Research Article

Protective Effect of *Piper Betel* Leaves Extract in the Treatment of Oral Submucous Fibrosis in Rats

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ABSTRACT

The aim is to study the Protective effect of aqueous extract of leaves of *Piper Betel* in the treatment of Oral Submucous fibrosis in rats. Rats were divided into three groups, group 1 and 2 five rats each and group 3 consisted of fifteen rats. Group 1 served as control and was not given any application. Group 2 rats were applied muco-adhesive paste of Gutkha and evaluated for the progress of OSMF for 3, 6 and 12 months respectively through biopsies. Group 3 rats were also applied muco-adhesive paste of Gutkha for 3, 6 and 12 months respectively, and on confirmation of OSMF induction through biopsies, muco-adhesive paste of aqueous extract of *Piper Betel* leaves was applied for 3, 6 and 12 months. Biopsies were taken on completion of the study. Treatment with aqueous extract of leaves of *Piper Betel* was found to restore the histological characteristics of normal mucosa in response to the duration of OSMF induction and treatment. Present study shows that aqueous extract of *Piper Betel* leaves holds promising result in the treatment of OSMF and other disorders requiring treatment with antioxidants.

Key words: *Piper Betel*, Oral Submucous fibrosis (OSMF), Gutkha, Aqueous extract.

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INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic disease of insidious onset featuring the deposition of fibrous tissue in the submucosal layer of the pharynx, palate, fauces, cheek and lips and esophagus.\(^1\) The increased deposition of collagen (fibrosis) in the potentially malignant oral disorder of oral submucous fibrosis (OSMF) is pathognomonic of the condition. Ever since its categorization as an oral disorder in the 1950s, the significance of this condition in causing widespread debility in the oral cavity and its propensity for malignant transformation has been well recognized.\(^2\)

Pindborg and Sirsat have defined OSMF as an “insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with epithelial inflammatory reactions followed by a fibro elastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus.
and inability to eat. The term oral submucous fibrosis (OSMF) follows from oral (meaning mouth), submucosal (meaning below the mucosa of the mouth) and fibrosis (meaning hardening and scarring).

OSMF has a multifactorial etiology. Several factors such as chilli consumption, nutritional deficiency states, areca nut chewing, genetic susceptibility, autoimmune & collagen disorders have been suggested to be involved in the pathogenesis of the condition. Clinical features of OSMF include burning sensation on taking spicy food, excessive salivation, dryness of the mouth, defective gustatory sensation and progressive restriction of mouth opening and the protrusion of the tongue. It is characterized by excessive production of collagen leading to inelasticity of the oral mucosa and atrophic changes of the epithelium, with the progress of the disease; fibrosis may extend from the lamina propria through entire submucosa to the muscle layer. Thick inelastic rope like fibrous bands appear vertically in the buccal mucosa, along the contours of the faucial pillars and around the entire circle of lips thus leading to difficulty in mouth opening and narrowing of the rima oris.

In order to understand the pathogenesis of the disease, the identification of the collagen type and content is necessary. Over deposition of unaltered collagen would indicate an overstimulation of normal fibroblasts with probable reduced or absent degradatory collagenase control. The most outstanding feature and the most reliable sign of oral submucous fibrosis is the presence of palpable bands in the oral mucosa, especially in the buccal mucosa. The disease, however, has other characteristic signs, such as diffuse blanching of the mucosa, occurrence of hyper pigmented areas adjacent to zones with loss of pigment, loss of tongue papillae, and a leathery consistency of the mucosa. A more serious complication of this disease is the risk of the development of oral squamous cell carcinoma (SCC), estimated to be 7.6 percent of cases over a 10 year period. The reasons for the rapid increase of the disease are reported to be due to an upsurge in the popularity of commercially prepared areca nut preparations (pan masala) in India and to an increased uptake of this habit by young people due to easy access, effective price changes and marketing strategies. OSMF has been reported in the Indian population and established in the Indian literature since the time of Sushruta. The various treatment modalities proposed for OSMF include nutritional support, immunomodulatory drugs, physiotherapy, local drug delivery, combined therapy and surgical management.

