Original Article

Study of oral lesions caused by different pan masala associated with proliferative marker study in experimental animals

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Abstract

Different panmasala available in the market because pre-malignant and malignant lesions like leukoplakia, oral submucus fibrosis, severe dysplasia and carcinoma. Addiction to panmasala is quite common in young generation in India. The prognosis and possible outcome of treatment vary in different stages. The early precancerous lesions lie in the grey zone and are potentially reversible. This sequence of events is studied in this work on experimental animal guinea pigs. Different brands of panmasala preparations are applied for specific periods of time on the guinea pigs and animals were sacrificed in specified periods of time. Then buccal mucosa of the animals are histopathologically examined with HandE stain and also studied by using proliferative marker PCNA and AgNOR.

Keywords: Panmasala, leukoplakia, oral submucus fibrosis, dysplasia, buccal mucosa of guinea pigs, HandE stain, proliferative markers PCNA and AgNOR, prickle cell layers.

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INTRODUCTION

Different panmasala preparations available in the market are predisposing factors for various pre-malignant and malignant lesions like leukoplakia, oral submucus fibrosis, severe dysplasia and carcinoma. Addiction to panmasala is quite common in India, particularly in young generation[1]. The prognosis and treatment modalities vary in different stages. The early precancerous lesions lie in the grey zone and are potentially reversible [2].This sequence of events is studied in this work on experimental animal guinea pigs. Different brands of panmasala preparations are applied for specific periods of time on the cheek of the guinea pig and animals were sacrificed in specified periods of time. The buccal mucosa of the animals retrieved is HandE stained[3]. The buccal mucosa is also studied by using two proliferative markers i) PCNA which characterized by DNA delta polymerase associated protein functions and is an essential component of eukaryotic chromosomal DNA replication[4] and in ii) AgNOR NORS' represents loops of DNA which codes for genes from RNA responsible for protein synthesis. NORS are located by silver nitrate under prescribed conditions. AgNOR counts differentiate between benign and malignant lesions[5].

In this study observation will be made on

1. Evolution of change in different layers of oral mucosa after application of pan masala by H and E staining.
2. Expressions of proliferate markers in various stages of evolution of the lesions.

MATERIALS AND METHODS

This experimental study was started with 12 guinea pigs of the species Cavia porcellus. Out of the 12 guinea pigs 8 were used as test and 4 were used as control. The pan masala were made in a paste by using normal saline and

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was applied thrice a day. Only normal saline were applied in the cheek of the control animals.1 year later from the start of the experiment 4 test animals and 2 control animals were sacrificed. Finally at the end of 18 months from the start of the experiment remaining 4 test animals and remaining 2 control animals were sacrificed. Each of the animals with their specific serial number sacrificed and their buccal mucosa were excised and they were processed for HandE staining, AgNOR staining and PCNA study.

RESULTS AND ANALYSIS

The study of the effects of pan masala in cheeks of guinea pigs and their histological examination with HandE staining and study of proliferative markers was undertaken.

Table 1: The status of the animals and time of sacrifice of animals were as follows

Status	Sacrificed after 1 year	Sacrificed after one and half year
Test Group	T1	T2
Control	C10	C2

Table 2: In all the groups the thickness of prickle cell layers was as follows (Fig.2):

Serial Number	Status	Thickness of the prickle cell layer in mm
1	T1	0.25mm
2	T1	0.24mm
3	T1	0.30mm
4	T1	0.31mm
5	C1	0.15mm
6	C1	0.14mm
7	T2	0.32mm
8	T2	0.31mm
9	T2	0.25mm
10	T2	0.28mm
11	C2	0.15mm
12	C2	0.13mm

Table 3: In all the groups the number of prickle cell layers was as follows

Serial Number	Status	Number of prickle cell layer
1	T1	16
2	T1	14
3	T1	21
4	T1	23
5	C1	13
6	C1	12
7	T2	27
8	T2	25
9	T2	16
10	T2	19
11	C2	13
12	C2	11

It is clear from these two charts that the prickle cell layer thickness and the number of prickle cell layer were increased in the T1 and T2 than C1 and C2 groups, thus suggestive of squamous hyperplasia in the T1 and T2 groups. Mild dysplastic changes were found in serial number 7 and 8 of the T2 group. In T2 layer the number of prickle cell and the thickness of prickle cell layers were increased, than the T1 group showing application (of pan masala) time has a relation with hyperplasia.

