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Local Anesthetic and Tissue Effects of the Leaf Extract and Fractions of *Sterculia tragacantha* Lindl

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ABSTRACT

The local anesthetic and tissue effects of the leaf extract and fractions of *S. tragacantha* were evaluated in this study. The extract 10 and 0.03 mg mL^{-1} produced 100 and 86% anesthesia, respectively while lignocaine 0.1 and 0.03 mg mL^{-1} produced 94.4 and 69.4% anesthesia respectively. The fractions showed concentration dependent local anaesthetic effect with fraction 5 being the most active fraction. Fractions 5 and 7 were more potent than lignocaine. Histological examination of skin sections of mice taken on days 1 and 5 post injection of *S. tragacantha* extract did not reveal any sign of tissue reaction. All fractions contained alkaloids and flavonoids. Three fractions (F_5, F_6 and F_7) contained saponins. This study showed that the extract and fractions of *S. tragacantha* exhibited local anesthetic activity. The alkaloids and saponins contained in the leaves of *S. tragacantha* may be responsible for this activity.

Key words: *S. tragacantha* extract, fractions, chromatography, Guinea pig wheal test, alkaloids

INTRODUCTION

Nociception is initiated by painful stimuli which stimulate afferent nociceptors (A-delta and C-fibres) located on the skin, muscles or viscera (Abdel-Salam, 2006). Nociception involves four physiologic processes namely transduction, transmission, modulation and perception (Busch *et al*., 2006). To abolish or interrupt these nociceptive processes in the peripheral nervous system, local anesthetics which are sodium channel blockers are infiltrated subcutaneously, intra dermally or epidurally (Nyborg *et al*., 2000; Abdel-Salam, 2006; Almeida *et al*., 2007).

Local anesthetics share similar molecular configuration consisting of a lipophilic aromatic ring connected to a hydrophilic amine ring (McLure and Rubin, 2005). These drugs are the safest and most effective drugs used in pain management in medicine (Ribeiro *et al*., 2003). However, they are not selective and may interfere with the homeostasis at the point of injection causing local tissue reaction (Burke *et al*., 1972; De Carvalho *et al*., 1976). This may lead to inflammatory reaction and subsequently pain during or after anesthesia (Aldrete and Johnson, 1970; Redd *et al*., 1990; Berto *et al*., 2011).

Other drugs such as atropine, meperidine and propranolol without the lipophilic and hydrophilic moieties have local anesthetic properties (Acalovschi and Cristea, 1995; Sudoh *et al*., 2003; McLure and Rubin, 2005). Also, extracts of *Corynanthe pachyceras*, *Picralima nitida*,

Mitragyna stipulosa, Pausinystalia johimbe, Cassia absus, Erythroxylum coca and *Voacanga africana* have been documented to possess local anesthetic properties (Bukhari and Khan, 1963; Odebiji, 1980; Tang *et al*., 1986; Oliver-Bever, 1986; Schmelzer, 2008). However, a wide range of medicinal plants used traditionally including *Sterculia tragacantha* are yet to be screened for this activity.

Sterculia tragacantha is a medium sized tree belonging to the genus *Sterculia* family Sterculiaceae. The ethno medicinal uses of this plant have been described (Iwu, 1993; Udegbunam *et al*., 2011). Recently the methanol leaf extract *Sterculia tragacantha* has been shown to possess analgesic activity (Udegbunam *et al*., 2011). Literature search did not reveal any report describing the ethno medicinal use of this plant for local anesthesia. This present study was therefore undertaken to investigate the local anesthetic effect of the methanol leaf extracts of *S. tragacantha*. The effects of its subcutaneous injection on the tissues were also investigated.

MATERIALS AND METHODS

Plant collection and identification: Fresh leaves of *Sterculia tragacantha* were collected in September, 2009 from Nsukka area. They were authenticated by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethno medicine and Drug Development, Nsukka.

Extraction of plant material: The fresh leaves were air dried, pulverized and cold macerated in 80% v/v methanol for 48 h. The extract obtained was concentrated using a vacuum rotary evaporator to afford a greenish brown extract (yield: 11.1%). Preliminary phytochemical tests of the extract showed the presence of carbohydrate, starch, glycosides, alkaloids, flavonoids, terpenes, tannins and saponin (Udegbunam *et al*., 2011).

Preparation of the fractions: The methanol extract was subjected to column chromatography to separate its components. Briefly, the column was prepared by adding slurry of silica in n-hexane gradually down a 1000 mL column. The column was allowed to stand for 24 h. A uniform mixture of silica and the extract in a ratio of 4:1 was introduced into the column. Different solvent systems of n-hexane, chloroform, ethyl acetate and methanol of different ratios were used for the separation (Table 1). Rate flow was 40 drops per minute and collections were at 10 mL. TLC of solvent system of 3:2:1 of chloroform, ethyl acetate and methanol, respectively was used to group the aliquots. Fractions were isolated under the UV lamp.

