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LOCAL ANAESTHETIC ACTIVITY STUDIES OF THE EXOTIC PLANT SPECIES, *CROTON BONPLANDIANUM BAILL*

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ABSTRACT: Pharmaceutical corporations have been heavily involved in the development of natural plant-based goods to create the most economical and cost-effective medicines for the underprivileged in recent years. Plants are abundant natural product suppliers. One of such natural sources having great medicinal value is *Croton bonplandianum*, commonly known as 'bantulasi'; Family: *Euphorbiaceae*. Because *Croton bonplandianum* is an alien plant species, the presence of alkaloids, flavonoids, saponins, steroids, resins, phenols, and rutin gives rise to its very useful medicinal value. It is used to treat skin problems, wounds, and high blood pressure. Three distinct guinea pig and frog experimental models were used in this investigation to screen the *Croton bonplandianum* extracts. The extract at dilutions 1:10, 1:50 and 1:100 showed the anesthetic activity in a dose-dependent manner in contrast to the typical medication xylocaine (0.2%). The techniques include lumbar plexus anesthesia, nerve block anesthesia, frog muscle twitch, guinea pig infiltration anesthesia, and surface anesthesia in rabbits. It was concluded that extracts of *Croton bonplandianum* possess local anesthetic activity.

INTRODUCTION: One of the exotic weed species, *Croton bonplandianum* (Family: *Euphorbiaceae*), is sometimes referred to as "bantulasi" and is primarily found in wastelands. This plant's leaves are extremely therapeutic and are used to cure skin conditions, wounds, and high blood pressure. They are also antiseptic and an antidote and plant parts are frequently employed in traditional medicine for a variety of purposes, including hepatic protection, body swelling, treating skin conditions and ringworms, hypertension, antioxidants, wound healing, antifungal, antibacterial properties, antifertility, antispasmodic, antiseptic, antidote, analgesic, antitumor, anticancer, acute constipation, abdominal dropsy, internal abscesses, insect repellent properties, nematicide, coronary-diseases, anti-inflammatory, larvicidal activity, anthelmintic; also used in the treatment of eye diseases, colds and coughs, epilepsy, gastric disorders, insanity, jaundice, liver complaints, scurvy, sprains, malaria, rheumatism, boils, bowel complaints, chicken pox, diarrhea, dysentery, *etc* [1].

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Alkaloids and terpenoids, which include irritating co-carcinogenic phorbol esters (Phillipson 1995), are abundant in cotyledon. The purpose of this study was to look at the plant's potential as a topical anesthetic given its extensive use and accessibility.

Pharmaceutical corporations have been heavily involved in the development of natural plant-based goods to create the most economical and cost-effective medicines for the Under-privileged in recent years. A review of the literature found that *Calotropis procera* latex demonstrated nerve block anesthesia, or local anesthetic activity, in frogs. However, extracts from *Croton bonplandianum Baill* have not yet been shown to have this effect by researchers [2]. Consequently, it is worthwhile to look into how effective *Croton bonplandianum Baill* extracts are as a topical anesthetic.

Local anesthetics are substances that stop nerve impulses from being sent, thereby preventing or relieving pain. They stop ions from passing through the sodium channel pore in neurons by binding to a particular receptor location within the channel. Their activity is confined to the application site, and as soon as it diffuses from the nerve, it quickly reverses [3]. Many delivery methods are used to administer them, including intravenous regional anesthesia, spinal anesthesia, epidural anesthesia, field block anesthesia, infiltration anesthesia, and nerve block anesthesia. And have the potential to produce deleterious side effects [4, 5, 6, 7, 8].

The present investigation was done to screen the local anesthetic activity of extracts derived from *Croton bonplandianum Baill.*

MATERIALSANDMETHODS:

Chemicals and Equipment: Ethanolic extract of *C. bonplandianum*, centrifuge, Distilled water, 0.1 NHCl, Normal saline (0.65% NaCl), Xylocaine (0.25w/v), Fixing solution, Hair depleting agent, Frog board, Frog stand, Hammer, Sharppins, Plastic tray, Sherrington recording drum, Drum-cylinder with smoked paper wrapped, Myographic lever, Surgical instruments and Dissection box.

