imaging due to their involvement in many neurodegenerative disorders. PET radiotracers were developed based on the structure of arecoline as mAChR agonist.[52]

4. Arecoline structure-activity relationship

The ester linkage of arecoline is predisposed to hydrolysis in the stomach.[53] The N-CH_3 group of arecoline is selective to M1 receptor.[54] Quaternization of nitrogen atom results in M1 receptor agonist with the same activity as arecoline itself. [55] Introducing another nitrogen in the arecoline ring gives derivatives less potent than arecoline itself as M1 receptor agonist.[56] The reduction of a double bond decreases the muscarinic agonist activity.[57] Moreover, substitution at position 4 antagonizes M1 receptor activity (Figure 2).[16]

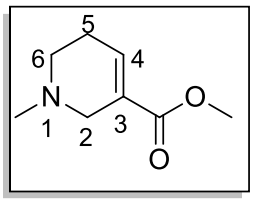

Figure 2. Chemical structure of arecoline.

5. Toxicology

It was reported that long-term and continuous high dose of arecoline is toxic depending on body weight, hematological parameters, and histopathological changes. Arecoline causes mutation of oral mucosal cells, which leads to oral cancer via producing reactive oxygen species (ROS).[58,59] Currently, increasing research has widely studied the toxicity of arecoline. The minimum lethal dose values of arecoline in mice, dog, and horse were 100, 5, and 1.4 mg/kg (p.o.), respectively.[60] Later, it was demonstrated that arecoline could induce hepatotoxicity and testicular toxicity via reactive oxygen species. [61] In 2013, it was reported by Lee et al that the most serious side effects of arecoline are oral toxicities. [62,63] Oral submucous fibrosis is a malignant disease mostly observed in Asian people chewing areca nut; epidemiological surveys afford valid evidence that chewing areca nut can lead to oral submucous fibrosis and arecoline is the main etiological factor.[64,65] Arecoline could affect the molecular processes of deposition and/or degradation of collagen.[66-68] Arecoline is an important factor in epithelial cell death due to oxidative stress via the production of reactive oxygen species in oral cancer and submucosal fibrosis which lead to acute cell trauma.[69, 70] Haque et al. (2000) reported that inflammatory cytokines (IL-1 b, IL-6, IL-8, TNF- a) significantly increased from oral submucous fibrosis in patients chewing betel quid. The genotoxic effects of arecoline were also studied using two different administration routes, including oral administration (p.o.) and intraperitoneal injection (i.p.).[71] Carcinogenicity of arecoline was observed through oral administration more than intraperitoneal injection.[72]

6. Biotransformation of arecoline

6.1. Metabolism of arecoline in mouse:

In 1989, Ohshima et al. identified *N*-nitrosonipecotic acid in rats as a major urinary metabolite of the areca-nut alkaloids.[73] In 2006, Giri, et al. studied the metabolic pathway of arecoline in the mouse. The study showed that arecoline was subjected to *N*-oxidation to give arecoline *N*-oxide and both compounds formed conjugates with mercapturic acid. Ester hydrolysis of arecoline to generate arecaidine was also detected. Further metabolism of arecaidine occurred by either *N*-oxidation to arecaidine *N*-oxide or by reduction of the double bond to yield *N*-methyl nipecotic acid followed by conjugation with glycine. Arecaidine also formed conjugates with glycine, glycerol, and mercapturic acid. The metabolic map of arecoline is illustrated in Figure 3.[74,75]

