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# Perfusion coefficient of areca nut and pan masala extracts in rat oral mucosa

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## ABSTRACT

Arecoline, a major constituent of areca nut extracts, is capable of causing deleterious effect, ranging from precancerous condition to carcinogenesis in oral tissue. These effects are dependent on the extent of permeability of oral mucosa to organic solutes present in areca nut and pan masala.

**Aims and Objectives :** Our study aims to evaluate the perfusion coefficient and diffusion rate of areca nut & pan masala extracts in oral mucosa using an animal model.

**Materials and Methods :** 24 (formalin fixed) rat buccal mucosal tissues (control: normal buccal mucosa tissue of rats) of 5sqmm, were perfused twice daily with 20µl of extracts of areca nut and pan masala in a specially designed perfusion chamber. Tissues were kept in-vitro condition alike to oral environment and assessed randomly at 24, 48 and 72 hrs for penetration levels of the solutions using stereo/light microscopes and images were analyzed using Lynx-Biolux Auto imaging software.

**Results and Discussion :** Average diffusion for areca nut is higher than pan masala extracts in per sq-mm of rat mucosal tissues respectively. Higher penetration potential of areca nut extracts causes deeper penetration into submucosa and muscles in 48 hrs in comparison to pan masala , which showed progressive penetrates into tissues in 72 hrs.

**Conclusion :** The areca nut showed elevated perfusion coefficient , which supported the fact that Areca nut and its commercial derivatives are detrimental for oral mucosa. Probably due to its ability of being more lipid soluble in nature, rapid penetration in oral mucosa tissue was characteristically seen in comparison to pan masala**.**

**Keywords: Areca nut, Perfusion Coefficient, Pan Masala, Rat buccal mucosa.**

## INTRODUCTION

Areca nut chewing in various forms, has become a trendy custom in the Indian subcontinent. Available Commercially as a readymade dry mixture, which mainly contains areca nut, lime, flavonoids. These mixtures are available either with tobacco known as Gutka or without tobacco available as Pan masala (fig.1).

Arecanut, a major constituent of pan masala is known to be mutagenic, genotoxic and carcinogenic in different experimental systems. As arecoline, a major constituent of areca nut extracts , is capable of causing deleterious effect, ranging from precancerous condition to carcinogenesis in oral tissue. Arecoline induces fibroblast proliferation and collagen synthesis. Arecoline is proficient in penetrating the oral mucosa to cause progressive cross- linking of collagen fibers [(6)] thus resulting in development of Oral Submucous Fibrosis (a precancerous condition) which is linked to the consumption of pan masala. OSMF affects to any part of the oral cavity and also the pharynx.

The lethal effect of arecoline is dependent on the rate of diffusion of the arecanut extract in the oral mucosa. The permeability of oral mucosa is a complex phenomenon and reflects the structure and pathologic status of the tissue as well as the nature of the penetrants. Permeability might play a role in the etiology of certain oral mucosal diseases, including premalignant conditions and cancer. Physical and chemical nature of a substance, plays an important role in determining the

extent of penetration of any substance in oral mucosa. Substances that dissolve readily in both types of solvent (i.e. non polar – lipid and polar – aqueous solvents) pass rapidly across mucosa, but maximum penetration occurs when substance share a slightly preferential lipid solubility [4].

## AIMS & OBJECTIVES

This study aims to evaluate the perfusion coefficient of areca nut & pan masala extracts in oral mucosa using an animal model.
Comparison of perfusion coefficient between the two solutions.

## MATERIALS & METHOD

Preparation of 24 (formalin fixed) Sprague Dawley rat buccal mucosal tissues of 5sq-mm were done. Later perfused by areca nut and pan masala extracts in a specially designed perfusion chamber. Rat buccal mucosal tissues: treated with areca nut extract -**10**, treated with pan masala extract -**10.** Control Group : normal buccal mucosa tissue of rats – **4.** Stereo / Light microscopes was used for evaluation . Lynx-Bioluxauto imaging software for analyses of images.

Preparation of the solutions - 30% solution of pan masala and arecanut: 2 gms of powdered arecanut and pan masala were dissolved separately in 6 ml of distilled water and the solutions were centrifuged for 30 mins at 15,000 rpm . The supernatant of both solutions were collected and used as shown in Fig.2 .


**Fig. 1 : Arecanut and pan masala**

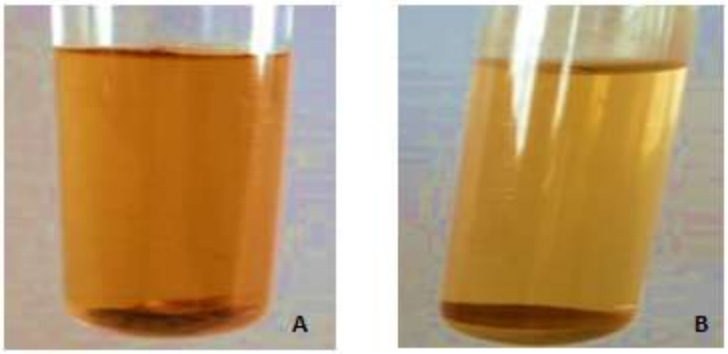


**Fig.2: Supernatant solution of (A) Pan masala (B) Arecanut**

Tissues were kept in-vitro condition simulating to oral environment. 5 sq-mm buccal mucosal tissues of rats were spread over a thyroform sheet and kept inside the specially designed perfusion chambers. The orientation of tissue was done with the epithelial surface outwards and the mucosa downwards to assist and assess perfusion flow. The buccal mucosal surface of the tissues was perfused twice daily with 20µl of the extracts. The perfusion chambers were placed in an Incubating chamber at 37 degree centigrade (Fig). The tissues were assessed randomly at 24, 48 and 72 hrs . The penetration levels of the solutions in the tissues was assessed by using stereo/ light microscopes. The images were analysed by image analysis software.

