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# Local Anesthetic Effect of *Citrullus colocynthis* on *Rana hexadactyla*

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## ABSTRACT

Local anesthetics are a well-known group of pharmaceutical agents used to relieve pain in specific parts of the organism, inhibiting propagation of signals along the nerves. The present study petroleum ether extract of *Citrullus colocynthis* was evaluated its local anesthetic activity in the animal frog *Rana hexadactyla*. Local anesthetics are intended to relieve pain by depressing or blocking the sensory nerves reversibly. Local anaesthetics block nerve conduction where applied locally to nerve tissue in appropriate concentration 1:10 and 1:100. They act on any part of the various systems and on every type of nerve fibre. Lumbar plexus anesthesia method (or) nerve block Anesthesia method is used to study the activity briefly. This study proves that the leaves are equally effective as that of the synthetic standard drug xyclocaine when placed in sciatic nerve. Further studies are warranted to compare the mechanism of action.

**Key words:** *Citrullus colocynthis,* local anesthetic, Gulf of Mannar, sciatic nerve, xyclocaine

## INTRODUCTION

Local anesthetics are known to inhibit voltage-dependent $Na^+$ channels, as well as $K^+$ and $Ca^{2+}$ channels, but with lower potency. Since, cellular excitability and responsiveness are largely determined by intracellular $Ca^{2+}$ availability, sites along the $Ca^{2+}$ signaling pathways may be targets of Las (Xu *et al*., 2003). The majority of work concerning the mechanisms of local anesthetics has focused on LA actions on the $Na^+$ and $K^+$ channels. LAs are known to reduce both $Na^+$ and $K^+$ currents (Hollmann and Durieux, 2000) which contribute to their anesthetic action. Alteration of those regulatory mechanisms by LAs can lead to changes in presynaptic transmission and postsynaptic excitability. A few studies have shown that bupivacaine, ropivacaine and lidocaine can affect the voltage-dependent $Ca^{2+}$ currents in different systems (Liu *et al*., 2001). Local anesthetics modulate inflammatory responses and may therefore be potentially useful in mitigating perioperative inflammatory injury (Hollmann *et al*., 2003). Lidocaine is one of the most common amide-type local anesthetics. In aqueous solution lidocaine usually exists as a mixture of charged and uncharged species, with a pKa value estimated as 7.9 (Matsuki *et al*., 2005). Surface application of the local anaesthesia to mucus membrane and cornea was studied in topical anaesthesia. In local anaesthetic penetrate frog's skin, for it possesses many properties of mucous membranes (Sollmann, 1918). Local anesthetics are drugs which upon topical application/local injection cause reversible loss of sensory perception, especially of pain, in a localized area of the body. They block generation and conduction of nerve impulse at all parts of the nervous where they come in contact, without causing any structural damage. Thus, not only sensory but also motor

impulses are interrupted where a local anesthetic is applied to a mixed nerve, resulting in muscular paralysis and loss of autonomic control as well. In older days local anesthesia was produced by cooling as well, e.g., application of ice, $CO_2$ snow, ethyl chloride spray. Local anesthetic drugs have a low pH and liberation free base, which occurs due to alkalinity of the body fluid essential for production of neural impulse formation and conduction. Hence at low pH, as influenced areas, where ionization is maintained anesthesia can develops. The clinically useful local anaesthetics are weak bases with amphiphilic property. Hydrophilic secondary or tertiary amines on one side and a hydrophilic aromatic residue on the other are joined by an alkyl chain through an ester (or) amide linkage (Tripathi, 1994). *Citrullus colocynthis* is a fruit commonly known as bitter apple or bitter cucumber, found in Sudan, Iran and India and in the deserts (Trease and Evans, 1970). The dried pulp of *Citrullus colocynthis* has been used for constipation, edema, bacterial infections, cancer and diabetes (Al-Ghaithi *et al*., 2004). Recently, the antioxidant effects and the effect of the aqueous extract of the pulp on kidney and liver functions were reported (Al-Hader *et al*., 1994). Nevertheless, to date, the scientific scrutiny of *Citrullus colocynthis* is insufficiently documented and warrants systematic analysis. In particular, the acute effect of aqueous extract of the leaf in vivo remains untested. The anti microbial and anti-inflammatory effects of leaf of *Citrullus colocynthis* were reported (Gurudeeban *et al*., 2010; Rajamanickam *et al*., 2010). In the present study, we have evaluated the effect of CLEt to the local anesthetic activity of the leaves in a suitable model.

## MATERIALS AND METHODS

**Collection of the plant:** Fresh *Citrullus colocynthis* leaves were collected from the Gulf of Mannar area, (Tamil Nadu) India. The specimen was certified by Botanical Survey of India (BSI) Coimbatore and documented in the Herbaria of C.A.S. in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu and India, during 2008. Mature leaves were separated manually from the aerial part of the plant. Then, the leaves was dried and minced with a grinder into a powder in preparation for extraction. The experiment was conducted in C.A.S. in Marine Biology and the chemicals were purchased from Sigma Chemicals (Mumbai).

**Preparation of extract:** The leaves were shade dried and subjected to size reduction to get a coarse powder. The powdered material was subjected to successive extraction in a Soxhlet apparatus, using petroleum ether as solvent at 50°C. The extract was then evaporated on a rotary evaporator.

**Animal model:** The adult frog (male or female) were collected from Parangipettai coast. The animals were divided into 6 groups and each group carries 4 frogs. Twenty four frogs were taken in to six groups consisting of 4 frogs each. The first group served as control which received 1 mg of xylocaine hydrochloride. The subsequent groups were tested with 5, 10, 20, 40 and 80 mg of *Citrullus colocynthis,* respectively.

