



Effect of Betel leaf extract on nerve impulse conduction through sciatic nerve in frog using a modified technique

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Abstract

Plain betel leaf extract is reported to produce local anesthesia by surface and infiltration techniques. **Objective:** (1) to explore the effect of betel leaf extract on conduction of nerve impulse and (2) to evaluate its potency as compared to 2% xylocaine. **Materials & Methods:** The extract (12 to 15 ml) was prepared by squeezing ground betel leaf paste. An in situ gastrocnemius–sciatic nerve preparation was made on both the legs of a pithed frog and necessary electrical and kymograph connections were made. Xylocaine or betel leaf extract (0.2 ml) was applied on the sciatic nerve of one side and the other side served as control. The response to the electrical stimulus given proximal to the site of application alternatively on both sides was recorded on the same drum, until there was no response. Then the drug was washed off and recovery recorded. **Results:** Betel leaf extract produced statistically significant conduction block anesthesia of slow onset (25 min as against 2 min with xylocaine) and fast recovery (2 min as against 44 min with xylocaine). The extract was found to possess 25% of the local anesthetic activity of xylocaine. **Conclusions:** Betel leaf extract produced a conduction block anesthesia of slow onset and quick recovery. It is less potent than xylocaine in this regard.

Key words: *Piper betel*, conduction block anesthesia and modified technique.

1. Introduction

Piper betel, commonly known as betel leaf, a plant extensively cultivated and consumed in western pacific basin and southern Asia, has a potential to find a place in modern medicine. Studies are going on to ascertain various pharmacological activities and pathological

consequences of betel chewing as betel quid [1]. The reports indicate that betel chewing as betel quid has mutagenic and carcinogenic potentials, while plain betel leaf has antimutagenic and anticarcinogenic potentials. The aqueous and alcoholic leaf extracts, as well as the essential

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oil from the leaf were screened for, and reported to have antibacterial, antifungal, antineoplastic and cardiac as well as respiratory depressant properties [2].

It is a common observation that betel chewing results in numbness in the mouth and the sensation of taste is lost for a brief period of time [3]. This mimics the mouth numbing properties of cocaine, an extract from the leaf of coca shrub. The potential for local anesthesia in betel leaf is thus apparent from the similarities to the history of cocaine, a potent local anesthetic [4].

It has been referred in Dr. Watt's Dictionary of Economic Products of India that an alkaloid named Arakene, with properties somewhat allied to that of cocaine, has been extracted from betel leaves [5]. Screening of betel leaves for local anesthetic activity needs a careful study by all usual routes of administration [6].

Three different procedures have been extensively used namely corneal anesthesia in rabbits [7], infiltration anesthesia in guinea pigs [8], and conduction block anesthesia in frogs using either lumbar plexus [8] or isolated gastrocnemius-sciatic nerve preparation [9].

The local anesthetic activity of betel leaf extract has already been demonstrated by the corneal and infiltration techniques [10]. Next step in this regard is to assess the local anesthetic potency of betel leaf extract on nerve impulse conduction. Plexus anesthesia in frogs is suitable only for assessing onset of local anesthesia and not for determining the duration of action of the drug since the procedure involves evisceration and the animal dies before recovery from the drug effect occurs [6].

On the other hand isolated gastrocnemius muscle-sciatic nerve preparation is viable enough for the study of duration of action of

the drug. As the local anesthetic activity is assessed by absence of response to a stimulus, it becomes mandatory to prove for each stimulus applied to the test set up, the control set up from the same animal is still eliciting a positive response.

But, by this method, the control and the test from the same animal cannot be run simultaneously under exactly similar experimental conditions, and thus giving scope for experimental errors. Hence in this study a modification of this method using an in-situ set up is resorted to.

2. Materials and methods

2.1 Preparation of various solutions for use

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, squeezed out the juice (yield 12 to 15 ml) and labeled as betel juice extract 'B'. Frog ringer was used for normal control 'C' and 2% xylocaine was used as drug control 'X'.