Medicinal Plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Large numbers of plant are constantly being screened for their chemical and pharmacological properties. By the application of modern techniques of isolation and pharmacological evaluation, many new plant drugs find their way to medicine as purified substances.

Piper betel, family Piperaceae, commonly known as the betel vine is a important medicinal and recreational plant in Southeast Asia. The most probable place of origin of betel vine is Malaysia but today the plants are also cultivated in India, Srilanka, Bangladesh, Burma and Nepal. Betel leaves are the most important plant part and are of medicinal, religious and ceremonial value in Southeast Asia. Piper betel is a medicinal plant that is traditionally used in catarhral and pulmonary affections, as a digestive and carminative and as a stimulant of pancreatic lipase. The aqueous extract of P. betel prevented formation of tumors when fed to rats in the initiation phase of induced-mammary carcinogenesis but could not inhibit tumor growth when fed to rats with induced mammary carcinogenesis.

Furthermore, the leaves of P. betel has strong anti-tumor promoting activities in Raji cells whereas the aqueous extract was reported to show anti-proliferative action towards kB cells, indicating their potential in treating oral cancer.

We isolated the extracts from the leaves of P. betel, and a study was designed using the extracts of the leaves of P. betel to assess the healing activity in OSMF induced rats.

**MATERIALS AND METHOD**

**Collection of Plant Materials**

Piper betel leaves were collected from local market. The leaves were identified and authenticated at Botany division, Osmania University and the voucher specimen was deposited.

**Preparation of Extract**

The freshly collected leaves were washed, shadow dried and then again dried in hot air oven at temperature not exceeding 50º C. The dried leaves were coarsely powdered mechanically. Powdered leaves 400 gms were then packed in soxhlet apparatus and successively extracted with methanol and water. The extracts were
concentrated in rotary evaporator at temperatures not exceeding 50°C and then dried under vacuum desiccators. The dried extracts were stored.

**Animals**

Male Wister rats weighing 175-200 gm were obtained from the animal house of Nizam Institute of Pharmacy and Research Centre, Hyderabad and housed in polycarbonate cages. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment with the exception of some experiments in which the animals were deprived of food, but not water, for 18-24 h before the experiments were performed. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to experimentation room and were maintained on standard conditions (23°C, 60%-70% relative humidity and 12 h photo period). The animals were divided into three groups of five animals each. Group 1 served as control and was not given any application. Group 2 rats were applied muco-adhesive paste of Gutkha and evaluated for the progress of OSMF for 3, 6 and 12 months respectively through biopsies. Group 3 rats were also applied muco-adhesive paste of Gutkha for 3, 6 and 12 months respectively, and on confirmation of OSMF induction through biopsies, muco-adhesive paste of aqueous extract of *Piper Betel* leaves was applied for 3, 6 and 12 months.

**Preparation of Muco-adhesive semisolid preparation of Gutkha:**

Mucoadhesive semisolid preparation of Gutkha was prepared using micro-adhesive polymers such as hydroxyethyl cellulose (HEC), carboxymethyl cellulose sodium salt (Na CMC) and equal mixture of HEC and NaCMC. (Table no. 1)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gutkha</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>2</td>
<td>HEC</td>
<td>4.0 gm</td>
</tr>
<tr>
<td>3</td>
<td>Glycerine</td>
<td>2.0 gm</td>
</tr>
<tr>
<td>4</td>
<td>Sodium metabisulfite</td>
<td>0.5 gm</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>15 ml</td>
</tr>
<tr>
<td>6</td>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Table 1:** Preparation of Muco-adhesive paste of Gutkha