Animals: Guineapig and Frog (Biggersize)

Methods: Nerve block anesthesia method, Musclet witch method (Frog), and Infiltration anesthesia method (guinea pig).

Preparation of Extracts: Apparently, healthy plants were collected around Hatgacha, Uluberia, West Bengal, India, and shade-dried at room temperature for 15 days and powdered. Fifty grams of dried powder was extracted insolvent ethanol by using the Soxhlet apparatus. Then the extract was concentrated to a gummy mass and stored at 4ºC for phytochemical and pharmacological screening and analysis. The gummy mass was employed to assess the effectiveness of local anesthetics.

Evaluation of Local Anesthetic Activity, Nerve Block Anesthesia: With the use of a pithing needle, the upper portion of the spinal cord was severed in four (150 grams) frogs. To create a bag composed of the abdominal walls, the abdomen was sliced open, and all of the organs were removed. The sciatic nerve was exposed to each of three dilutions of the test drug (1:10, 1:50 and 1:100 dilutions of extract) separately.

Application of Drugs: A cotton piece was inserted into the first frog's abdominal pouch after being submerged in a 0.2% xylocaine solution. Similarly, separate pieces of cotton were immersed in 1:10, 1:50, and 1:100 dilutions of extract and then placed in the abdominal pouch of the 2nd, 3rd and 4th frogs respectively.

Leg Withdrawal Action: The frog board was positioned vertically to allow the frog's hind legs to swing loose. The beakers holding 0.1 N HCl and regular saline were filled with the rear legs of the right and left animals. Both before and after the medicine was administered, there was a sudden retraction of the legs.

Muscle Twitch Method: The frog is put to death by euthanasia, and its gastrocnemius sciatic muscular nerve is prepared for dissection. It was put in the Lucas wet chamber, which was filled with the saline of frogs. The muscular tendon was fastened to the lever's hook, and the knee joint was secured to one of the chamber's corks. The lever was set in a horizontal line, the drum speed was maximized, and the after-load screw was adjusted such that it touched the hook lever. After positioning the nerve on the electrodes, cotton soaked in saline was placed over it to prevent it from drying out.

Lever contact with the drum was permitted. It was re-positioned approximately one inch above the base. By running the drum until a straight-forward muscle curve was produced, the baseline was established.

To investigate the effects (local anesthetic activity) of standard and test medicines (1:10, 1:50, and 1:100 dilutions), the position of contact was altered [10].

Infiltration Anaesthesia: Using depleting chemicals, the fur on the back of a healthy adult male guinea pig (CPCSEA regd. No. 1458/PO/a/11/CPCSEA) was removed. After being cleaned with regular saline, the depleted region was left to dry.

Four separate guinea pigs were then intradermally injected with the reference and test medication (1:10, 1:50, and 1:100 dilutions). Animal responses, such as squeaks or twitches, to the sharp pin pricking the injection site, were noted with a (+) or a (-) if the animal did not react at all [11].