2.2 Technique adopted for conduction block anesthesia

Conduction block anesthesia was tested in gastrocnemius-sciatic nerve preparation in situ, with appropriate modifications on the method described [9] as given below:-

Pithed frog was laid on its back on the board. The skin of the abdomen around was cut through and stripped down to the toes to expose the muscles of the thigh and leg. The gastrocnemius muscle on one side was freed from its insertion by cutting off the Achilles tendon and a thread was tied to its free end. The leg bone was cut, a short distance away from the knee joint using a bone cutter. The muscle was wrapped with a small piece of gauze wetted with ringer.

Next the sciatic nerve running along the thigh muscles was exposed and displayed using a small X-ray sheet to serve as the site of drug application. The sacral region of the vertebral column was then held and lifted using a forceps. Keeping intact the spinal origin of the sciatic nerve, the overlying bone below the origin as well as the muscles of the back were cut. A blunt glass rod was used for teasing the nerve. To this part of the nerve an electrode from the stimulator was attached.

The free end of the muscle was tied to the recording device so that the response for the electrical stimulus can be recorded on smoked kymograph moving with the slowest speed. The entire set up on one side was done is shown (Fig. 1).

In the same animal similar set up was done on the other side and attached to the same recording device so that responses to the electrical stimulation of the nerve on both sides can be recorded alternatively on the same drum. The

stimuli were applied at min intervals. One side was kept as control and the other was used for either xylocaine or betel juice extract. The INCO MEDICARE Research Stimulator was used to give single square pulses.

A single stimulus of 25 volts strength and 0.5 m seconds pulse duration was used [11]. Stimulation of nerve was continued till there was no response. Then the drug was washed off by repeated washing with ringer (3 times). Application of stimulus was continued to record recovery from anesthesia.

3. Results

The control side in each animal was responding to all stimuli from the start till the end. Figure 2 shows the effect of xylocaine and betel leaf extract on onset of conduction block anesthesia. The results of observations on onset, peak effect, recovery and duration of anesthesia after xylocaine treatment (n: 7) and betel leaf extract treatment (n: 10) are shown as bar chart in Fig. 3.

IN-SITU TECHNIQUE

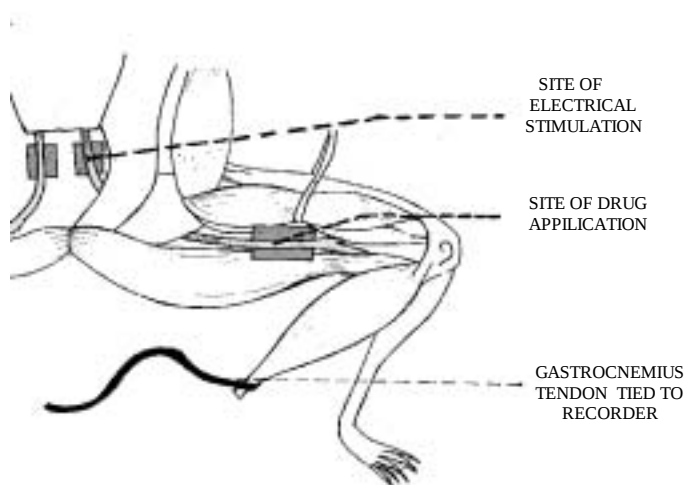


Figure 1. Setup for conduction block anesthesia technique using gastrocnemius muscle and sciatic nerve preparation in situ

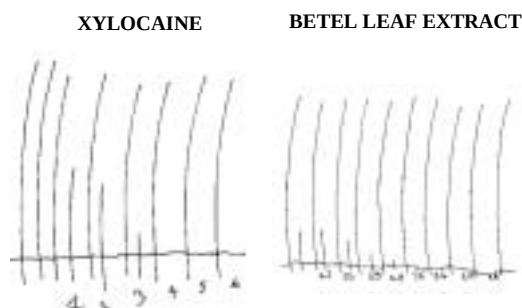


Figure 2. Effect of xylocaine and betel leaf extract on conduction block anesthesia.

The numbers indicate the time in minutes of stimulus application on the nerve corresponding to test side. The unnumbered responses correspond to stimulus on the control side of the same animal.

