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Molecular Modelling and Biochemical Evidence to Explore Potentials of Chewing Tobacco in Drug Resistance and Cancer Induction

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Abstract—*Industrial workers have exposure to several composite mixtures, heavy organo-metallic complexes and they also consume tobacco (by smoking and chewing). The habit of smoking or chewing tobacco is linked to an unprecedented rise in lung cancer cases in labour class. The carcinogenic effects of tobacco were initially thought to be restricted to the lungs and the adjoining areas, but recent studies indicate that tobacco also aggravates cancer of the liver, stomach and cervix. In the present study, we explored the molecular mechanism of the role of chewing tobacco in the development of drug resistance and cancer induction. Tobacco leaves are consumed in Indian society, and the samples of these leaves were procured from 20 different cities of India. Interestingly, culturing breast cancer cells in tobacco leave extract (100µg/ml) for several generations follow a trend towards acquiring resistance for anticancer drug mitomycin C., In addition, these cells are showing a higher level of matrix metalloprotease gelatinase activity. Secretion of MMPs is the hallmark of metastatic and invasion phenotype of cancer cells. Protein kinase C is the master regulator of several signalling pathways contributing into metastasis and invasion of cancer cells. Tobacco contains ~120 biologically active phytochemicals, and virtual screening was performed with the C1b domain of PKC-α as the drug target. Several phytochemicals fit well into PKC with high affinity. Phytosterols are found in the prominent candidate molecules, and these molecules might have a crucial role in potentiating the metastatic activity of cancer cells. Hence, a virtual screening experiment complemented with in-vitro studies have assigned an additional role to the phytochemicals present in the tobacco, and these finding may help clinicians to redesign their anti-cancer therapy.*

Keywords*: Cancer, Drug, Gelatinase, Metastasis, Protein kinase C, Tobacco.*

1. INTRODUCTION

Tobacco which is the main ingredient associated with smoking is directly linked to lung cancer [1]. The habit of smoking or chewing toboacco in India is linked to an unprecedented rise in lung cancer cases [2, 3]. The carcinogenic effects of tobacco were initially thought to be restricted to the lungs and the adjoining areas, but recent studies indicate that tobacco also aggravates cancer of the liver, stomach and cervix [4]. Many of the phytochemicals present in tobacco are known to be regulators of specific signalling molecules (transcription factors, kinases, phosphatases, etc.) depending on the nature of signalling pathways that they target [1-3]. One such important signalling molecule is Protein Kinase C (PKC). PKC is a serine-threonine kinase that is present in the cell as a family of 10 isozymes divided into three classes [4, 5]. PKC is a crucial enzyme that is known to control key signalling pathways in cancer cell biology which include cellular proliferation, carcinogenesis and apoptosis [6-10]. Tobacco leaves were procured from 20 different cities of India. Interestingly, culturing breast cancer cells in tobacco leave extract (100µg/ml) for several generations follow a trend towards acquiring resistance for anticancer drug mitomycin C., In addition, these cells are showing a higher level of matrix metalloprotease gelatinase activity. Secretion of MMPs is the hallmark of metastatic and invasion phenotype of cancer cells. Tobacco contains ~120 biologically active phytochemicals, and virtual screening was performed with the C1b domain of PKC-α as the drug target. Several phytochemicals fit well into PKC with high affinity. Phytosterols are found in the prominent candidate molecules, and these molecules might have a crucial role in potentiating the metastatic activity of cancer cells. Hence, a virtual screening experiment complemented with in-vitro studies have assigned an additional role to the phytochemicals present in the tobacco, and these finding may help clinicians to redesign their anti-cancer therapy.

2. MATERIAL AND METHODS:

Cell lines: MDAMB-231 and MCF-7 cells were procured from national cell culture facility, Central Drug Research Institute, Lucknow.

Chemicals and reagents: 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT), DMEM: F12 media was purchased from Hyclone (Logan, USA). Other reagents and chemicals were of analytical grade purity.

Collection of Tobacco leaves: Tobacco leaves were purchased from local market of 20 different cities within India. These leaves are been consumed by indian either by smoking or by chewing along with Betal nut. The leaves samples were verified as tobacco by a taxonomist.

Treatment of cells with Phytochemicals for experiments: In all of the experiments, cells were seeded for overnight in DMEM: F12 complete media. Next morning, cells were treated with different concentrations of the phytochemicals prepared in serum-free media and incubated for various time-periods in CO_2 incubator.

Virtual screening of Tobacco phytochemicals: Virtual Screening to identify the potent phytochemicals from tobacco is performed as described previously (Ref).

Interaction analysis of ligand-PKC complexes: The phytochemical-PKC molecular models were visualised using PyMOL v0.99 [11]. The interaction analysis between the ligand and the amino acid residues of the C1b domain were visualised using Ligplus^{+} software [12].

Viability assay and morphological analysis: Breast cancer cells were treated with tobacco leaf extract and viability were measured by MTT assay. Post treatment, cells were visualised for morphological characteristics by observing under a Nikon Eclipse TS-100F inverted microscope.