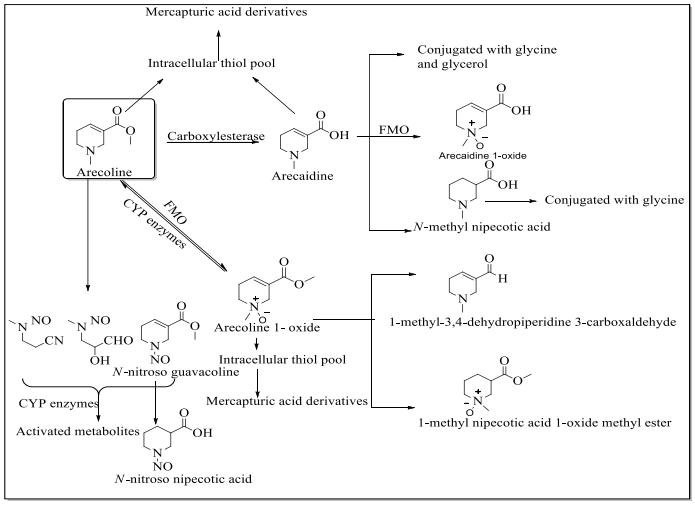

Figure 3. Arecoline metabolic pathway in the mouse

6.2. Microbial transformation of arecoline

It was reported that arecoline was transformed in vitro by *Cunninghamella blackesleeana* NRRL 1369, four major metabolites were isolated and identified as illustrated in Figure 4. Moreover, it was converted by *Aspergillus niger* ATCC 10549, and two major metabolites were isolated and identified as illustrated in Figure 5.[76]

The cytotoxic activity of arecoline and its isolated pure metabolites was determined using MTT assay against cancer as well as normal cell lines of lung and blood cells. This assay was used for screening the cytotoxic activity of tested compounds against non-small cell lung cancer A549 and leukemia cancer K562 cell lines using staurosporine and doxorubicin as references. The selectivity index of these metabolites was evaluated. Furthermore, the effect on cell cycle and induction of apoptosis was studied.

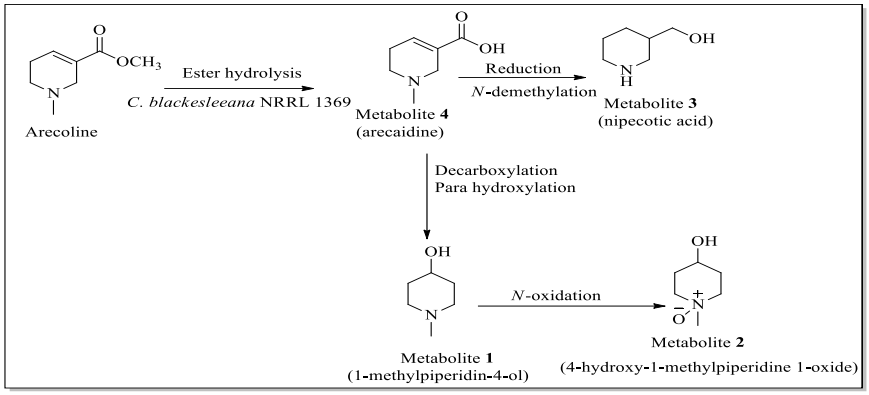

Figure 4. Microbial transformation of arecoline by C. blackesleeana NRRL 1369.

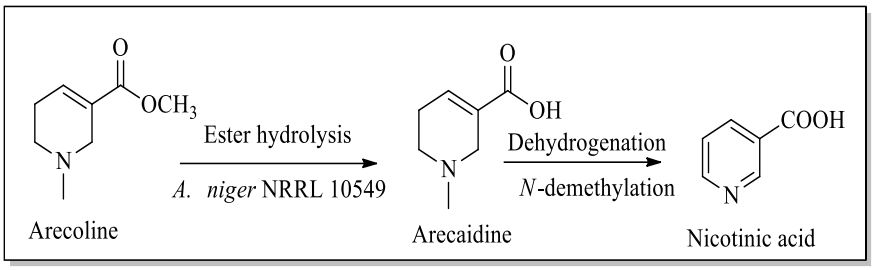

Figure 5. Microbial transformation of arecoline by *A. niger* NRRL 10549.

The obtained results revealed that 1-methylpiperidin-4-ol, nipecotic acid, and nicotinic acid, possess very strong *in vitro* cytotoxic activity against non-small cell lung cancer A549 and leukemia cancer K562 with selectivity index values of more than 3. It was found that metabolite **1** induced cell cycle arrest in non-small cell lung cancer A549 at G2/M phase. Nipecotic acid and nicotinic acid-induced cell cycle arrest in leukemia cancer K562 at S-phase. The cytotoxicity results were confirmed by good protein-ligand binding affinity to M3-mAChR (PDB ID: 4U15) using MVD software.

