

Evaluation of Surface anesthetic action of Aqueous Extract of Piper Betel leaf On Rabbit Cornea

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Abstract

Aim: Piper betel Linn. (Piperaceae) commonly known as betel leaf and the habit of betel chewing is widely prevalent in most parts of India. It is claimed to have aphrodisiac, laxative, antimicrobial, mucolytic, anti-inflammatory and euphoric properties and proven antimutagenic and anti-carcinogenic effect. It is commonly observed that chewing of betel leaf produces numbness in the mouth, suggesting a possible local anesthetic effect. This observation prompted us to take this study . The aim of the study was to evaluate the local anesthetic activity of betel leaf extract after autoclaving the extract of betel leaf. **Materials and methods:** Extract of plain betel leaf, was tested for surface anesthetic activity using rabbits. Aqueous extraction of Piper betel leaf (AEPBL) done by Soxhelt's apparatus .Twenty male Rabbit's were taken and divided in to four groups each group contain 5 animals, Group I - Standard (2% xylocaine), Group II - Test 0.3% (AEPBL), Group III - Test 0.6 % (AEPBL),Group IV - Test 12 % (AEPBL) was instilled in conjunctival sac of right eyes. Standard protocol was followed to elicit light reflex, corneal reflex and to measure pupillary size after instilling the test drugs in the eye. There was dose dependent increase in onset and duration of local anesthetic activity with 6% and 12% doses of alcoholic extract of Piper betel leaf. **Results:** Betel leaf showed significant surface anesthetic activity comparable to that of Xylocaine. **Conclusion:** As a surface anesthetic, the onset was as quick as xylocaine and the duration was shorter than xylocaine

Keywords: Corneal reflex, Piper betel (betel leaf), Light reflex, local anesthetic activity, Rabbit cornea, Soxhelt's apparatus. Xylocaine.

Introduction

Piper betel L belongs to the family Piperaceae, i.e. the Black Pepper family. Fresh leaves are chewed with other adjuncts (betel quid) and the betel chewing is a habit in most parts of India. Betel leaf has astringent, aphrodisiac, laxative, antimicrobial, mucolytic, anti-inflammatory and euphoric properties. The Essential oil isolated from the leaves is useful in treatment of the respiratory catarrhs and as an antiseptic [1]. Eugenol, one of the principal constituents of betel leaf has also been shown to possess anti-inflammatory effects in various animal model studies with various inflammogens [2]. One study revealed that Piper betel has properties to decrease mitochondrial activity in human sperm and ability to work as contraceptive [3]. Ethanolic extract of Piper betel leaf and essential oil possess antihistaminic activity [4]. Marked antinociceptive activity was seen with the extracts of Piper betel leaf in the hot plate and the formalin tests but not in the tail-flick test [5]. Piper betel leaf has antimutagenic and anti-carcinogenic effect [6]. Recently research is focused mainly on the carcinogenic and mutagenic potential of the betel quid [7, 8]. The betel leaf has anti-inflammatory action and is used as a common household remedy for inflammation in the oral cavity [1, 9]. It is observed that chewing of betel leaf causes numbness of buccal mucus membrane temporarily. This observation leads us to take up this study. Local anesthetic effect of betel leaf due to betel leaf consumption and consequences [10] has no scientific study report so far.

Material and methods

Preparation of Betel leaf extract

The Betel leaf was collected from a local market in month of April 2014, and authenticated in department of Botany at Government Degree College, Khammam

Preparation of extract: The leaves of piper betel about 50gms were taken cleaned with distilled water and were shade dried and reduced to coarse powder in a mechanical grinder. The aqueous extract of the powdered material was obtained using Soxhlet extractor. The extract obtained was evaporated at 45°C to get a semisolid mass. Phytochemical analysis of the extract was done. The study was performed between the months of March and April 2014, in the department of pharmacology, Mamata Medical College (MMC), Khammam, Andhra Pradesh. The water soluble extractive value of aqueous extract of piper betel leaf was 33.07gms w/w.

Animals used:

Twenty albino rabbits of either sex weighing between 2.5-3kg were obtained from central animal house of MMC. Approval of Institutional Animal Ethics Committee for the experimental protocol was taken. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were housed in Polypropylene cages and maintained at 24°C ± 2°C under 12h light/dark cycle and were fed with standard pellet diet and had free access to water.

Drugs used: Xylocaine 2% Plain Injection Manufactured by Astra Zeneca, Aqueous extract of *leaves of piper betel*.

Preparation of doses of aqueous extract of *piper betel leaves* (AEPBL)

0.3% AEPBL, 0.6% AEPBL and 12% AEPBL prepared from the stock solution. Commercially available 2% Xylocaine is used as standard

Delivery of the test solutions to experimental groups:

The animals were placed in rabbit holding cages and randomly allocated into four groups. The upper and lower eye lashes were carefully clipped off to avoid the corneal reflex initiated by accidental touching of the eye lashes. Left eye serves as control and right eye as test. The conjunctival sac of right eye held open to form a pocket. In to these pockets Group I animals were delivered one drop of 2% Xylocaine. Group II, Group III and Group IV were delivered one drop of 0.3% AEPBL, 0.6% AEPBL and 12% AEPBL respectively. 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. The corneal reflex was tested by touching cornea from the side using a wet cotton wisp. The test was started five minutes after application of the drug in all groups and repeated every five minutes until corneal reflex was lost (blinking lost) followed by reappearance of corneal reflex (blinking reappearance). The time between disappearance and reappearance of corneal reflex (corneal reflex) was registered. The results obtained were recorded and tabulated. The time between disappearance and reappearance of corneal reflex (corneal reflex) was registered. The results obtained were recorded and tabulated.