3. RESULTS AND DISCUSSION:

Tobacco extract reduces the anticancer potential of mitomycin-C: Breast cancer cells MDAMB-231 were treated with different concentration of tobacco leave (0-500µg/ml) MTT assay measured extract and cellular viability. Interestingly as we were growing cancer cells for several generations, these cells were acquiring resistance towards the anticancer activity of Mitomycin-C (Fig. 1). After seven generations, the anticancer activity of Mitomycin-C was diminished by 70%, but these cells got contaminated so we could not be able to continue experiments to verify the observation. Imaging of cells after seven generations indicate that cells are projecting pseudopodia. In-directly these observations are exhibiting mobile phenotype. The trend indicates that cancer cells are using phytochemicals present in tobacco leaves to overcome apoptogenic activity of Mitomycin-C[13].

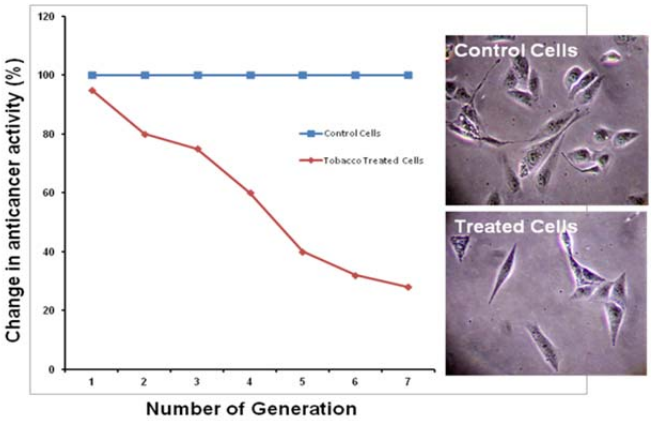

Fig. 1: Tobacco extract reduces the anticancer potential of mitomycin-C.

Cancer Cells pre-conditioned with tobacco extract secretes gelatinase activity: Breast cancer cells MDAMB-231 were treated with different concentration of tobacco leave extract (0-500µg/ml) and gelatinase activity was determined. As expected, cells grow in the presence of tobacco leave extract secreted gelatinase in the culture supernatant (Fig. 2). Secretion of gelatinase activity is associated with

Phytochemicals from tobacco fit well into PKC C1b domain: Tobacco contains several phytochemicals with potent biological effects. To explore the role of PKC into the effect of tobacco on cancer cells, phytochemicals from tobacco were docked into the C1b domain of PKC using Autodock 4.1 as described in **"Material and Methods"**. Phytochemicals were fitting well into the C1b domain with high affinity as exhibited by binding energy (Table 1). The phytochemicals which are showing high affinity are Phytosterols, Xanthin and nicotine derivatives (Table 1). These molecules are fitting well and exhibiting molecular interactions with the residues present within the C1b domain of PKC. The analysis of molecular models of PKC-phytochemical clearly indicates that the phytochemical are binding into the C1b domain in the similar conformation as the phorbol ester such as PMA (Fig. 3A).

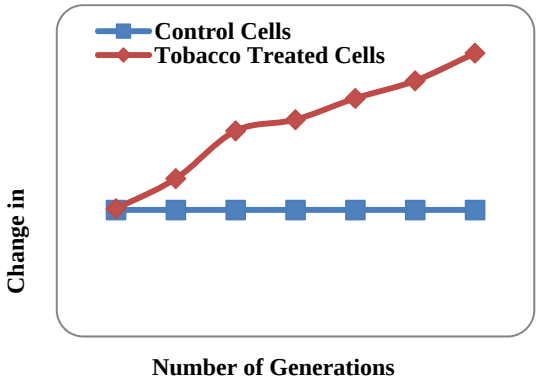

Fig. 2: Tobacco extract pre-conditioning allow cancer cells to secrete gelatinase in the culture supernatant.

Phorbol ester binds into the C1b domain into the extended conformation, and it involves polar and non-polar interactions with the residues of the PKC. PMA is a well-known tumour inducer in different mouse models [10]. Molecular interaction analysis of Xanthin further validates the idea that phytochemicals present in tobacco mimic binding mode and molecular interactions (Fig. 3B). Hence, our preliminary study using molecular modelling and biochemical tools provide insight into the carcinogenic potentials of tobacco present in cigarette smoke or chewing Pan Masala.

Table 1: Tobacco Phytochemicals fit into PKC C1 Domain.