Nipecotic acid, arecaidine, and nicotinic acid showed permeability values near that of arecoline. Online prediction tools were used to predict the cellular permeability of the metabolites. 1-Methylpiperidin-4-ol presented the highest value of permeability compared to arecoline and other metabolites using the colorectal carcinoma (Caco-2) cells model. On the other hand, the study revealed that metabolite **2** (4-hydroxyl-1-methylpiperidine 1-oxide) showed low *in vitro* cellular cytotoxicity against non-small cell lung cancer A549 and leukemia cancer K562 cell lines. Metabolite **2** also showed the lowest value of permeability using Caco-2 cells model compared to arecoline and other metabolites, which would affect its activity. The study also showed that all metabolites do not violate the drug-likeness classifiers identified by Lipiniski, Veber, and Egans. Therefore, they would be considered orally active drugs.

CONCLUSION

Arecoline exhibits many pharmacological activities due to its effect on mAChRs. The biotransformation of arecoline is of interest due to its good biological activities. This review includes mammalian as well as fungal transformation. Biotransformation of arecoline involved ring unsaturation, decarboxylation, hydration of double bond, ester hydrolysis, and formation of *N*-oxides. Further studies are strongly recommended to understand the mechanism of arecoline biotransformation using other fungi as well as bacterial and plant cell cultures.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

REFERENCES

1. Baiping M, Bing F, Hongzhi H, Yuwen C. Biotransformation of Chinese herbs and their ingredients. World Science and Technology. 2010;12(2):150-154. doi:10.1016/s1876-3553(11)60012-4

2. Parkinson A, B. Buckley D, Kazmi F, Parkinson O. Biotransformation of xenobiotics. In: W. Ogilvie B, ed. Casarett & Doull’s Toxicology: The Basic Science of Poisons. Vol 7. 9th ed. ; 2008:161-304.

3. Fura A, Shu YZ, Zhu M, Hanson RL, Roongta V, Humphreys WG. Discovering drugs through biological

transformation: role of pharmacologically active metabolites in drug discovery. *J Med Chem.* 2004;47(18):4339-4351. doi:10.1021/jm040066v

4. 1. Manske RHF, Holmes HL. Alkaloids: Chemistry and Physiology. Elsevier Science & Technology Books; 2014.

5. Qiu S, Sun H, Zhang AH, et al. Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chin J Nat Med.* 2014;12(6):401-406. doi:10.1016/S1875-5364(14)60063-7

6. Bribi N. Pharmacological activity of Alkaloids: A Review. AJB. 2018;1(1):1-6. doi:https://doi.org/10.63019/ajb.v1i2.467

7. 1. Aniszewski T. Alkaloids: Chemistry, Biology, Ecology, and Applications. Elsevier Science; 2015.

8. Lord GA, Lim CK, Warnakulasuriya S, Peters TJ. Chemical and analytical aspects of areca nut. *Addict Biol.* 2002;7(1):99-102. doi:10.1080/13556210120091455

9. Liu YJ, Peng W, Hu MB, Xu M, Wu CJ. The pharmacology, toxicology and potential applications of arecoline: a review. *Pharm Biol.* 2016;54(11):2753-2760. doi:10.3109/13880209.2016.1160251

10. WINDHOLZ M (Ed.). The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 10th ed. Merck; 1983.

11. Dewick PM. Medicinal Natural Products: A Biosynthetic Approach. 2nd ed. Wiley; 2002.

12. Funayama S, Cordell GA. In: Alkaloids: A Treasury of Poisons and Medicines. 1st ed. Elsevier; 2015:181-192.

13. Xie DP, Chen LB, Liu CY, Zhang CL, Liu KJ, Wang PS. Arecoline excites the colonic smooth muscle motility via M3 receptor in rabbits. *Chin J Physiol.* 2004;47(2):89-94.

