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ORIGINAL ARTICLE **OPEN ACCESS**

Inhibitory efficacy of bioactive Compounds extracted from *Areca catechu* L. nut on Matrix Metalloproteinases an *In-Silico* study

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ABSTRACT

Arecoline is a nicotinic acid-based mild parasympathomimetic stimulant alkaloid and Nonanaldehyde or Nonanal is well known for its fragrance property (in the form of nonanoic acid), found in Areca catechu L. nut. The effect of Arecoline and nonanaldehyde against matrix metalloproteinase (MMPs) 2 and 9 to investigate its biological properties through systems biology based approach. Arecoline and nonanaldehyde are capable of inhibiting MMP 9 and MMP 2 in a significant level. Corneal healing may be due to the presence of arecoline and nonanoicacid found in Areca catechu L. nut. This may leads to various studies related with eye disorders.

Keywords: Inhibitory efficacy, Molecular docking, Areca catechu L.nut, MMP 9,MMP 2

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INTRODUCTION

Computational methods have been developed for the easy prediction and interpretation of a metabolic network under a particular range of conditions [1]. Areca palm is a medicinal plant with numerous pharmacological activities [2]. Areca catechu L. nut belonging to Arecaceae family [3]. Areca catechu L. nut used for the treatment of wounds for several years [4]. This nut is also good for the treatment of Alzherimer's disease [5]. Arecoline is the inevitable constituent of Areca catechu L. nut [6].

The alkaloids of Areca catechu L. follows the order as root, unripe nut, spike, leaves and vein [7]. Areca catechu L. nut is a powerful antibacterial and antioxidant due to its content of tannin [8]. Molecular docking studies also favour the protective role of some compounds against the arecoline induced toxicity [9]. In the area of molecular modeling, docking is methods which interpret preferred orientation of one molecule to a second when found to each other to form a stable complex [10]. The naturally existing alkaloid arecoline shows orthostreic partial agonist properties [11]. The unavailability of approved treatment for some diseased cells, the scientific community to choose new compounds with capacity to treat it [12].

MATERIAL AND METHODS

Healthy un ripened Areca catechu L. nuts were collected from Kollam district of Kerala, India. It was dehusked and dried for three weeks. The dried seeds were powdered. The plant Areca catechu and Areca catechu L. nut were authenticated by JNTBGRI, Thiruvananthapuram, Pin 695 562, Kerala, India and voucher specimens (Specimen Numbers TBGT/95955 & TBGT/95956) are deposited at the herbaria of the same research institute.

The docking of proteins and ligands has been extensively utilized to predict the binding mechanisms and affinities of ligands. The CB-Dock is carried out over the whole surface of the protein to improve the sampling efficiency and detect the integrated cavity of the targeted docking module for discovering residues that potentially interact with ligands [13].

The Arecoline ($C_8H_{13}NO_2$) and Nonanaldehyde ($C_9H_{18}O$) structures were downloaded from the PubChem compound database, belonging to the National Center for Biotechnology Information. The structure of the compounds is shown in Figure 1, 2.

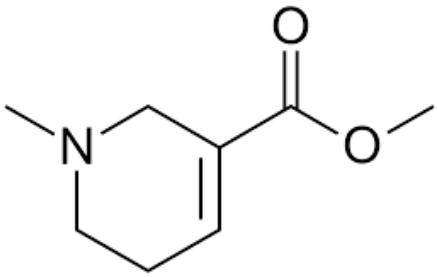

Figure 1. Arecoline ($C_8H_{13}NO_2$)

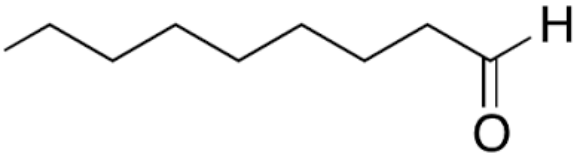

Figure 2. Nonanaldehyde ($C_9H_{18}O$)

Absorption, distribution, metabolism and excretion (ADME) for finding the properties of drug (describes the disposition of a pharmaceutical compound within an organism). ADME properties allow drug developers to understand the safety and efficacy of a drug candidate, and are necessary for regulatory approval [14].