Animals: The local anesthetic effect of the extract and fractions were evaluated using Guinea pigs (*Cavia porcellus*) weighing 300-400 g. The tissue effect of the extract was evaluated using mice

Table 1: Solvent systems for column chromatography of the methanol extract of *S. tragacantha*

N-hexane	Chloroform	Ethyl acetate	Methanol
100	-	-	-
80	20	-	-
30	60	10	-
-	80	20	
-	-	60	40
-	-	40	60
-	-	20	80
-	-	-	100

weighing 28-30 g. The experimental animals were housed in standard experimental conditions in the laboratory unit of the Department of Veterinary Physiology and Pharmacology University of Nigeria, Nsukka. They were fed standard commercial diet. Water was provided free choice. All experiments were approved by the Animal ethical Committee University of Nigeria, Nsukka.

Phytochemical analysis of the fractions: The fractions were screened for the presence of tannins, saponins, alkaloids, flavonoids, glycosides, reducing sugars, terpenes, polyuronides and anthroquinones as described by Harborne (1984).

Determination of the pH of extract: Two different solutions (10 mg mL^{-1}) of the extract were prepared using distilled water and 10% Tween 20, respectively. The pHs of the solutions as well as those of the control solutions [lignocaine (10 mg mL^{-1}) and distilled water] were determined using a pH meter.

Test for local anesthetic effect: Two Guinea pig wheal tests were carried as described by Shetty and Anika (1982). In the preliminary study, the extract (10 and 0.03 mg mL^{-1}) and lignocaine (0.1 and 0.03 mg mL^{-1}) were injected intra dermally. In the second study, the extract (0.15 and 0.015 mg mL^{-1}), fractions (0.15 and 0.015 mg mL^{-1}) and lignocaine (0.15 and 0.015 mg mL^{-1}) were injected intra dermally. The wheals formed were tested for sensitivity every 5 min for a period of 30 min. The total score for each wheal was added at the end of the experiment and expressed as the sum of negative responses out of 36 possible. The percentage anesthesia was determined as the number of negative responses over the number of 36 possible responses multiplied by 100.

Evaluation of the tissue effect of the extract: Mice weighing 28-30 g were assigned to two treatment groups (n = 5). The areas for injections were marked with indelible ink. The animals in groups 1 and 2 were injected subcutaneously (s.c.) with 0.02 mL of distilled water and extract respectively. On days 1 and 5 post injection, 2 mice were euthanized per group and skin sections taken for histology. Skin sections were fixed immediately in 10% formal saline. These tissues were later processed and embedded in paraffin wax. Sections were cut and stained with haematoxylin and eosin and examined under the light microscope.

RESULTS

Phytochemical test: All fractions contained alkaloids and flavonoids. Three fractions (F_5, F_6 and F_7) contained saponins.

Determination of the pH: As shown in Table 2, the solutions formed from the extract were acidic.

Local anesthetic effect: The result of the preliminary Guinea pig wheal test presented in Fig. 1 showed that the extract at 10 and 0.03 mg mL^{-1} produced 100% and 86% anesthesia respectively while lignocaine (0.1 and 0.03 mg mL^{-1}) produced 94.4% and 69.4% anesthesia respectively. The result of the second Guinea pig wheal experiment as presented in Fig 2 showed that the different fractions produced varying degrees of anesthesia with F_5 being the most active fraction. Three fractions (F_2, F_5 and F_6) were more potent than lignocaine. Also five fractions (F_2, F_3, F_5, F_6 and F_7) were more potent than the extract.

Table 2: pH of solutions

Solutions	pH
Distilled water (DW)	7.1
1% lignocaine	5.1
10% tween+MEST (1%)	4.6
DW+MEST (1%)	4.5

