



Determination of local anesthetic action of Betel leaf extract alone and with Betel nut using infiltration and surface anesthesia

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Abstract

Objective: (1) To evaluate the local anesthetic activity of betel leaf and assess the effect of betel nut on such effect and (2) to observe local anesthetic activity after autoclaving the extracts of betel leaf. **Materials and methods:** Extracts of plain betel leaf with betel nut, with and without autoclaving, were tested for surface and infiltration anesthetic activities using rabbits and guinea pigs. The results were compared with normal saline control and xylocaine drug control. **Results:** Betel leaf showed dose-dependant infiltration anesthetic activity comparable with xylocaine. As a surface anesthetic, the onset was as quick as xylocaine and the duration was shorter than xylocaine. Betel nut significantly reduced the infiltration activity and abolished the surface anesthetic activity of betel leaf. Autoclaving did not result in any loss of activity. **Conclusion:** Betel leaf has potent local anesthetic action both by surface and infiltration techniques. This effect is reduced by the addition of betel nut but not lost on autoclaving.

Keywords: *Piper betel*, *Areca catechu*, local anesthetic activity

1. Introduction

Piper betel Linn. (Piperaceae) commonly known as betel leaf is a native to southern India and Malaysia. It is cultivated and consumed in western pacific basin and southern Asia. The use of betel leaf can be traced as far back as two thousand years. Fresh leaves are chewed with betel nut, *Areca catechu* (Arecaceae) and other adjuvants (betel quid) in most parts of India and the habit of betel chewing is so widely prevalent that it ranks next to consumption of alcohol, coffee and smoking [1]. Many studies have been undertaken based on this habit from

ancient times till date to elucidate the chemical composition of betel leaf and assess the pharmacological effects and pathological changes produced by betel leaf use. Betel leaves are reported to contain an aromatic oil [2], minerals [3], glycosides [4], enzymes, vitamins, essential amino acids [5] and tannins [6]. A literature search for various pharmacological effects of betel revealed that it is claimed to have astringent, aphrodisiac, laxative, antimicrobial, mucolytic, anti-inflammatory and euphoric properties.

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Most of these alleged actions have been scientifically tested for their worthiness and reported [7-8]. Recently research is focussed mainly on the carcinogenic and mutagenic potential of the betel quid [9-10] and on the anti-mutagenic and anti-carcinogenic effect of plain betel leaf extract [11].

It is a common observation that chewing of betel leaf produces numbness in the mouth, suggesting a possible local anesthetic effect. It has been mentioned that the perception of taste and sensibility of the buccal mucous membrane becomes temporarily dulled after betel chewing [12]. Use of betel leaf has been perceived to have dysesthetic properties and is discouraged in the west [1].

The juice of the leaves was used as drops in painful afflictions of eye and ear. It is stated in Dr. Watt's Dictionary of the Economic products of India that an alkaloid named Arakene, with properties somewhat allied to that of cocaine, has been extracted from leaves [13].

Literary search for a local anesthetic effect of betel leaf among more than one hundred articles related to betel consumption and consequences [1], revealed that this aspect was not looked for and no scientific study had been reported so far. Earlier study in this department with cold extract of betel leaf has demonstrated local anesthetic effect using frog plexus anaesthesia (unpublished data).

Pharmacological actions of betel leaf alone and in combination with betel nut have been studied for various effects in this department.

It has been found that addition of betel nut modifies the action of betel leaf (unpublished observations). The present study aims at exploring the possible local anesthetic effect of betel leaf extract alone and in combination with betel nut using infiltration and surface anesthetic techniques. The effect of sterilizing the extracts by autoclaving for any loss of activity is also assessed.

2. Material and methods

2.1 Preparation of Betel leaf extract

Fresh betel leaves from a single twining plant cultivated in local garden were procured, cleaned and wiped. 20 g of leaves were weighed, ground to a paste without adding water in a mortar and pestle, transferred to a muslin cloth, the juice was squeezed out and divided into two portions. One portion was labeled as 'B' - representing plain betel juice extract. The other portion was autoclaved and labeled as 'AB' - representing autoclaved betel juice extract. 20 g of betel leaves were again weighed out and ground to a smooth paste with one gram of betel nut and extracted with 100 ml of distilled water.

This extract was again divided into two portions. One portion served as betel leaf with betel nut extract and labeled as 'BP'. The other portion was

Table 1a.

The failure of response to stimulus expressed as percentage in each group in infiltration anaesthesia model.

