

Bio-flavonoids and Garcinoic Acid from *Garcinia Kola* Seeds with Promising Local Anesthetic Potentials.

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ABSTRACT

Local anesthetics are a well-known group of pharmaceutical agents used to relieve pain in specific organism, inhibiting propagation of signal along the nerves. In the present study ethanol extract, kolaviron, GB1, GB2 and garcinoic acid of *Garcinia kola* seeds were evaluated for local anesthetic activity. Xylocaine the reference drug induced 100% local anesthesia on the guinea pig skin at 1mg/ml. GB1 caused 92% local anesthesia at 10mg/ml, which was similar to the effect of Xylocaine (0.66mg/kg).

Key words: *Garcinia kola*, local anesthetic, xylocaine

INTRODUCTION

Nociception is initiated by painful stimuli which stimulate afferent nociceptors (A-delta and C-fibres) located on the skin, muscles or viscera¹. Nociception involves four physiologic processes namely transduction, transmission, modulation and perception². To abolish or interrupt these nociceptive processes in the peripheral nervous system, local anesthetics which are sodium channel blockers are infiltrated subcutaneously, intradermally or epidurally^{3,4,5}.

Local anesthetics share similar molecular configuration consisting of a lipophilic aromatic ring connected to a hydrophilic amine ring⁶. These drugs are the safest and most effective drugs used in pain management in medicine⁷. However, they are not selective and may interfere with the homeostasis at the point of injection causing local tissue reaction^{8,9}. This may lead to inflammatory reaction and subsequently pain during or after anesthesia^{10,11,12}.

Other drugs such as atropine, meperidine and propranolol without the lipophilic and hydrophilic moieties have local anesthetic properties^{6,13,14}. However, a wide range of medicinal plants used traditionally including *Garcinia kola* are yet to be screened for this activity.

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 ethanolic seed extract of *G. kola*, kolaviron, GB1, GB2 and garcinoic acid.

MATERIAL AND METHOD

General experimental procedures

The UV spectra were obtained with a shimadzu 3101 PC instrument and IR spectra determined with a jasco FT-IR

410 apparatus. ¹H (400.6MHz) and ¹³C (100.13 MHz) nmr spectra were recorded in CDC₁₃ (with its signals at δ 7.25 and 77.0 ppm as reference) TLC was carried out on silica gel 60 GF₂₅₄ pre-coated plates with detection by UV light or by spraying with 50% H₂SO₄ followed by heating at 100°C.

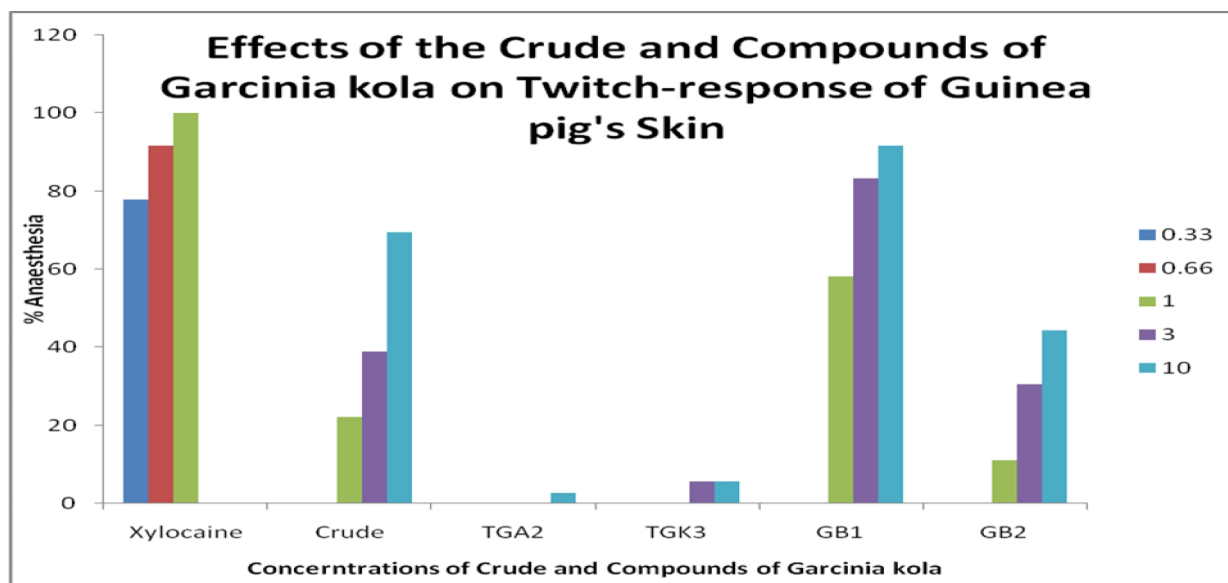
Plant material, preparation of extract, fractions and compounds

Garcinia kola seeds were collected within the surrounding of Orba, Nsukka, Enugu State, Nigeria in March 2010, Nigeria, and was identified and authenticated by Mr. Alfred Ozioko of International Centre for Ethnomedicine and Drug Development. The voucher specimen (INTERCEDD 022010) is deposited at the same center.