A B C D

**Fig. 3 : (A-C) Assembled specially designed perfusion chamber (D : Incubating chamber )**

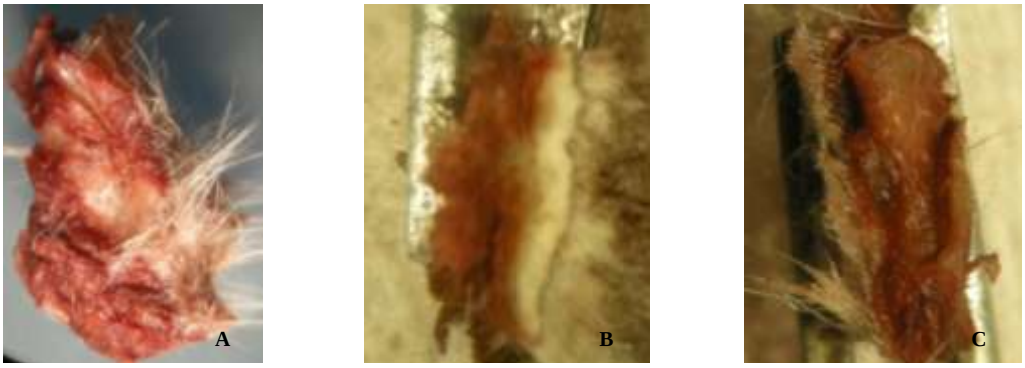


**Fig 4: Macroscopic pictures of penetration of Pan masala extract at (A) 24 hrs (B) 48hrs (C) 72 hrs**


**Fig 5: Macroscopic pictures of penetration of Arecanut extract at (A) 24 hrs (B) 48hrs (C) 72 hrs**

## RESULT

**Table. 1 : Comparison Of Coefficient Of Perfusion ( Micron/Sq-mm) Stereomicroscope - Post Image Analysis**

| | Coefficient Of Perfusion ( micron/ sq.mm) | | |
|---|---|---|---|
| | Stereo Microscope | | |
| | 24hrs | 48hrs | 72hrs |
| Areca nut | 6.94 | 7.19 | 7.37 |
| Pan masala | 2.6 | 4.5 | 7.17 |

**Table . 2 : Comparison Of Coefficient Of Perfusion ( Micron/Sq-mm) light microscope - Post Image Analysis**

| | Coefficient Of Perfusion ( Micron/Sqmm) | | |
|---|---|---|---|
| | Light Microscope | | |
| | 24hrs | 48hrs | 72hrs |
| Areca Nut | 10.93 | 11.28 | 11.51 |
| Pan Masala | 3.5 | 8.3 | 10.95 |

**Table. 3 : Comparison of Average Diffusion Rate Of Solutions (micron/sq-mm)**

| | Average Diffusion Rate Of Solutions |
|---|---|
| Areca nut | 11.24µ/sqmm |
| Pan masala | 7.58µ/sqmm |

**Table. 4 : Comparison of Average Diffusion Rate Of Solutions single table (micron/sq-mm)**

| | Coefficient Of Perfusion ( micron/sqmm) | | | | | | Average Diffusion Rate Of Solutions |
|---|---|---|---|---|---|---|---|
| | Stereo Microscope | | | Light Microscope | | | |
| | 24hrs | 48hrs | 72hrs | 24hrs | 48hrs | 72hrs | |
| Areca nut | 6.94 | 7.19 | 7.37 | 10.93 | 11.28 | 11.51 | 11.24µ/sq.mm |
| Pan masala | 2.6 | 4.5 | 7.17 | 3.5 | 8.3 | 10.95 | 7.58µ/sq.mm |

## DISCUSSIONS

The results obtained shows that the arecanut extracts have better rate of diffusion than the pan masala solution. The penetration of pan masala was restricted only to the epithelium and continued down to the connective tissue after 48 hrs ,further penetration into the muscles and submucosa was only observed after 72 hrs of incubation.
Penetration potential of areca nut extracts reveals higher than pan masala , as areca nut penetrated deeper into submucosa and muscles in 48 hrs in comparison to pan masala. Thus we observed that
Areca nut has elevated perfusion coefficient, as it might be more lipid soluble, which attributes for its rapid penetration in tissues.

## CONCLUSION

Both arecanut and pan masala are deleterious to oral mucosa. But the destructive potential of arecanut extracts are much higher than pan masala as areca nut has higher rate of diffusion in tissue and thus it has higher perfusion coefficient.

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