TABLE 1: NERVE BLOCK ANESTHESIA METHOD: LOCAL ANESTHETIC ACTIVITY OF TEST DRUGS

Group: Standard vs. Test (herbal extract)	Response	
	Left (Leg)	Right (Leg)
Before the Application of the Standard drug (Xylocaine)	+	+
After the Application of the Standard drug (Xylocaine)		
0 min	+	+
2 min	+	+
4 min	+	+
6 min	+	+
8 min	-	-
10 min	-	-
12 min	-	-
14 min	-	-
Before Application of Test drug (herbal extract)	+	+
After the Application of the Test drug (herbal extract) 1:10 concentration		
0 min	+	+
2 min	+	+
4 min	+	+
6 min	-	-
8 min	-	-
10 min	-	-
12 min	-	-
14 min	-	-
Before Application of Test drug (herbal extract)	+	+
After the Application of the Test drug (herbal extract) 1:50 concentration		
0 min	+	+
2 min	+	+
4 min	+	+
6 min	+	+
8 min	-	-
10 min	-	-
12 min	-	-
14 min	-	-
Before Application of Test drug (herbal extract)	+	+
After the Application of the Test drug (herbal extract) 1:100 concentration		
0 min	+	+
2 min	+	+
4 min	+	+
6 min	+	+
8 min	+	+
10 min	-	-
12 min	-	-
14 min	-	-

+ =leg withdrawal. - =leg not withdrawal.

TABLE 2: MUSCLE TWITCH METHOD: LOCAL ANESTHETIC ACTIVITY OF TEST DRUGS

	Time in Minutes	Response in cm
Control (Before Application of drug)	0 min	1.6±0.01
Test (After Application of the drug)	05 min	0.9±0.02
1:10 Conc.	10 min	0.7±0.03
	15 min	0.6±0.02
	20 min	0.5±0.01
	25 min	0.4±0.02
	30 min	0.3±0.01
	35 min	0.4±0.02
	40 min	1.3±0.01
NS (Reproduced)	05 min	1.3±0.02
Test (After Application of the drug)	05 min	1.0±0.02
1:50 Conc.	10 min	0.8±0.01
	15 min	0.7±0.03
	20 min	0.6±0.02
	25 min	0.5±0.01
	30 min	0.4±0.02
	35 min	0.5±0.02
	40 min	1.4±0.01
NS (Reproduced)	05 min	1.3±0.03
Test (After Application of the drug)	05 min	1.2±0.02
1:100 Conc.	10 min	1.1±0.02
	15 min	1.0±0.03
	20 min	0.9±0.01
	25 min	0.8±0.02
	30 min	0.7±0.03
	35 min	0.6±0.03
	40 min	1.2±0.01
NS (Reproduced)	05 min	1.3±0.01

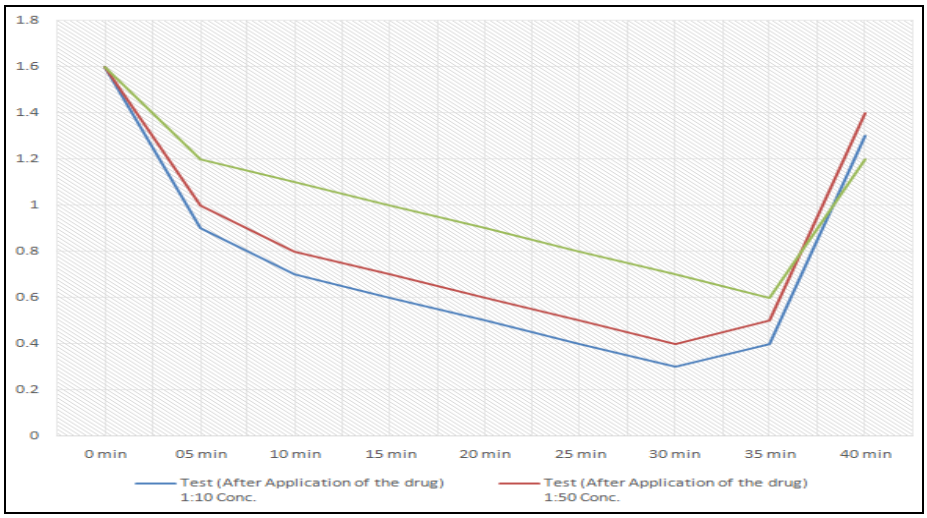

FIG. 1: GRAPH OF LOCAL ANESTHETIC ACTIVITY OF TEST DRUGS USING MUSCLE TWITCH METHOD

TABLE 3: INFILTRATION METHOD: COMPARISON BETWEEN STANDARD AND TEST DRUGS FOR LOCAL ANESTHETIC ACTIVITY

Standard vs. Test (herbal extract)	Response	
	Standard (0.2% Xylocaine)	Test
Before the Application of the drug		
1 min	+	+
After the Application of the standard and test (1:10 Conc.)		
1 min	+	+
5 min	+	-
10 min	-	-
15 min	-	-