Table 4: AgNOR count was made for all the specimens of T1, T2, C1 and C2 group's results were as follows

Serial Number	Status	AgNOR Count
1	T1	4.2
2	T1	3.3
3	T1	3.2
4	T1	4.1
5	C1	1.1
6	C1	1.2
7	T2	4.2
8	T2	3.3
9	T2	4.2
10	T2	4.3
11	C2	1.2
12	C2	1.1

It is seen that the proliferative marker AgNOR count were higher in the T1 and T2 groups than in the C1 and C2 groups. Highest counts were found in serial 7 and 8 of the T2 group were mild dysplastic charges were seen. Average count of T2 was found to be greater than T1; signifying greater application time (of pan masala) has a relation with proliferation.

Table 5: PCNA Counts are as follows

Serial Number	Status	PCNA Count
1	T1	33%
2	T1	31%
3	T1	39%
4	T1	40%
5	C1	15%
6	C1	14%
7	T2	45%
8	T2	41%
9	T2	35%
10	T2	38%
11	C2	14%
12	C2	10%

It is thus found that the PCNA labeling index was higher in the specimens of T1 and T2 groups than in the C1 and C2 groups. PCNA LI was highest in the 7 and 8 serials where dysplasia was found. Average PCNA LI of T2 was also found to be greater than T1 signifying that the increased application time (of pan masala) has a relation with proliferation.

DISCUSSION

The buccal mucosa of the animals after proper processing was stained with haematoxylin and eosin. These slides when examined under microscope gave a clear assessment of the proliferative nature of the lesions produced on test animals. In the first parameter under HandE staining the thickness of pickle cell layer of all the test and control animals were measured with help of occulometer. It was found that the thickness of the prickle cell layer of the test animals was definitely increased than the control group of animals. It was also seen that the average thickness of the prickle cell layer of T1 group of animals which were exposed to the application of pan masala for a period of 1 year, were lesser than the average thickness of T2 group of animals which were exposed to pan masala application period of one and a half year. This gave an indication of squamous hyperplasia which evolved over a period of time and had a relation to the period of application of pan masala.

Table 6: The squamous hyperplasia was seen more in the T2 group than in the T1 group. The controls both C1 and C2 groups did not show any squamous hyperplasia

Status	Average prickle cell layer thickness	Average number of prickle cell layer
T1	0.27mm	18.5
T2	0.29mm	21.7
C1	0.15mm	12.5
C2	0.14mm	12.0

Next in our study was the study of proliferative markers. In the first study we had used AgNOR to see the extent of proliferative activity of the squamous epithelium in response to the application of panmasala. AgNOR was visualized as clearly defined dark brown dots or blots in a yellow background with the nuclei. The area occupied by the NORs varied in size and shape. All these areas both intra-cellular and extra-cellular were counted under oil immersion (5x100). 100 cells were counted in a systematic manner. The average count was noted. The result showed that the AgNOR count in the test cases of the T1 and T2 were higher than C1 and C2 groups. Thus the proliferative activity of the T1 and T2 was distinctly higher than the C1 and C2 group. It was also noted that the average AgNOR count of all the animals in the T1 group which were exposed to the application of pan masala for 1 year were slightly lower than the average AgNOR counts of the T2 group.

Table 7: Average AgNOR Count

Status	Average AgNOR Count
T1	3.7
T2	4.0
C1 7	1.1
C2 8	1.1

In our study it was found that the PCNA labeling was higher in the test animals than the control animals. It was also found that the average PCNA LI was higher in the T2 group where pan masala was applied for one and a half years than the T1 group where the pan masala was applied for a period of one year. Thus it may be said that the PCNA-LI a sensitive marker of proliferation was also directly proportional to the period of application of pan masala.

Table 7: Showing PCNA LI was highest in serial 7 and 8 sections with mild dysplasia

Status	PCNA LI Average	Comment
T1	35	
T2	39	
T2 Serial 7	45	Mild dysplasia was found in HandE
T2 Serial 8	41	Mild dysplasia was found in HandE
C1	15	
C2	12	

The ranges of PCNA values (considering all the cases) are wider than the AgNOR counts. So interpretation of any change can be detected easily. So, PCNA is better as proliferative marker. Thus it is seen that pan masala has some agents that produce proliferation of the squamous cell of buccal mucous membrane of the experimental animals. This agent had caused the proliferation of the prickle cell layer by increase in the number of prickle cell layer and the thickness of the prickle cell layer and showed features of mild dysplasia with atypical cells.

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