**Preparation of Muco-adhesive paste of aqueous extract of *Piper Betel* leaves:**

Mucoadhesive semisolid preparation of aqueous extract of *Piper Betel* leaves was prepared using micro-adhesive polymers such as hydroxyethyl cellulose (HEC), carboxymethyl cellulose sodium salt (Na CMC) and equal mixture of HEC and NaCMC. (Table no. 2)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract of <em>Piper Betel</em> leaves</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>2</td>
<td>HEC</td>
<td>2.0 gm</td>
</tr>
<tr>
<td>3</td>
<td>Na CMC</td>
<td>2.0 gm</td>
</tr>
<tr>
<td>4</td>
<td>Sodium metabisulfite</td>
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</tr>
<tr>
<td>7</td>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Table 2:** Ingredients of Muco-adhesive paste of aqueous extract of *Piper Betel* leaves

**In vivo experimentation**

There were three groups of rats for the study. All experimental protocols described below were approved by the ethical board.

**Group 1:** Control group comprising 5 rats which will not be given any application during the study course.

**Group 2:** Induction group comprising of 15 rats which will be further divided into three sub groups for Gutkha application for duration of 3, 6 and 12 months.

**Group 3:** Treatment group comprising of 15 rats which will be further divided into three sub groups for Gutkha application for duration of 3, 6 and 12 months followed by treatment for a period of 3, 6 and 12 months. The rats were anesthetized and sacrificed after the experimental period by cervical decapitation. The tissue was examined histopathologically.

**RESULTS**

The grading of OSMF was one according to Utsimomiya H, Tilakratne Wm, Oshiro K et al (2005) and graded as early, intermediate and advanced.

**Figure 1:** Normal mucosa

Mean value of right and left OSMF induced buccal mucosa was taken and early OSMF was established in the three months of Gutkha application group of rats (Figure 2). Figure 2, 3 and 4 represents Gutkha induction.
Aqueous extract of *Piper Betel* leaves in this group for three months reversed the early OSMF changes to normal mucosa (Figure 5).

In the twelve months of Gutkha application group of rats the mean value of right and left OSMF induced buccal mucosa showed advanced OSMF development (Figure 4). Treatment with Aqueous extract of *Piper Betel* leaves in this group for twelve months reversed the advanced OSMF to intermediate OSMF (Figure 7).

**DISCUSSION**

OSMF has affected millions of individuals and is likely to reach an alarming proportion in the near future. The patients initially complain of burning sensation in the oral cavity while consuming spicy food. As the disease progresses the oral mucosa becomes blanched, slightly opaque and fibrous bands appear leading to difficulty in opening the mouth, inability to whistle and difficulty in swallowing.24 Pindborg JJ et al (1984) in their article evaluated the malignant potential of submucous fibrosis in 89 patients. At the time of first examination oral cancer was found in 9 patients (10%) and for the rest of the cases the rate of malignant transformation was 4.5%.35 Murti PR et al (1985) observed the malignant transformation rate in oral submucous fibrosis over a 17 year period and their study showed that oral submucous
fibrosis possesses a high degree of malignant potential, within 2 years of observation the malignant transformation rose from 4.5 to 7.6%.

*Piper betel*, family Piperaceae, commonly known as the betel vine is an important medicinal and recreational plant in Southeast Asia. Our earlier study showed that the aqueous extract of *Piper Betel* leaves contains highest amount of phenolic compounds and exhibited the greatest antioxidant activity.

In the present study OSMF induction was done for 3, 6, 12 months respectively and gradual changes were observed during 3 months to 12 months duration of induction. OSMF treatment was carried out using application of the aqueous extract of leaves of *Piper Betel*, and it was found to restore the histological characteristics of normal mucosa in response to the duration of OSMF induction and treatment.

**CONCLUSION**

Present study shows that aqueous extract of *Piper Betel* leaves holds promising result in the treatment of OSMF and other disorders requiring treatment with antioxidants.

**REFERENCES**