14. Volgin AD, Bashirzade A, Amstislavskaya TG, et al. DARK Classics in Chemical Neuroscience: Arecoline. ACS Chem *Neurosci.* 2019;10(5):2176-2185. doi:10.1021/acschemneuro.8b00711

15. 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. *PLoS One.* 2015;10(10):e0140907. Published 2015 Oct 21. doi:10.1371/journal.pone.0140907

16. Kumar YC, Malviya M, Chandra JN, et al. Effect of novel N-aryl sulfonamide substituted 3-morpholino arecoline derivatives as muscarinic receptor 1 agonists in Alzheimer's dementia models. *Bioorg Med Chem*. 2008;16(9):5157-5163. doi:10.1016/j.bmc.2008.03.019

17. Frucht H, Jensen RT, Dexter D, Yang WL, Xiao Y. Human colon cancer cell proliferation mediated by the M3 muscarinic cholinergic receptor. *Clin Cancer Res.* 1999;5(9):2532-2539.

18. Oppitz M, Möbus V, Brock S, Drews U. Muscarinic receptors in cell lines from ovarian carcinoma: negative correlation with survival of patients. *Gynecol Oncol.* 2002;85(1):159-164. doi:10.1006/gyno.2002.6597

19. Rayford W, Noble MJ, Austenfeld MA, Weigel J, Mebust WK, Shah GV. Muscarinic cholinergic receptors promote growth of human prostate cancer cells. *Prostate.* 1997;30(3):160-166. doi:10.1002/(sici)1097-0045(19970215)30:3<160::aid-pros3>3.0.co;2-q

20. Song P, Sekhon HS, Jia Y, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. *Cancer Res.* 2003;63(1):214-221.

21. Song P, Sekhon HS, Lu A, et al. M3 muscarinic receptor antagonists inhibit small cell lung carcinoma growth and mitogen-activated protein kinase phosphorylation induced by acetylcholine secretion. *Cancer Res*. 2007;67(8):3936-3944. doi:10.1158/0008-5472.CAN-06-2484

22. E. R. Spindel, in *Muscarinic Receptors*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, **208**, pp. 451–468.

23. Alessandrini F, Cristofaro I, Di Bari M, Zasso J, Conti L, Tata AM. The activation of M2 muscarinic receptor inhibits cell growth and survival in human glioblastoma cancer stem cells. *Int Immunopharmacol*. 2015;29(1):105-109. doi:10.1016/j.intimp.2015.05.032

24. Chen YJ, Chang LS. Arecoline-induced death of human leukemia K562 cells is associated with surface up-modulation of TNFR2. *J Cell Physiol.* 2012;227(5):2240-2251. doi:10.1002/jcp.22963

25. Tseng SK, Chang MC, Su CY, et al. Arecoline induced cell cycle arrest, apoptosis, and cytotoxicity to human endothelial cells. *Clin Oral Investig*. 2012;16(4):1267-1273. doi:10.1007/s00784-011-0604-1

26. Huang LW, Hsieh BS, Cheng HL, Hu YC, Chang WT, Chang KL. Arecoline decreases interleukin-6 production and induces apoptosis and cell cycle arrest in human basal cell carcinoma cells. *Toxicol Appl Pharmacol*. 2012;258(2):199-207. doi:10.1016/j.taap.2011.11.001

27. JOHNSTON, G., KROGSGAARD-LARSEN, P. & STEPHANSON, A. Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. *Nature* 258, 627–628 (1975). https://doi.org/10.1038/258627a0

28. Maiese K, Holloway HH, Larson DM, Soncrant TT. Effect of acute and chronic arecoline treatment on cerebral metabolism and blood flow in the conscious rat. *Brain Res.* 1994;641(1):65-75. doi:10.1016/0006-8993(94)91816-3

29. Gilani AH, Ghayur MN, Saify ZS, Ahmed SP, Choudhary MI, Khalid A. Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. *Life Sci.* 2004;75(20):2377-2389. doi:10.1016/j.lfs.2004.03.035