Solution tested	Doses in ml (Intradermal)				
	0.1	0.2	0.3	0.6	0.9
Saline control(C)	0.0	0.0	0.0	0.0	0.0
Xylocaine(X)	100	100	100	100	100
Betel leaf (B)	69.5 ± 35.6	91.7 ± 20.5	83.3 ± 25.8	91.7 ± 20.4	100
Betel leaf + Betel nut (BP)	0.0	0.0	2.8 ± 6.8	8.33 ± 20.4	11 ± 27.2
Autoclaved Betel leaf (AB)	5.6 ± 13.6	41.7 ± 49.2	61.1 ± 49.1	66.7 ± 51.6	77.8 ± 40.4
Autoclaved Betel leaf + Betel nut (ABP)	0.0	0.0	0.0	0.0	0.0

n=6 animals/group/dose, values are mean ± SD

Table 1b.

Local anesthetic effect: Infiltration anesthesia
(Comparison by group)

Groups Compared	Doses (ml)				
	0.1	0.2	0.3	0.6	0.9
B vs. C	**	****	****	****	****
B vs. X	NS	NS	NS	NS	NS
B vs. AB	**	**	NS	NS	NS
B vs. BP	**	****	****	****	****
B vs. ABP	**	****	****	****	****

** p<0.01; **** p<0.0001 ; NS p>0.05 (not significant)

autoclaved and labelled as 'ABP'. Normal saline 'C', served as normal control whereas xylocaine 2% 'X' served as reference standard.

2.2 Testing for infiltration anesthesia

Infiltration anesthetic activity was tested in guinea pigs by the intradermal method of Bulbring and Wajda [14]. 30 healthy guinea pigs weighing 400-500 g were selected and divided into 5 groups of 6 each, for testing the extracts at a dose of 0.1, 0.2, 0.3, 0.6 and 0.9 ml respectively. The fur on the dorsal side of each animal was shaved to prepare six testing sites, distributed as three in a row on either side of the mid-dorsal line and marked as shown in fig. 1.

Each animal belonging to the same group received one particular dose of each of the solutions, intradermally, in a pattern as shown in the fig. 1, and tested one by one. This allowed testing of one dose of each of the solutions in the same animal, minimising the error due to biological variation.

Each animal was tested for reflex skin retraction and / squeaking for pinpricks. The responses were checked at every minute, by recording the number of pricks out of 6, not felt by the animal. Six such observations were made in each animal for six minutes with one minute interval between stimuli, after each injection. That is, each site was tested for a total of 36 stimuli and the total number of stimuli that failed to evoke response were summed up and represented as N. Local anesthetic activity was regarded complete when the animal failed to respond to all the 36 pin-pricks at the site of injection.

2.3. Testing of surface anesthesia

This was tested on the cornea of the rabbits following the procedure of Shapero and Southgate [15]. Rabbits (1.5-2.0 kg) and guinea pigs (400-500 g) were selected. Each animal was put in the animal restrainer. Their eyelashes were trimmed, taking care not to damage the eyelids. A cotton wool served as the source of touch stimuli. Without the knowledge of the animal, the cornea was touched with the cotton wool and closure of the eye lids (blinking response) was looked for. The animals giving a positive response to the corneal reflex were included in the study.

The selected animals were divided into 6 groups of 6 animals each. The groups were named as C, X, B, AB, BP and ABP, based on the solution tested on them. The lower eyelid of the animal was pulled to form a pouch. Two drops of the test solution were instilled into the eye. The eyelids were drawn closer to ensure uniform distribution of the solution. The medial canthus was pressed simultaneously to avoid removal of the solution through the nasolacrimal duct. The right eye of each animal received the control drug X while the left eye received the test solution.

Corneal reflex as above was checked every minute. Surface anesthesia was regarded complete if the animal failed to blink in response to all the 6 touch stimuli and the time taken to achieve this was taken as time of onset of local anesthetic effect. The procedure of checking for corneal reflex was continued every minute for at least 10 minutes to check for reappearance of corneal reflex. The time taken for onset and recovery were thus recorded.

2.4 Statistical analysis

The values are expressed as mean \pm SD. The results were analysed statistically using Student's *t*-test. A probability value of $p < 0.05$ was considered as statistically significant.

3. Results

3.1 Infiltration anesthesia

The data for the 6 doses of solutions C, X, B, AB, BP and ABP, obtained from the 5 groups of animals

Table 2a.