Table 1: ADME properties of Arecoline and Nonanaldehyde

Ligands	**Arecoline**	**Nonanaldehyde**
Molecular Weight	155.19 g/mol	142.24 g/mol
Num. H-bond acceptors	3	1
Num. H-bond donors	0	0
Log *P*	.80	2.78
Log *S*	-0.80	-3.02
Solubility	Water soluble	Water soluble
GI absorption	High	High
BBB permeant	No	Yes
Lipinski	Yes; 0 violation	Yes; 0 violation
PAINS	0 alert	0 alert

The MMP 9 (PDB ID 1GKC) and MMP 2 (PDB ID 1J7M) experimental structures were downloaded from the PDB database (Protein DataBank) (http://www.rcsb.org). For docking studies, the inhibitors and other chemical compounds attached to the protein structure were separated and removed from the data file. The water molecule, hetero atom coordinates, included in the co-crystallized protein structure, were also removed. Finally, the protein is prepared by using the software Discover Studio Visualizer software.

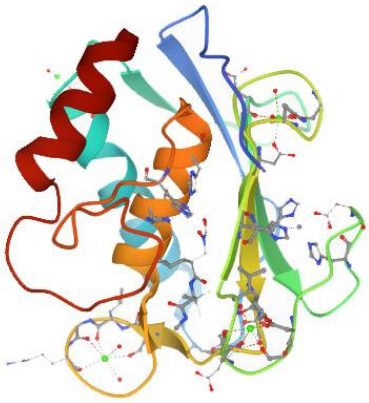

Figure 3. MMP 9

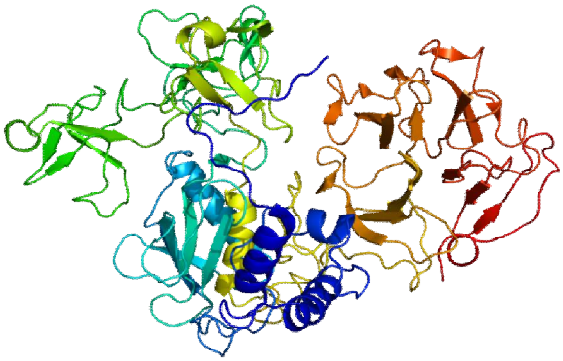

Figure 4. MMP 2

Upcoming docking methods are based on blind docking, which refers to docking a ligand to the whole surface of a protein. It requires multiple energy calculations, several runs, and trials. So the blind docking method is utilized for identifying valid ligand binding sites.

In this study, docking is accomplished by using CB-Dock (Cavity-detection guided Blind Docking-http://cao.labshare.cn/cb-dock/). CB-Dock generates a set of points to represent the solvent-accessible surface and calculates the curvature factor of each point [13]. CB-Dock needs a protein file in the PDB format and a ligand file in the MOL2, MOL, or SDF formats as input. Following submission, CB-Dock verifies the input files and uses MGL Tools and Open Babel to convert them to pdbqt formatted files [15]. The CB-Dock uses a density-peak-based clustering method which is used to group the locations on the concave surface. As a consequence, we obtained many clusters of dots corresponding to holes on the protein's surface. Unlike other techniques for predicting binding sites, this method attempted to identify as many actual binding cavities [16].

RESULTS

The docking results are analysed and visualised by the BIOVIA Discovery Studio Visualizer. Additionally, this programme was utilised to verify the proteins and ligands employed in this study. This programme also includes a large collection of protein ligand plots and other graphical representations of data. Henceforth, this programme will be utilized to visualise the three-dimensional structure of protein-ligand hydrogen bond interactions, aromatic interactions, and two-dimensional ligand-protein interactions. Docking was done by CB-Dock tool

Table 2: Docking results of Arecoline and Nonanaldehyde

SI.NO	PROTEIN	COMPOUND
1	MMP 9-inhibitor complex PDB ID 1GKC	Arecoline ($C_8H_{13}NO_2$)
2	Metalloproteinase 2 PDB ID 1J7M	Arecoline ($C_8H_{13}NO_2$)
3	MMP 9-inhibitor complex PDB ID 1GKC	Nonanaldehyde ($C_9H_{18}O$)
4	Metalloproteinase 2 PDB ID 1J7M	Nonanaldehyde ($C_9H_{18}O$)