Statistical analysis: All the values were expressed as Mean ± SEM. The differences were compared using one way Analysis of variance (ANOVA). The p values <0.05 were considered significant.

Results

The time of disappearance of corneal reflex and the time between the disappearance and reappearance of corneal reflex were recorded in 20 albino rabbits after the administration of standard 2% xylocaine and three different doses of aqueous extract of *leaves of piper betel*. At baseline (0 min), all the rabbits in four groups (n = 20) showed the presence of a normal corneal reflex, light reflex and the size of the pupil being normal. Light reflex and size of the pupil remained normal in all groups till the end of the experiment. Group I animals which were delivered one drop of 2% Xylocaine had loss of corneal reflex at 3.2 ± 0.1 minutes. Group II which was delivered one drop of 3% AEPBL did not show any loss of corneal reflex even after 20 minutes. Group III and Group IV which were delivered one drop of 6% AEPBL and 12% AEPBL showed loss of corneal reflex at 11 ± 1.1 and 5 ± 0.7 minutes respectively. Reappearance of corneal reflex has been observed at 34 minutes with 2% Xylocaine, and at 5.6 and 22 minutes, with 6% AEPBL and 12% AEPBL respectively. Duration of surface anesthesia with 6% AEPBL and 12% AEPBL was 6 and 17 minutes. Duration of surface anesthesia with 12% AEPBL which was 17 minutes, and was significant when compared with standard 2% Xylocaine (33 ± 0.47) as shown in Table 1

Table: 1 Duration of Surface Anesthesia in (Minutes) with Different doses of aqueous extract of *Piper Betle Leaf* (AEPBL) on Rabbit cornea

Groups n= 20	Pupil Size	Light Reflex	Loss of Corneal Reflex Mean \pm SEM	Reappearance of Corneal Reflex Mean \pm SEM	Duration of Surface Anesthesia. Mean \pm SEM
Group I 2% Xylocaine	6.5 mm	+	3.2 ± 0.1	34.4 ± 0.6	33 ± 0.47
Group II 3% AEPBL	6.5 mm	+	0	0	nil
Group III 6% AEPBL	6.5 mm	+	$11 \pm 1.1^*$	5.6 ± 1.1	6
Group IV 12% AEPBL	6.5 mm	+	$5 \pm 0.7^*$	$22 \pm 2.5^*$	17.18*

AEPBL - Alcoholic extract of Piper Betle Leaf, n-Number of animals, * p<0.05.

Discussion

Piper betel Linn. (Piperaceae) commonly known as betel leaf is a native to southern India and Malaysia. Apart from chewing habit, these leaves have traditional use in the Indian society for religious purpose. A perennial dioecious creeper. Stems semi woody, climbing by means of short adventitious roots. Leaves 10-20 cm long, broadly ovate, slightly cordate and often unequal at the base, shortly acuminate, glabrous, and glaucous on sides, bright green or yellowish, petiole stout 2.0-2.5 cm long. Male spikes are cylindrical dense. Female spikes are 2.5-5.0 cm long, pendulous. Fruits rarely produced, often sunk in the fleshy spike, forming nodule-like structures [11]. Betel leaf is very often used as home remedy for many common illnesses such as Sore Throat, Problem of Breast milk secretion, Constipation, Scanty or Obstructed Urination, Headache and Weakness of Nerves. The leaves, soaked in mustard oil and warmed, may be applied to the chest to relieve cough and difficulty in breathing and in the treatment of inflammation such as arthritis and orchitis that is inflammation of the testes also used as home remedy for treatment of boils. The study is about local anesthetic action. In our study 12% AEPBL produced significant surface anesthetic action on rabbit cornea. In one of the study on surface anesthetic activity of betel leaf, the onset was as quick as xylocaine and the duration was shorter than xylocaine and showed that betel leaf has potent local anesthetic action [12]. There were no side effects like conjunctival injection, corneal abrasion, etc when rabbits were observed for more than ten days in our experiment. The chief constituent of the leaves is a volatile oil varying in the leaves from different countries and known as betel oil. It contains two phenols, betel-phenol (chavibetol) and chavicol, Cadinene [13], and also contains high amount of tannins [14], sugar and diastases. The best oil is a clear yellow colour with aromatic odor and sharp burning in taste. Chavicol has powerful antiseptic properties. The alkaloid arakene in it has properties resembling cocaine in some respect. Its minerals and vitamin contents are calcium, carotene, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 44 [15]. The alkaloid arakene, tannins and phenols present in the beetle leaf may be the cause for local anesthetic action. Betel leaf juice in the form of ointment, cream or drops can be evaluated and developed as topical herbal-local anesthetic. Further research has to be done for isolation of the active

principle responsible for local anesthetic activity can lead to addition of a valuable, an effective, safe and cost effective herbal drug for various diseases. A new molecule of local anesthetic activity like xylocaine can be developed as they have higher safety margins with minimum or no side effects.

Conclusion:

This study reveals that betel leaf exhibits local anesthetic activity along with other properties such as, antidiabetic, antifungal, antimicrobial, anti-inflammatory, antihistaminic, antiulcer, etc actions. The betel leaves being natural, abundant and easily available, we can develop many cost effective molecules for various diseases. Further research has to be done to investigate the mechanism of actions with other therapeutic activities. Our study supports the local anesthetic activity of betel leaf extract.

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