30. Wan X. X., Du Z. M., Chen Y. Y. Effect of arecoline on spontaneous peristalsis of isolated ileum. *J Pla Jr Coll Med*. 1999;27:87-88.
31. Si C., Wei & nbsp; M. A study of the effects of arecaline hydrobromide on the dispersed colonic smooth muscle cells in rats. *J Tradit Chin Med* .2004;38:48-50.
32. Wan X. X., Du Z. M., Chen Y. Y. Effect of arecoline on spontaneous peristalsis of isolated ileum. *J Pla Jr Coll Med*. 1999;27:87-88.
33. Y. Ni, J. Wang and R. Wang, Comparative study of the effect of areca nut and arecoline on gastrointestinal motility, *Pharmacol Clin Chin Mater Med*, 2004, **20**, 11–12.
34. Liu Z, Huang Y, Cui Z, et al. Application of Traditional Chinese Medicine in medical practice: a survey of community residents in Beijing, China. *J Tradit Chin Med.* 2017;37(2):261-268. doi:10.1016/s0254-6272(17)30053-5
35. Zheng Z. Modern Research and Application of Traditional Chinese Medicine. Academic press; 1999.
36. Feng Q, Li G, Yang Y, Gao J. Studies on the increasing-effect components for molluscicides in nut of Areca catech *Zhong Yao Cai.* 1999;22(11):572-574.
37. Li G, Feng Q, Yang Y, Gao J. Studies on the effect-increasing components for molluscacides in nut of Areca catech L. *Zhongguo zhongyao zazhi* . 2000;25:160-162.
38. Shao-lin W. Concentration-effect relationship of arecoline on rat portal vein and calcium channel current in guinea pig ventricular myocytes. Chinese Journal of Parasitic Disease Control. 2001;14:139-141.
39. W. Z, Z. L, B. W. Morphological observation of semen areca and white pepper against cysticercus cellulosae in vitro. *Modern J Integr. Trad Chin Western Med*. 2003;12:237-238.
40. Zha C, Chen G, Wu. M. The role of Magnolia officinalis Rehd et Wils and betel nut on Fasciola hepatica in vitro. 1990;6:266-269.
41. S. S. . Luo, H. D. Z, X. L. L, L Z. Study on antimicrobial activity of arecoline from betel nut in vitro. Innov Ed Farm Prod Process. 2010;10:47-50.
42. Dongmei C, Shaofeng M, Hai WANG. Antithrombosis through activating endothelial target for acetylcholine and its molecular mechanism. *Chinese Pharmacological Bulletin*. 1986;12.
43. Goto H, Tanaka N, Tanigawa K, Shimada Y, Itoh T, Terasawa K. Endothelium-dependent vasodilator effect of extract prepared from the seeds ofareca catechuon isolated rat aorta. *physiother. res.*1997;11(6):457-459. doi:10.1002/(sici)1099-1573(199709)11:6<457::aid-ptr123>3.0.co;2-j
44. Ling HY, Wang G, Zhang W, Li X, Zhou SH, Hu B. Arecoline improves vascular endothelial function in high fructose-fed rats via increasing cystathionine-γ-lyase expression and activating K(ATP) channels. *Acta Pharmacol Sin.* 2012;33(8):1023-1029. doi:10.1038/aps.2012.63
45. Chen D, WANG. H, Mu S. Antithrombosis through activating endothelial target for acetylcholine and its molecular mechanism. *Chinese Pharmacological Bulletin.* 2002;18:527-531.
46. Wei Z, Shou-Hong Z, Hong-Yan L, et al. Arecoline Repressed Inflammation Factor Expression of Macrophages Stimulated by Oxidized Low Density Lipoprotein and Its Mechanism. *Chinese J. of Arteriosclerosis*. 2009;17(4):269-272.
47. Yao Q, Qi Z, Wang G, et al. Arecoline improved glucose and lipid metabolism in type 2 diabetic rats. *Chinese Pharmacology Bulletin.* 2009;25:1477-1481.
48. Hsu H-F, Tsou T-C, Chao H-R, et al. Effects of arecoline on adipogenesis, lipolysis, and glucose uptake of adipocytes—a possible role of betel-quid chewing in metabolic syndrome. Toxicology and Applied Pharmacology. 2010;245(3):370-377. doi:10.1016/j.taap.2010.04.008
49. Qi Z, Yao Q, Wang G, et al. Effect of arecoline on PDX-1 mRNA expression in rats with type 2 diabetes meilitus. *Int J Pathol Clin Res*.2010;30:14-19.
50. Huang LW, Hsieh BS, Cheng HL, Hu YC, Chang WT, Chang KL. Arecoline decreases interleukin-6 production and induces apoptosis and cell cycle arrest in human basal cell carcinoma cells. *Toxicol Appl Pharmacol*. 2012;258(2):199-207. doi:10.1016/j.taap.2011.11.001
51. Chen YJ, Chang LS. Arecoline-induced death of human leukemia K562 cells is associated with surface up-modulation of TNFR2. *J Cell Physiol.* 2012;227(5):2240-2251. doi:10.1002/jcp.22963
52. Ozenil M, Pacher K, Balber T, et al. Enhanced arecoline derivatives as muscarinic acetylcholine receptor M1 ligands for potential application as PET radiotracers. *Eur J Med Chem.* 2020;204:112623. doi:10.1016/j.ejmech.2020.112623
53. Nieschulz O, Schmersahl P. Zur Pharmakologie der Wirkstoffe des Betels. 2. Umnoandlung des Arecolin in Arecaidin [On the pharmacology of active materials from betel. 2. Transformation of arecoline to arecaidine]. *Arzneimittelforschung*. 1968;18(2):222-225.
54. Moltzen EK, Pedersen H, Bøgesø KP, et al. Bioisosteres of arecoline: 1,2,3,6-tetrahydro-5-pyridyl-substituted and 3-piperidyl-substituted derivatives of tetrazoles and 1,2,3-triazoles. Synthesis and muscarinic activity. *J Med Chem.* 1994;37(24):4085-4099. doi:10.1021/jm00050a006
55. 1. P. K-L, H. B. A Textbook of Drug Design and Development. Harwood Academic Pub; 1991.
56. Messer WS, Dunbar PG, Rho T, et al. Synthesis, biochemical activity and behavioral effects of a series of 1,4,5,6-tetrahydropyrimidines as novel ligands for M1