Tobacco Phytochemicals	Binding Energy (Kcal/mol)
γ-Sitosterol	-7.51
Ergosterol	-7.25
(11E,13R)-LABDA-11,14-DIENE-8,13-DIOL	-6.79
FLAVOXANTHIN	-6.56
Scopoletin	-6.51
α-Cyperone	-6.49
(11E,13S)-LABDA-11,14-DIENE-8,13-DIOL	-6.42
DRIMAN-8-OL	-6.41
15-NOR-8ALPHA-HYDROXY-12E-LABDEN-14-OL	-6.28
4-(2',2',6'TRIMETHYL-6'-VINYL-CYCLOHEXYL)-2-BUTANONE	-6.21
DRIMAN-8,11-DIOL	-6.2
(E)-5-ISOPROPYL-8-HYDROXYNON-6-EN-2-ONE	-6.11
EXO-1-(1-METHYL-4-ISOPROPYL-7,8-DIOXABICYCLO-{3,2,1}-OCT-6-YL)-ETHANOL	-6.03
(E)-5-ISOPROPYL-8-HYDROXY-8-METHYLNON-6-EN-2-ONE	-5.96
Neoxanthin	-5.95
CICHORIIN	-5.89
CAPSIDIOL	-5.88
ANATABINE	-5.85
3,3,5-TRIMETHYL-8-ISOPROPYL-4,9-DIOXABICYCLO-(3,3,1)-NONAN-2-OL	-5.83
Oxynicotine	-5.81

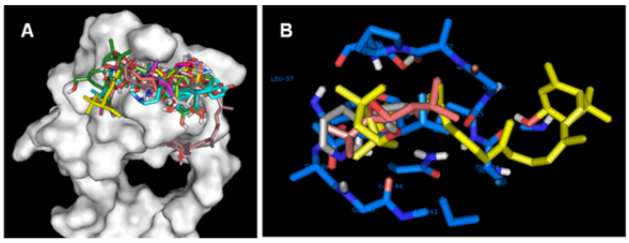

Fig. 3: Molecular Modeling of Tobacco phytochemicals into the PKC C1b domain. (A) Binding mode of phytochemicals and (B) Molecular interactions of Xanthin with residues present within PKC C1b domain.

4. ACKNOWLEDGEMENTS

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REFERENCES

1. Pratheeshkumar, P., et al., *Cancer prevention with promising natural products: mechanisms of action and molecular targets.* Anticancer Agents Med Chem, 2012. **12**(10): p. 1159-84.
2. Deka, S.J., et al., *Alkyl Cinnamates Induce Protein Kinase C Translocation and Anticancer Activity against Breast Cancer Cells through Induction of the Mitochondrial Pathway of Apoptosis.* J Breast Cancer, 2016. **19**(4): p. 358-371.
3. Deka, S.J., et al., *Danazol has potential to cause pkc translocation, cell-cycle dysregulation and apoptosis in breast cancer cells.* Chem Biol Drug Des, 2016.
4. Nishizuka, Y., *Protein kinase C and lipid signaling for sustained cellular responses.* The FASEB Journal, 1995. **9**(7): p. 484-96.
5. Ways, D.K., et al., *MCF-7 breast cancer cells transfected with protein kinase C-alpha exhibit altered expression of other protein kinase C isoforms and display a more aggressive neoplastic phenotype.* Journal of Clinical Investigation, 1995. **95**(4): p. 1906-1915.
6. Grumont, R., et al., *The mitogen-induced increase in T cell size involves PKC and NFAT activation of Rel/NF-kappaB-dependent c-myc expression.* Immunity, 2004. **21**(1): p. 19-30.
7. Schonwasser, D.C., et al., *Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes.* Mol Cell Biol, 1998. **18**(2): p. 790-8.
8. Goode, N., et al., *Differential regulation of glycogen synthase kinase-3 beta by protein kinase C isotypes.* Journal of Biological Chemistry, 1992. **267**(24): p. 16878-82.
9. Santiago-Walker, A.E., et al., *Protein Kinase C δ Stimulates Apoptosis by Initiating G1 Phase Cell Cycle Progression and S Phase Arrest.* Journal of Biological Chemistry, 2005. **280**(37): p. 32107-32114.
10. Furstenberger, G., et al., *Skin tumor promotion by phorbol esters is a two-stage process.* Proc Natl Acad Sci U S A, 1981. **78**(12): p. 7722-6.
11. Schrodinger, LLC, *The PyMOL Molecular Graphics System, Version 1.3r1*. 2010.
12. Wallace, A.C., R.A. Laskowski, and J.M. Thornton, *LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions.* Protein Engineering, 1995. **8**(2): p. 127-134.
13. Elmore, S., *Apoptosis: a review of programmed cell death.* Toxicol Pathol, 2007. **35**(4): p. 495-516.
14. Marcus, A. and J.I. Maletic. *Recovering Documentation-to-Source-Code Traceability Links using Latent Semantic Indexing*. in *25th IEEE/ACM International Conference on Software Engineering (ICSE'03)*. 2003. Portland, OR.
15. Maletic, J.I., M.L. Collard, and A. Marcus. *Source Code Files as Structured Documents*. in *10th IEEE International Workshop on Program Comprehension (IWPC'02)*. 2002. Paris, France.
16. Marcus, A., *Semantic Driven Program Analysis*, in *Department of Computer Science*. 2003, Kent State University: Kent, OH, USA.
17. Salton, G., *Automatic Text Processing: The Transformation, Analysis and Retrieval of Information by Computer*. 1989: Addison-Wesley.
18. Briand, L.C., J. Daly, and J. Wüst, *A unified framework for coupling measurement in objectoriented systems.* IEEE Transactions on Software Engineering, 1999. **25**(1): p. 91-121.