20 min	-	-
25 min	-	-
30 min	-	-
35 min	- +	- +
NS (Reproduced)		
1 min	+	+
After the Application of the standard and test (1:50 Conc.)		
1 min	+	+
5 min	+	+
10 min	-	-
15 min	-	-
20 min	-	-
25 min	-	-
30 min	-	-
35 min	- +	- +
NS (Reproduced)		
1 min	+	+
After the Application of the standard and test (1:100 Conc.)		
1 min	+	+
5 min	+	+
10 min	-	+
15 min	-	-
20 min	-	-
25 min	-	-
30 min	-	-
35 min	- +	- +
NS (Reproduced)		
1 min	+	+

+ = shows twitch response to pinprick. - = does not show any response to pinprick.

RESULTS AND DISCUSSION: The local anaesthetic activity was studied by the Nerve block anesthesia method, Muscle twitch method (Frog), and Infiltration anesthesia method (guinea pig) with minor modifications, While Xylocaine (0.25w/v) was used as the standard drug in both models. The nerve-block anesthesia method, Muscle twitch method (Frog), and Infiltration anesthesia method (guinea pig) models are used to determine the duration of an aesthesia simultaneously.

The extracts made from *Croton bonplandianum Baill* were determined using 0.1N HCl in a beaker after dilutions of the extract 1:10, 1:50 and 1:100. Before the medication was administered in the nerve block anesthesia approach, the hind leg abruptly withdrew from the beaker containing the 0.1N HCl when the leg came into contact with the acid. When the medication was tested with HCl after administration, the leg did not withdraw from the acid. Thus, it was demonstrated that the *Croton bonplandianum Baill*. extracts exhibited local anesthetic action. In contrast to the 1:50 and 1:100 concentrations, the test drug (1:10) concentration demonstrated equivalent action to that of the standard medication (xylocaine).

In the Muscle twitch method, control shows a height of response (normal animal) of 1.6 cm and after administration of the test drug, the height gradually decreased up to 35 minutes. The test drug (1:10) concentration exhibited good activity when compared to 1:50 and 1:100 concentrations.

Thus, it was discovered that the local anesthetic action lasted for 35 minutes. Before medication administration, there was a response for pinprick in the Infiltration procedure for up to one minute. For 35 minutes following the test drug's (1:10) administration, there was no reaction to pinprick testing. When compared to 1:50 and 1:100 concentrations, the test medication concentration (1:10) showed good activity. Thus, it was discovered that the local anesthetic action lasted for 35 minutes.

CONCLUSION: Employing a Soxhlet apparatus, *Croton bonplandianum Baill* was extracted using ethanolic extraction. Three different ratios of extraction were prepared (1:10, 1:50, and 1:100). Subsequently, the diluted solution was examined using the frog muscle twitch method, the Guinea pig infiltration anesthesia method, and the nerve

block anesthesia method. From the above observation, we conclude that the onset of action time was 6 minutes for 1:10 concentration, 8 minutes for 1:50 concentration and 10 minutes for 1:100 concentrations in the Nerve block method. The duration of local anesthetic activity in muscle twitch and infiltration method for 1:10 concentration was approximately 35 minutes. After the examination, we concluded that extracts of *Croton bonplandianum Baill* possess local anesthetic activity since the action lasted for 35 minutes. Furthermore, we propose that the extracts of *Croton bonplandianum Baill* can be utilized instead of synthetic local anaesthetics as a natural local anaesthetic.

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