Table 3: Vina scores of Arecoline and Nonanaldehyde

	PROTEIN	LIGAND	Vina SCORE
1	MMP 9-inhibitor complex PDB ID 1GKC	Arecoline ($C_8H_{13}NO_2$)	-5.9
2	MMP 9-inhibitor complex PDB ID 1GKC	Nonanaldehyde ($C_9H_{18}O$)	-5.3
3	Metalloproteinase 2 PDB ID 1J7M	Arecoline ($C_8H_{13}NO_2$)	-4.3
4	Metalloproteinase 2 PDB ID 1J7M	Nonanaldehyde ($C_9H_{18}O$)	-3.9

Table 4: Docking results of MMP 9-inhibitor complex (PDB ID: 1GKC) and ARECOLINE $C_8H_{13}NO_2$)

Vina score	**Cavity size**	**Center**			**Size**		
		x	**y**	**z**	**x**	**y**	**z**
-5.9	446	62	31	114	17	17	17

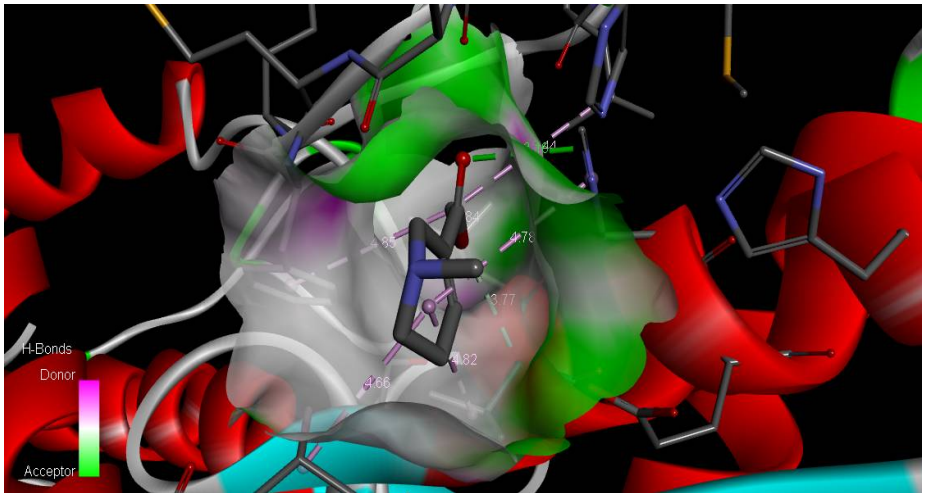

Figure 5. 3D Docking results of MMP9-inhibitor complex (PDB ID: 1GKC) and Arecoline ($C_8H_{13}NO_2$)

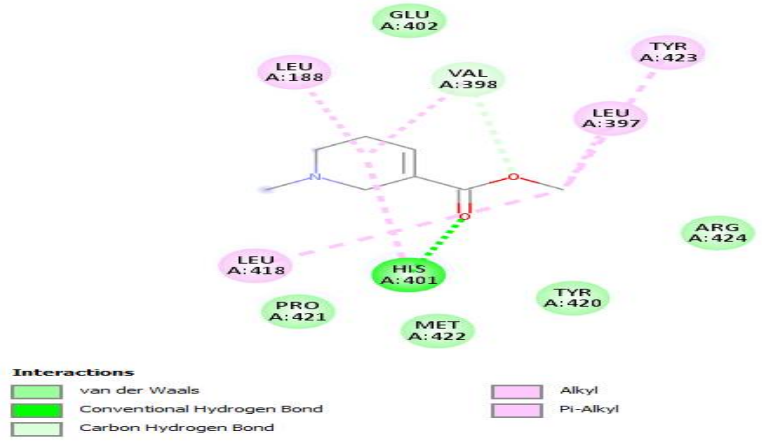

Figure 6. 2D Docking results of MMP 9-inhibitor complex (PDB ID: 1GKC) and ARECOLINE $C_8H_{13}NO_2$)

Table 5: Docking results of Metalloproteinase 2 PDB ID 1J7M and Arecoline $C_8H_{13}NO_2$