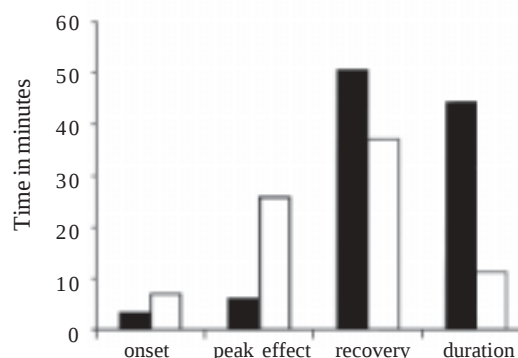


Figure 3. Effect of xylocaine and betel leaf extract on nerve impulse conduction.

With xylocaine the response started decreasing by 3.3 ± 1.80 min (T1). Complete absence of response resulted by 6.1 ± 2.55 (T2). The time taken for the development of complete absence of response (T2-T1), was found to be 2.8 ± 0.75 min. After washing off the drug there was no response to stimulus for the next 37 min.

After that the response again reappeared and gradually increased to reach original intensity by 50.4 min. The duration of action of 2 % xylocaine in this set up was thus found to be 44 ± 9.38 min. Regarding the responses to betel leaf extract (n: 10), the first sign of decrease in response started by 6.8 ± 3.29 min (T1). The complete absence of response resulted by 25.8 ± 14.44 min (T2).

The latency of on set, T2-T1, was 19.0 ± 11.14 min. After washing off the betel leaf extract using ringer the response started reappearing by 27.8 ± 14.44 (within 2 min) and complete recovery resulted by 36.8 ± 14.80 (within 8 min), indicating that the effect was only short lived and the duration of action of betel leaf was 11 ± 1.49 min.

4. Discussion

The *in-situ* nerve-muscle preparation used here is a modification of the isolated nerve-muscle preparation is described [9] for assessing local anesthetic activity.

It has the following advantages over the original method: one, here it is possible to check the normal response simultaneously with the test response throughout the test period under exactly similar experimental conditions, in the same animal at the same time.

Two, it is easy to keep the site of stimulation and the muscle isolated, away from the site of application of the drug along the nerve so that error due to spillage of drug on the muscle or on the site of stimulation on the nerve, is nullified. Three, the viability of the *in-situ* setup is longer than the isolated preparation. Thus the modified technique permits accurate evaluation of potency with minimum of subjective interference.

Local anesthetics (LA) are sodium channel blockers that halt impulse conduction reversibly

in excitable tissues such as peripheral nerves and spinal roots. When applied to a peripheral nerve they prevent conduction of impulse generated proximal to the block and thus abolishes response to the impulse in the innervated part distal to the block. The betel leaf extract has thus prevented the muscle contraction in response to the electrical stimulus, given proximal to the area of extract application.

On the control side, the conduction of impulse was intact throughout and the amplitude of response was also not altered significantly (Fig.2). This proves that the absence of response on the test side is not due to fatigue, but due to betel leaf extract.

Regarding the onset of LA activity there was a progressive decrease in the amplitude of contraction culminating in total absence. The time taken for initiation of any LA effect itself was more for betel than for the xylocaine.

This is statistically significant ($p \leq 0.0001$) suggesting that the LA effect of betel is weaker when compared to xylocaine. The time taken for the development of full LA effect was more for betel leaf extract than for xylocaine and this difference is statistically significant ($p \leq 0.0001$), indicating that the spread of LA effect is slower for betel when compared to xylocaine.

A comparative evaluation of the local anesthetic potency of betel leaf can be calculated indirectly as follows: -

If we consider the effect of 2% xylocaine as is 100%, then how much of xylocaine's effect is exhibited by betel leaf can be assessed in percentage.

Since local anesthetic potency and rate of diffusion are inversely proportional, the following formula can be used to calculate this:

$$100 \times \frac{\text{Onset time for xylocaine}}{\text{onset time for betel leaf extract}} =$$

$$100 \times \frac{6}{26} = 23.07 \%$$

On an average, the local anesthetic effect lasted for 44 min in the case of xylocaine and 11 min only with betel leaf extract. This difference is highly significant statistically ($p \leq 0.0001$). This shows that the LA effect with betel was retained as long as it was in contact with the nerve and when the exposure was terminated by washing, the effect quickly wears off unlike xylocaine.