receptors. *Bioorg Med Chem Lett* 1992;2(8):781-786. doi:10.1016/s0960-894x(00)80531-4

57. Moser U, Lambrecht G, Mutschler E, Sombroek J. Struktur- und Konformations-Wirkungs-Beziehungen heterocyclischer Acetylcholinanaloga, 18. Mitt. Synthese und muskarinartige Wirkung von Estern des Sulfoarecaidins und Sulfoisoarecaidins [Structure- and conformation-activity relationships of heterocyclic acetylcholine analogs, 18. Synthesis and muscarine-type activity of sulfoarecaidine and sulfoisoarecaidine esters]. *Arch Pharm (Weinheim).* 1983;316(8):670-677. doi:10.1002/ardp.19833160805

58. Lin KH, Lin CY, Liu CC, Chou MY, Lin JK. Arecoline N-oxide: its mutagenicity and possible role as ultimate carcinogen in areca oral carcinogenesis. *J Agric Food Chem.* 2011;59(7):3420-3428. doi:10.1021/jf104831n

59. Wei X, Zhang J, Niu J, Zhou X, Li J, Li B. Evaluation of arecoline hydrobromide toxicity after a 14-day repeated oral administration in Wistar rats. *PLoS One*. 2015;10(4):e0120165. Published 2015 Apr 16. doi:10.1371/journal.pone.0120165

60. X. Z, C. L, L. H, L. J, S. W, Z. C. Experimental study on increasing toxicity of Areca catechu and Artemisia annua together. *Shizhen Guo Yi Guo Yao*. 2013;24:2608-2609.