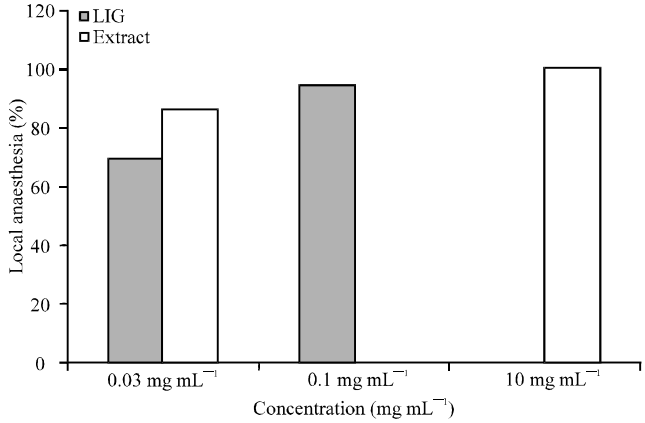

Fig. 1: Percentage local anesthesia of *S. tragacantha* extract and lignocaine

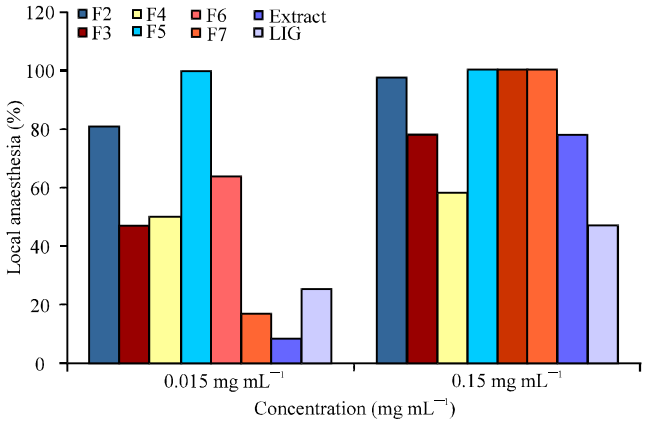

Fig. 2: Percentage local anesthesia of fractions of *S. tragacantha*

Tissue effect of the extract: Histological examination of the skin sections did not reveal any sign of tissue reaction in the extract treated group on days 1 and 5.

DISCUSSION

The extract of *S. tragacantha* showed local anesthetic activity following intra dermal injection in Guinea pigs. This prompted the fractionation of the methanol extract and the fractions obtained were also tested for local anesthetic activity. The fractions showed anesthetic activity of varying degrees. The results of both Guinea pig wheal tests suggest that both the extract and fractions of *Sterculia tragacantha* were able to inhibit nerve impulse conduction in the skin of Guinea pigs.

It was noted that the solution of the extract formed in Tween 20 and distilled water were acidic in nature. These solutions on subcutaneous injection did not irritate the tissue. Acidic solutions of local anesthetics are known to cause tissue irritation, inflammation and pain following injection

(Aldrete and Johnson, 1970; Redd *et al*., 1990; Berto *et al*., 2011). We therefore suggest that the absence of inflammation after injection of this solution may be due to its anti-inflammatory and anti-oxidant properties (Udegbunam *et al*., 2011). The acute inflammatory process involves the activity of inflammatory mediators such as neutrophil-derived free radicals, Reactive Oxygen Species (ROS), Nitric Oxide (NO•), prostaglandins and cytokines (Valko *et al*., 2006; Gusdinar *et al*., 2011). ROS play an important role in the pathogenesis of local and systemic inflammatory disorders (Valko *et al*., 2006; Gusdinar *et al*., 2011). For this reason, agents that can effectively inhibit prostaglandins, cytokines and the oxidative burst of activated leukocytes contribute to the prevention of inflammation (Tanas *et al*., 2010).

Plants are known to contain several phytochemical constituents which exhibit complex interactions producing synergistic or antagonistic responses (Savelev *et al*., 2003). Thus, it was not surprising that the fractions which showed potent local anesthetic activity contained both alkaloids and saponins. Plants rich in alkaloids have been shown to possesses local anesthetic properties. The pharmacologic study of two diterpene alkaloids, 3 acetylaconitine (ACC) and aconitine isolated from the root of *Aconitum flavum* by Tang *et al*. (1986) showed that both alkaloids had local anesthetic action. The local anesthetic effect of *Cassia absus* was attributed to the presence of two alkaloids-chaksine and isochacksine (Bukhari and Khan, 1963). Also, the local anesthetic effect of *Erythrophleum guineense* was attributed to the presence of cassine while an indole alkaloid was incriminated as being responsible for the local anesthetic effect of *Mitragyna stipulosa* (Oliver-Bever, 1986). Saponins have been shown to have analgesic effects (Calixto *et al*., 2000; Wang *et al*., 2008; Ma *et al*., 2011).

CONCLUSION

The results of this study showed that the extract and fractions of *S. tragacantha* possessed local anesthetic activity. Injection of the extract subcutaneously did not cause tissue reaction. Thus, it can be concluded that the alkaloids and saponins contained in the leaves of *S. tragacantha* may be responsible for this activity. The extract of *S. tragacantha* and its fractions might be useful for local anesthesia prior to minor surgical procedures. Further experiments will be carried out to explore the use of this extract for local anesthesia in goats.

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