Vina score	Cavity size	Center			Size		
		x	y	z	x	y	Z
-4.3	37	-4	-4	-6	17	17	17

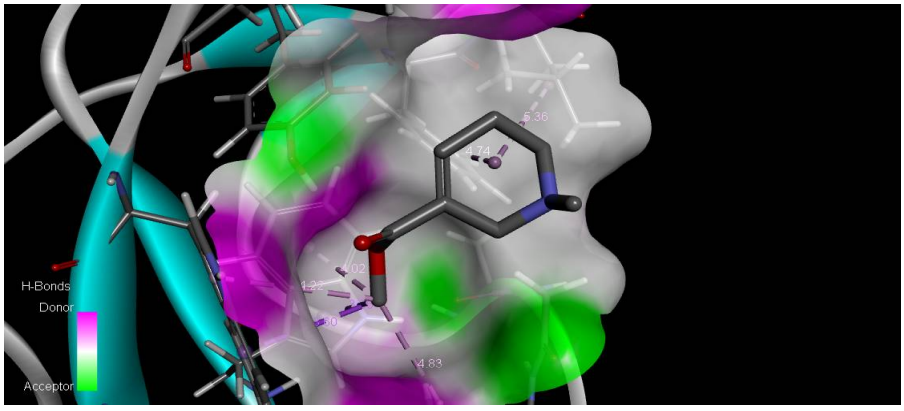

Figure 7. 3D Docking results of Metalloproteinase 2 PDB ID 1J7M and Arecoline ($C_8H_{13}NO_2$)

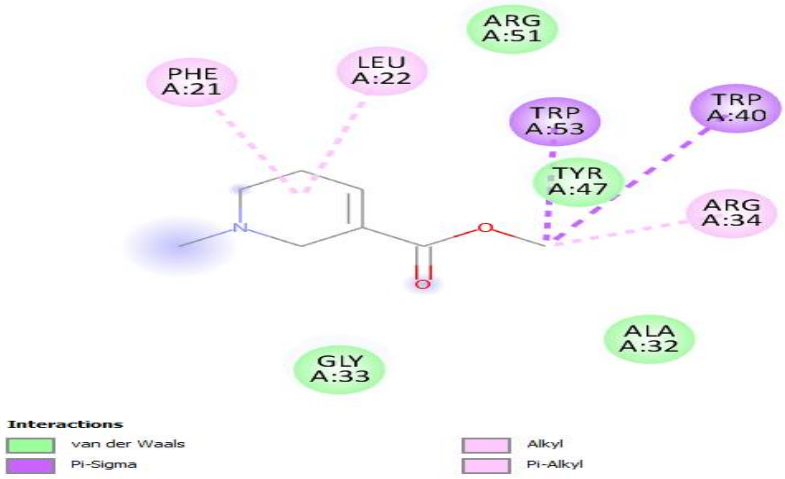

Figure 8. 2D Docking results of Metalloproteinase 2 PDB ID 1J7M and Arecoline ($C_8H_{13}NO_2$)

Table 6: Docking results of MMP 9-inhibitor complex (PDB ID: 1GKC) and Nonanaldehyde ($C_9H_{18}O$)

Vina score	Cavity size	Center			Size		
		x	y	z	x	y	z
-5.3	446	62	31	114	20	20	20

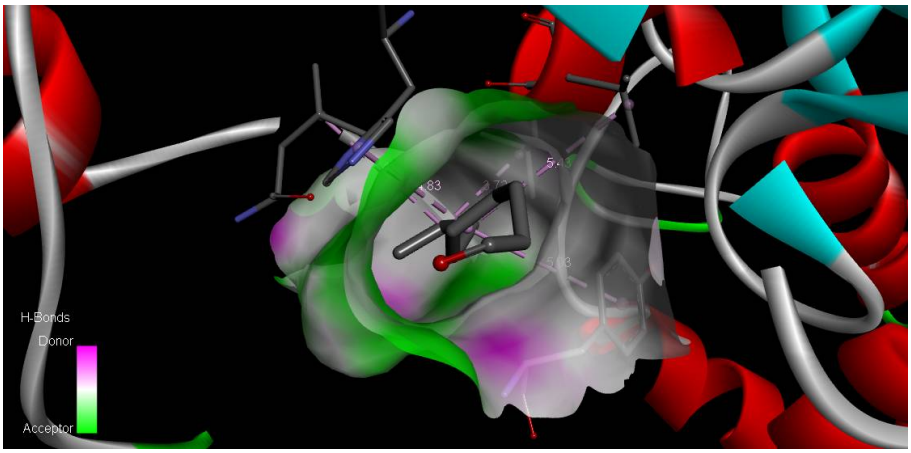

Figure 9. 3D Docking results of MMP9-inhibitor complex (PDB ID: 1GKC) and Nonanaldehyde ($C_9H_{18}O$)