To offer a measure of relative potency, the minimum blocking concentration (C_m) of LA is defined as the drug concentration that just halts impulse traffic [12]. For xylocaine it is found to be 2% (concentrations $< 2\%$ did not produce any effect). C_m represents a dynamic equilibrium between channel bound and channel released drugs such that the net sodium current is decreased below the firing threshold level.

C_m is considerably dependent not only on speed of drug diffusion and concentration but also on volume of local anesthetic solution. In this study the betel leaf extract was obtained by squeezing the leaves without adding water and so no possibility of getting a solution of higher concentration. But isolation of the active principle harboring the LA effect can be attempted and quantified to get the C_m .

The chemical composition and its characteristics contained in fresh leaves [13-16], benzene extracts and alcoholic extracts [17] of betel leaves have been studied. Betel leaf has been reported to contain high concentration of essential oil [18-21]. The fraction in which the LA effect resides has to be identified first. This should be followed by the isolation and characterization of the active principle.

5. Conclusion

From this study it is evident that betel leaf extract produces conduction block anesthesia of slow onset, delayed peak effect and fast recovery. It also roughly estimates its potency as one fourth that of standard drug xylocaine. The fact that the effect is retained as long as the extract is in contact with the nerve but fades off quickly on washing unlike

xylocaine, needs further exploration. As an extension of this study, quantitative evaluation using the technique of spinal anesthesia [22] will be more appropriate with respect to its clinical use.

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References

1. Norton SA. (1998) *J. Am. Acad. Dermatol.* 38 : 81.
2. Sathyavathi GV, Ashok KG, Neeraj T. (1987) *Medicinal plants of India*, Vol. II, Indian Council of Medical Research: New Delhi, 437
3. Chopra RN, Chopra IC, Handa KL, Kapur LD. (1958) *Chopra's Indigenous Drugs of India*. UN Dhur & Sons Pvt. Ltd.: Calcutta; 371.
4. Wiedsmith JAW. (1984) *Reg. Anesth* 9: 161
5. Dey, K.L. (1984) *Indigenous drugs of India*, II Edn. International Book Distributors: Dehradun; 239
6. Laurence DR, Bacharach AL. (1964) *Evaluation of drug activities: Pharmacometrics*. Academic Press, London & New York, 205
7. Shapero M, Southgate PJ. (1970) *Br J. Pharmacol.* 38: 265
8. Bulbring E, Wajda I. (1945) *J. Pharmacol.* 85: 78.
9. Muralikrishna R. (1999) *Vademecum of Experimental Pharmacology* ESPE Media & Printing: Visakhapatnam; India, 30.
10. Krishnakumar S, Geetha VS, Kuruvilla A. (2001) *J. Nat. Rem.* 1: 28.
11. *Pharmacological Experiments on Isolated Preparations* (1968) by the staff of the Department of Pharmacology, University of Edinburgh, E. & S. Livingstone Ltd., Edinburgh and London, 118
12. Rudolph H. de jong. (1996) *Local anesthetic Pharmacology*, Regional anesthesia and analgesia, W.B. Saunders Company: Pennsylvania; 124
13. Anantha Samy TS, Kamat VN, Pandya HG. (1960) *Curr. Sci.* 29: 133.
14. Lakshmiah N, Rama Shastri BV. (1969) *J. Nutr. Dietet.* 6: 200.
15. Nanda RS, Kapoor K. (1971) *Ind J. Med. Res.* 59: 1966.
16. Gopalan C, Rama Shastri BV, Balasubramaniam SC. (1984) *Nutritive value of Indian foods*, National Institute of Nutrition, Hyderabad, Indian Council of Medical Research: New Delhi; 85.
17. Deshpande SM, Upadhyay RR, Singh RP. (1970) *Curr. Sci.* 39: 372.
18. Nigam SS, Purohit RM. (1962) *Relchstoffe Aromen*. Koerperpflagemittel 12: 185.
19. Baslas RK, Bbaslas KK. (1970) *Flavour Ind.* 1: 473.
20. Sharma ML, Rawat AKS, Balasubramaniam VR, Singh A. (1982) *Indian Perfum.* 26: 134.
21. Sharma ML, Rawat AKS, Balasubramaniam VR, Singh A. (1983) *Indian Perfum.* 27(2) 91.
22. Robert AT. (1965) *Screening methods in Pharmacology* Academic Press: New York & London; 268.