DEVELOPMENT OF AN ANIMAL MODEL TO STUDY ORAL SUBMUCOUS FIBROSIS USING AQUEOUS ARECA NUT (*Areca catechu*) EXTRACT

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Oral Submucous Fibrosis (OSF), a potentially malignant condition of the mouth, has an insidious onset inducing progressive fibrotic changes of the oral sub-mucosa resulting restricted mouth opening. Its aetiology and pathogenesis remain obscure to date. However, areca nut (*Areca catechu*) has been implicated for the development and progression of the disease as evidenced by numerous epidemiological and experimental studies.

Non-availability of a reproducible animal model of OSF may have hindered uncovering the pathogenic mechanisms involved in this disease process. In addition, it may also account for the paucity of research related to the development of specific therapeutic agents to treat this disease. Therefore, the present study was undertaken to develop an animal model of OSF which would be confirmed by qualitative and as well as quantitative histopathological analysis.

BALB/c mouse was selected as the experimental animal. The corresponding test (*n=20*) and control (*n=20*) animals were allotted to four different treatment intervals i.e. 300, 350, 450 and 600 days, maintaining 5 animals in each group. A drop (~ 35 µl) of areca extract in normal saline (265g dry weight/L) was administered to the oral mucosa of each of the test group mice using a transfer pipette and a similar amount of normal saline was used for the control. The

buccal and tongue mucosae of the animals were harvested at the allotted time intervals, fixed in para formaldehyde and processed for routine histology. Tissue sections of 4-5μm were stained with haematoxylin and eosin. Selected tissue sections were stained with van Gieson and Masson's trichrome to confirm the fibrotic changes in the connective tissues. Histopathological criteria described by Pindborg and Sirsat (1996) were employed to confirm the diagnosis of OSF in the animal model. Quantitative histomorphometric measurements were taken for both buccal and tongue mucosae to confirm excessive deposition of collagen in the lamina propria and atrophy of the epithelium in the corresponding tissue.

Histopathological examination of tissues obtained from animals of every treatment interval clearly demonstrated cellular changes which were pathognomonic of OSF. Buccal mucosa of the test group showed excessive fibrotic changes both at qualitative and quantitative levels. Epithelial atrophy was increased throughout the treatment intervals except in the papillary epithelial compartment at 300-day interval. Polynomial regression plots showed that atrophy of the inter-papillary epithelial compartment occurred prior to the atrophy of the papillary epithelial compartment. Similar patterns of fibrosis and atrophy were observed in the tongue mucosae of the treated animals. Connective tissue changes preceded atrophic changes of the epithelium and a similar sequence of events has been observed in human subjects affected by this condition.

The results obtained in the series of experiments with BALB/c mice clearly demonstrated the classical histological features seen in OSF in human subjects. Concerning the site-specificity for the development of OSF, the buccal mucosa ranked above the lingual mucosa. Scanning the literature, it appeared that this is the first successful attempt in inducing oral submucous fibrosis in an animal model substantiating the causative role of areca nut in the pathogenesis of this disease.