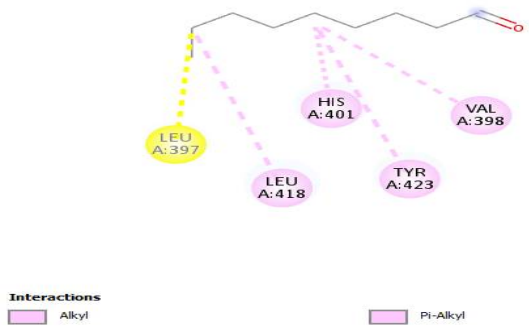

Figure 10. 2D Docking results of MMP 9-inhibitor complex (PDB ID: 1GKC) and Nonanaldehyde ($C_9H_{18}O$)

Table 7: Docking results of Metalloproteinase 2 PDB ID 1J7M and Nonanaldehyde ($C_9H_{18}O$)

Vina score	Cavity size	Center			Size		
		x	y	z	x	y	z
-3.9	37	-4	-4	-6	20	20	20

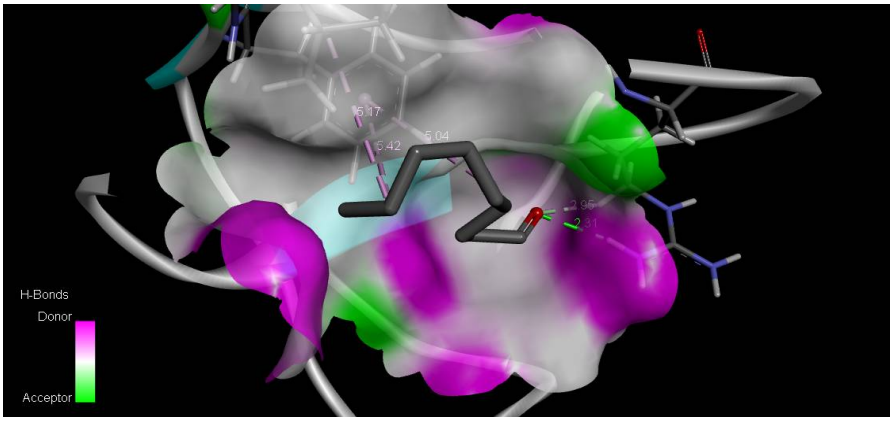

Figure 11. 3D results of Docking results of Metalloproteinase 2 PDB ID 1J7M and Nonanaldehyde ($C_9H_{18}O$)

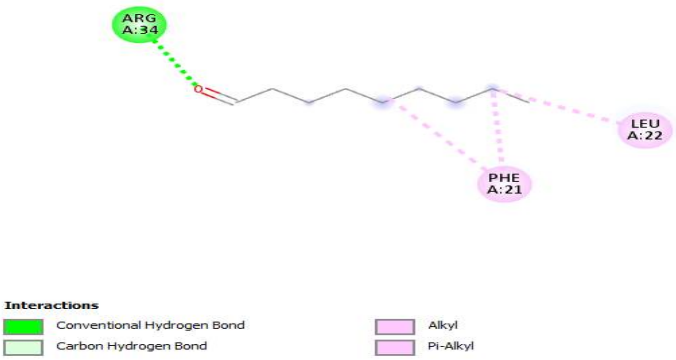

Figure 12. 2D results of Docking results of Metalloproteinase 2 PDB ID 1J7M and Nonanaldehyde ($C_9H_{18}O$)