**Apparatus and chemicals:** Frog board, Frog stand, Hammer, pins, Normal saline, Lignocaine (xylocaine), 0.1% HCl.

**Local anesthetic activity:** Adult *Rana hexadactyla* of either sex was used. The method of nerve block anesthesia method suggested by Sollmann (1918). The leaves collected only from the young

stems of *Citrullus colocynthis* were used. The extracts of leaves applied directly is not good because of it high concentration and poisonous nature. Hence it is diluted (1) 1:10 depending upon the concentration reaction and (2) 1:100. The anesthetic time deviations also vary. The method suggested by Sollmann (1918) and Kulkarni (1999) were adopted. The frog is decelerated and the upper part of the spinal cord was destroyed with the help of pithing needle. The abdomen was cut opened and all the abdominal organs are removed so that a pouch (sac) was made of abdominal walls. The sciatic nerve was exposed to the local anesthetic actions of the drug. 0.1 N HCl in beaker was used to find out the anesthetic activity of the Leaves extract from *Citrullus colocynthis* at various concentrations viz., 5, 10, 20, 40 and 80 mg, respectively was taken in the cotton by immersing the piece of cotton inside the 1:10% /1:100 solutions. The cotton was placed exactly on the sciatic nerve. The drug was allowed to act for 5 min. After the incubation time the hind leg dipped into 0.1% HCl containing beaker and measured the local anesthetic activity.

## RESULTS AND DISCUSSION

In the present investigation results showed when the acid has been contacted with the hind leg before administration of the drug, there was a sudden withdrawal of leg from the HCl beaker. However, after application of the drug, when tested with HCl, there was absence of withdrawal of leg from the acid. The foot-withdrawal reflex in frog was carried out. The onset of anaesthesia was determined by the loss of foot-withdrawal reflex against 0.1 N HCl (1:10 dilution; 1:100 dilutions). The onset of anaesthesia in frog was found to be 15, 12.5, 12, 8 and 5 min against the dose of 5, 10, 20, 40 and 80 mg of *Citrullus colocynthis,* respectively. In the xylocaine hydrochloride control group the onset of anaesthesia was 5 min. Hence, it proves that the leaves from *Citrullus colocynthis* have local anesthetic activity (Table 1). Animal receiving only distilled water did not show any anaesthesia. From the present study it is revealed that the *Citrullus colocynthis* possesses good local anaesthetic property. When the onset time was compared with the standard xylocaine hydrochloride it revealed delayed onset as compared to the standard drug even when the quantity of the test drug was much higher. The duration of anaesthesia was also found to be shorter than the standard drug. The reason might be due to impurities present in the crude extract. To rule out the possibility of having any anaesthetic activity of the solvent, wheals were produce with distilled water, but no anaesthetic activity could be revealed with it suggesting that the anaesthetic property was of the extract's own. Martin *et al.* (2008) reported that a low-dose perioperative IV lidocaine after total hip surgery offers no beneficial effect on postoperative analgesia and does not modify pressure and tactile pain thresholds. A ventricular arrhythmia developed 15 min after local anesthetic injection to 13-yr-old girl scheduled for knee surgery under

Table 1: Local anesthetic activity of *Citrullus colocynthis*

| Groups | 1: 10 dilution | 1: 100 dilution | Time required for complete blockade (min) |
|---|---|---|---|
| Control | - | - | - |
| Forg treated with CLEt (5 mg/100 mL) | ++ | ++ | 15.0 |
| Forg treated with CLEt (10 mg/100 mL) | +++ | +++ | 12.5 |
| Forg treated with CLEt (20 mg/100 mL) | ---- | ---- | 12.0 |
| Forg treated with CLEt (40 mg/100 mL) | ----- | ----- | 10.0 |
| Forg treated with CLEt (80 mg/100 mL) | ++++ | ++++ | 4.5 |
| Forg treated with std. drug xylocanie (2 %) | +++-- | +++-- | 5.0 |

The given test drug cause loss of foot withdrawal reflex, when it is applied to the lumber plexus. The test drug exhibits an equal effect like that of lignocaine

general anesthesia and posterior lumbar plexus block. A 20% lipid emulsion was successful in converting the ventricular arrhythmia to a sinus rhythm. This is consistent with previous reports suggesting that lipid emulsion is an effective emergency treatment of local anesthetic toxicity (Ludot *et al*., 2008). Carruthers *et al*. (2010) the safety of lidocaine 15%/prilocaine 5% topical anesthetic ointment used as anesthesia for Intense Pulsed Light (IPL) treatment. Under the conditions reported, topical lidocaine 15%/prilocaine 5% produces low levels of systemic absorption. Epidural anaesthesia in the cat with lignocaine and amethocaine, amethocaine was distinctly more potent and more toxic than lignocaine. The criteria for measuring the responses in the cat and in man differ and concluded that epidural anaesthesia in the two species is remarkably similar (Duce *et al*., 1969). The milky latex from *Calotropis proceru* produces the nerve block anesthesia (Ramanathan, 2008). The anaesthetic property of the extract is similar to that of xylocaine hydrochloride except for the time of duration.

## CONCLUSION

The leaves from *Citrullus colocynthis* produces the nerve block anesthesia. The onset of action of time is 4.5 min for 80 mg/100 mL and 2% standard drug onset of action of time is 5 min. Further investigation will find out the therapeutic compound responsible for local anesthetic isolated from *Citrullus colocynthis*.

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