Onset and duration of surface anasthesia in different groups

Groups	Onset (minutes)		Duration (minutes)	
	Rabbit	Guinea pig	Rabbit	Guinea pig
B	2.6 ± 0.82	2.67 ± 1.21	2.8 ± 0.75	3.67 ± 1.21
AB	2.8 ± 0.98	1	3.0 ± 0.63	3.83 ± 2.79
BP	0	0	0	0
ABP	0	0	0	0
Xylocaine	1	1	6.0 ± 1.1	5.67 ± 1.63
Control	0	0	0	0

n=6 ; values are mean ± SD

were computed for each dose and each solution. For saline control 'C' no response was missed out and N = 0, indicating absence of local anesthetic activity. For the drug control Xylocaine 'X', N = 36 i.e., 100% absence of response to stimuli, indicating potent local anesthetic activity. For B, AB, BP and ABP, the response failure was computed as % failure in comparison with Xylocaine control 'X' (Table 1a). The values for 'B' at all 5 doses were compared with those of C, X, AB, BP and ABP (Table 1b).

3.2 Surface anasthesia

The values for onset and duration of corneal anasthesia, with their mean and standard deviation in rabbits and guinea pigs for various groups were tabulated in Table 2a. The duration of corneal anasthesia was calculated as a difference between time of onset and time of recovery. The values of animals belonging to different groups were compared and analysed statistically (Table 2b)

4. Discussion

In infiltration anasthesia, control group 'C' responded to all stimuli retaining 100% reflex and 0% infiltration anasthesia. Standard drug control group 'X' did not respond to any stimuli, showing 100% infiltration anasthesia. Betel leaf extract treated group 'B' showed 69.5% to 100% infiltration anesthetic property of xylocaine, depending on the dose used. In fact, B equalised with X at 0.9 ml, and proved equipotent. Statistically, the local anesthetic activities between the two could not be differentiated ($p > 0.05$).

Autoclaved extract of Betel leaf (AB) also had infiltration anesthetic activity that could not be differentiated from that of xylocaine from doses 0.3 ml through 0.9 ml, though in lower doses the effect was less, but still significant. Presence of betel nut in BP, reduced the infiltration anasthesia of betel leaf, to a statistically significant degree. Autoclaving the leaf extract BP abolishes the infiltration anasthesia of betel leaf, indicating that autoclaving actually potentiates this effect of betel nut.

In case of surface anasthesia, the normal control group C, as well as betel nut and betel leaf combination whether autoclaved or not, showed no sign of corneal anasthesia till 10 minutes. The drug control group X, plain betel juice treated group B, as well as autoclaved betel juice treated group AB, all showed corneal anasthesia within 10 minutes.

The onset of effect was quick with xylocaine, i.e., within 1 minute where as it was delayed till 2.6 to 2.8 minutes in B and AB. Comparison of groups indicated that there was no significant difference in the onset of effect between B and X ($p > 0.05$). Autoclaving the extract did not alter the effect of betel leaf ($p > 0.05$). Addition of betel nut to betel leaf significantly abolished the local anesthetic effect

Fig 1: Sites for intradermal injection in Guinea pig

C	X
B	BP
AB	ABP

Table 2b.

Comparison by groups for onset and duration of corneal anesthesia

Groups Compared	Onset		Duration	
	Rabbit	Guinea pig	Rabbit	Guinea pig
B vs.C	****	****	****	****
B vs.X	NS	NS	***	NS
B vs.AB	NS	NS	NS	NS
B vs.BP	****	****	****	****
B vs.ABP	****	****	****	****
AB vs.X	NS	NS	***	NS

*** p<0.001; **** p<0.0001 ; NS p>0.05 (not significant)

of betel leaf ($p < 0.0001$). Autoclaving did not affect this effect of betel nut ($p < 0.0001$)

Duration of corneal anesthesia was longer in the case of xylocaine when compared to betel leaf this difference was statistically significant in the case of rabbits ($p < 0.001$). In the case of guinea pig, both xylocaine and betel leaf seem to have more or less same duration of effect ($p > 0.05$) and the onset of effect was quicker compared to that of rabbit. Addition of betel nut significantly reduced the

duration of action of plain betel leaf and autoclaving retained this effect of betel nut.

From this study it is evident that plain betel leaf is comparable to xylocaine in its surface and infiltration anesthetic property. Addition of betel nut to betel leaf extract markedly reduces the local anesthetic effect of betel leaf and this effect of betel nut is retained even after autoclaving. Autoclaving of the leaf extract does not have any significant loss of activity, thus permitting its use as a herbal medicine. Compared to rabbits, the cornea of guinea pigs seems to be more permeable as evidenced by statistical data.

As an extension of this study, tests for possible local irritancy using betel leaf juice is mandatory. Uses of betel leaf juice as topical herbal-local anesthetic in the form of ointment or cream can be explored. Pharmacokinetic studies using betel leaf juice for penetration of cornea in different animal models and in human will throw more light on its clinical use. Further, isolation of the active principle responsible for local anesthetic activity can lead to addition of a valuable drug to the therapeutic armamentarium.

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