DISCUSSION

Arecoline is a nicotinic acid-based mild parasympathomimetic stimulant alkaloid found in the areca nut, the fruit of the areca palm (*Areca catechu*) endemic to south and southeast Asia. Arecoline posses partial agonist effect on nicotinic and muscarinic acetylcholine receptors, arecoline evokes multiple effects on the central nervous system (CNS), including stimulation, alertness, elation, and anxiolysis [17]. Even though Arecoline is believed to have the mild adductive effect, it also proved that Arecoline is a potent drug against many diseases like nervous, cardiovascular, digestive and endocrine systems and anti-parasitic effects [18]. Excessive usage of Arecoline was reported to have several adverse effects includes the development of oral cancer, oral fibrosis and other type of cancers [19]. Even though the effect of Arecoline is Controversial, the dose dependent activity, disease pathology and site of action may support the pharmacological activity of the Arecoline. In present study, we investigated the effect of Arecoline against Matrix Metalloproteinase (MMPs) 2 and 9 to investigate its biological properties through systems biology based approach. MMPs are a family of zinc-dependent endoproteases with multiple roles in tissue remodeling and degradation of various proteins in the extracellular matrix (ECM). MMPs promote cell proliferation, migration, and differentiation and could play a role in cell apoptosis, angiogenesis, tissue repair, and immune response. MMPs may also affect bioactive molecules on the cell surface and modulate various cellular and signaling pathways. Alterations in MMP expression and activity occur in normal biological processes e.g. during pregnancy and wound healing, but have also been observed in cardiovascular diseases such as atherosclerosis, aneurysms and varicose veins, musculoskeletal disorders such as osteoarthritis and bone resorption, and in various cancers. MMPs have also been implicated in tumor progression and invasiveness [20]. Among all subclasses, MMP 9 and 2 are more active as far as biological degradations are concerned. In some conditions, the beneficial effects of MMPs are overtaken by its adverse effects. Many disorders includes muscular dystrophy, Alzheimer's disease and in Parkinson's disease, MMP 9 over activity leads to the degradation of many intracellular components [21-23]. Recent reports suggest that inhibition of MMP s play a significant recovery in the disease progression [24].

Many experiments shows that corneal ulcerations are always associated with excessive release and activity of MMP 2 and MMP 9 [22]. Inhibition of MMP 2 and 9, reported to have a better recovery of macular degeneration, diabetic retinopathy, and corneal neovascularization [25]. Many drugs in the

treatment of cancer shows potential activity when introduced along with the MMP 2 and 9 inhibitors [26,27]. But many synthetic inhibitors and other drugs posses lot of adverse effects during the administration.

In present study, we noticed a significant inhibition of MMP 2 and 9 by Arecoline. MMP 9 bound with Arecoline with a binding score of -5.9, which is more significant than MMP 2 which posses -4.3. in total, MMP 9 was predicted to have 6 bond with different amino acids as shown in Figureure. Whereas, MMP 2 could able to form 5 bonds with Arecoline. In addition to Arecoline, we investigated the effect of nonanoic acid, in its aldehyde form-Nonanaldehyde. Nonanaldehyde or Nonanal is well known for its fragrance property and widely used for the production of fragrances with the chemical formula $C_9H_{18}O$. Nonanal is present in wide verity of natural oils includes cinnamon, rose, citrus and in pine [28]. Nonanaldehyde inhibited MMP 9 with the binding score of -5.3, which is nearly a good significant range compare to Arecoline. If produced 5 different bonds with MMP 9. Nonanaldehyde bound with MMP 2 in a score of 3.9 and with 4 different bonds. The above results clearly justifies that both Arecoline and Nonanaldehyde are capable of inhibiting MMP 2 and MMP 9 in a significant level. Even though both the compounds possess inhibitory effects, clear activity and drugability of both compounds can be identified only after performing the in vitro and in vivo experiments. GC-MS of different solvent extracts (methanol, toluene, ethyl acetate, chloroform and n-hexane) of Areca catechu L.nut were noted (Private communications) .

CONCLUSION

Matrix Mettaloproteinase (MMPs) 2 and 9 bound with Arecoline with a finding score of -5.9 and -4.3 respectively. and that of nonanalydehyde, -5.3 and -3.9. Arecoline has more significant effect with MMP 9 than MMP 2 nonanalydehyde also has more significant effect with MMP8 than MMP 2. From these we can clearly justifies that bot Arecoline and nonnanaldehyde are capable of incivility MMP 2 and MMP 9 in significant level. Corneal healing may be due to the presence of arecoline and nonanoicacid in Areca catechu L. nut. This may leads to the study of various treatments related with eye problems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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