61. Zhou J, Sun Q, Yang Z, Zhang J. The hepatotoxicity and testicular toxicity induced by arecoline in mice and protective effects of vitamins C and e. *Korean J Physiol Pharmacol*. 2014;18(2):143-148. doi:10.4196/kjpp.2014.18.2.143

62. Lee SS, Tsai CH, Yu CC, Chang YC. Elevated snail expression mediates tumor progression in areca quid chewing-associated oral squamous cell carcinoma via reactive oxygen species. *PLoS One.* 2013;8(7):e67985. Published 2013 Jul 10. doi:10.1371/journal.pone.0067985

63. Chen PH, Huang B, Shieh TY, et al. The influence of monoamine oxidase variants on the risk of betel quid-associated oral and pharyngeal cancer. *ScientificWorldJournal.* 2014;2014:183548. doi:10.1155/2014/183548

64. Prabhu RV, Prabhu V, Chatra L, Shenai P, Suvarna N, Dandekeri S. Areca nut and its role in oral submucous fibrosis. *J Clin Exp Dent*. 2014;6(5):e569-e575. Published 2014 Dec 1. doi:10.4317/jced.51318

65. Hosein M, Mohiuddin S, Fatima N. Association Between Grading of Oral Submucous Fibrosis With Frequency and Consumption of Areca Nut and Its Derivatives in a Wide Age Group: A Multi-centric Cross Sectional Study From Karachi, Pakistan. *J Cancer Prev*. 2015;20(3):216-222. doi:10.15430/JCP.2015.20.3.216

66. Utsunomiya H, Tilakaratne WM, Oshiro K, et al. Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J Oral Pathol Med*. 2005;34(8):498-507. doi:10.1111/j.1600-0714.2005.00339.x

67. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol.* 2006;42(6):561-568. doi:10.1016/j.oraloncology.2005.08.005

68. J. B. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. British Journal of Oral and Maxillofacial Surgery. 2014;52:673-682.

69. Thangjam GS, Kondaiah P. Regulation of oxidative-stress responsive genes by arecoline in human keratinocytes. *J Periodontal Res.* 2009;44(5):673-682. doi:10.1111/j.1600-0765.2008.01176.x

70. Chang MC, Ho YS, Lee PH, et al. Areca nut extract and arecoline induced the cell cycle arrest but not apoptosis of cultured oral KB epithelial cells: association of glutathione, reactive oxygen species and mitochondrial membrane potential. *Carcinogenesis.* 2001;22(9):1527-1535. doi:10.1093/carcin/22.9.1527

71. Haque MF, Meghji S, Khitab U, Harris M. Oral submucous fibrosis patients have altered levels of cytokine production. *J Oral Pathol Med.* 2000;29(3):123-128. doi:10.1034/j.1600-0714.2000.290304.x

72. Chatterjee A, Deb S. Genotoxic effect of arecoline given either by the peritoneal or oral route in murine bone marrow cells and the influence of N-acetylcysteine. *Cancer Lett.* 1999;139(1):23-31. doi:10.1016/s0304-3835(98)00364-4

73. Ohshima H, Friesen M, Bartsch H. Identification in rats of N-nitrosonipecotic acid as a major urinary metabolite of the areca-nut alkaloid-derived nitrosamines, N-nitrosoguvacoline and N-nitrosoguvacine. *Cancer Lett.* 1989;44(3):211-216. doi:10.1016/0304-3835(89)90063-3

74. Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. *Chem Res Toxicol.* 2006;19(6):818-827. doi:10.1021/tx0600402

75. Giri S, Krausz KW, Idle JR, Gonzalez FJ. The metabolomics of (±)-arecoline 1-oxide in the mouse and its formation by human flavin-containing monooxygenases. *Biochem. Pharmacol.* 2007;73(4):561-573. doi:10.1016/j.bcp.2006.10.017

76. Ragab AE, Badawy ET, Aboukhatwa SM, Kabbash A, Abo El-Seoud KA. In Vitro Characterization of Inhibitors for Lung A549 and Leukemia K562 Cell Lines from Fungal Transformation of Arecoline Supported by In Silico Docking to M3-mAChR and ADME Prediction. *Pharmaceuticals*(Basel). 2022;15(10):1171. Published 2022 Sep 21. doi:10.3390/ph15101171