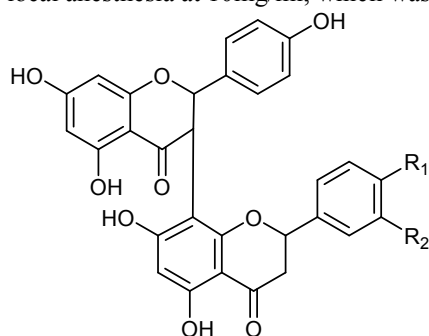
The air-dried and powdered plant material (5Kg) was macerated in a mixture of CH₂Cl₂-MeOH (1:1) for 48h. Removal of the solvent *in vacuo* in a rotary evaporator provided an organic extract (600g).

Kolaviron was isolated according to Iwu et al¹⁵ as modified by Farombi et al¹⁶. Briefly, the powdered seeds were extracted with light petroleum ether (b.pt 40-60 o C) in a soxhlet for 24h. The defatted, dried marc was repacked and extracted with acetone (Me₂CO). The extract was concentrated and diluted twice its volume with water and extracted with ethyl acetate. The concentrated ethyl acetate fraction gave a yellow solid known as Kolaviron (TGA) Further purification of TGA using silica gel as stationary phase and mixture of CH₂Cl₂/ acetone afforded GB1 and GB2. The fraction obtained with EtOAc/nhexane (8:2) was further purified using silica gel as stationary phase and EtOAc/nHexane mobile phase yielded garcinoic acid (TGK3).

Identification of GB1, GB2 and TGK3



Effects of the crude and fractions of *Garcinia kola* on Twitch response of guinea pigs skin (Local Anesthetic Test) Xylocaine the reference drug induced 100% local anesthesia on the guinea pig skin at 1mg/ml. GB1 caused 92% local anesthesia at 10mg/ml, which was similar to the effect of Xylocaine (0.66mg/kg)

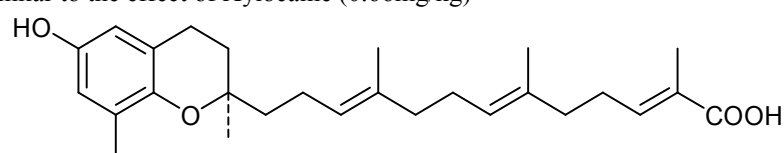


GB1, R1= OH, R2= H

GB2, R1=R2= OH

TGA2= Kolaviron

Crude = Ethanolic extract of *Garcinia kola* seeds



TGK3= Garcinoic acid

The known compounds GB1, GB2 and garcinoic acid were identified by comparison of NMR data with published data.

Animals

Adult male guinea pigs (178-205 g), from the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria Nsukka were kept in stainless steel cages and fed *ad libitum* with standard laboratory animal chow (vital feed®), except where fasting was required. They were maintained in accordance with the recommendation in the Guide for Care and Use of Laboratory Animals (DHH, NIH Publication No. 85-23, 1985). The experiments were conducted with the permission of the Institution's Animal Ethics Committee.

Brine shrimp lethality test (BSLT)

The effect of the extract on brine shrimps was evaluated using the method of McLaughlin *et al*¹⁷. Briefly, brine shrimp eggs were hatched in culture tank containing sea water under bright light for 48 h. Ten nauplii were counted into bijou bottles in triplicates and were incubated with graded concentrations of the extract (10, 100 and 1000 ppm) at room temperature for 24 h. The

mean surviving nauplii was determined for each concentration of the extract and compared with that of the control. The result was analyzed using probit analysis (minitab for windows release 12.21) to determine the LC50 at 95% confidence interval.

Local anesthetic test

Twitch response test (TRT)

The local anesthetic effect of the extract, fraction and compounds were investigated using the method described by Anaga *et al*¹⁸. The lower back of a guinea pig was shaved 24 h before the test. Three different concentrations of extract, kolaviron, GB1, GB2, and garcinoic acid (1, 3 and 10) and xylocain (0.33, 0.66 and 1.00) were used. A volume of 0.25 ml each of the concentrations of the extract and xylocain were injected intradermally to form wheals. Therefore six different sites on the lower back of the guinea pig were used. Five minutes after the injection, the sensitivity of the site was tested by pricking lightly with a needle six times at each of the injection sites and as control; a site far away from injection sites was pricked. The response at the site of

injection indicated the degree of desensitization which was expressed as the number of negative responses i.e. of failure to twitch; 36/36 indicates maximum anesthesia while 0/36 indicates no anesthesia. The test was repeated at 5 min intervals for a period of 30 min after the injection. The total score for each group was summed up and expressed as the total number of negative responses out of the 36 possible responses and expressed as percent anesthesia.

RESULTS AND DISCUSSIONS

The ethanolic extract of *G. kola* seeds showed 69.4% local anesthetic activity at the concentration of 10 mg/ml following intra dermal injection in Guinea pigs, while kolaviron, garcinoic acid, GB2 exhibited 2.8%, 5.3% and 44.4% at the same concentration. Furthermore Xylocaine the reference drug induced 100% local anesthesia on the guinea pig skin at 1mg/ml. GB1 caused 92% local anesthesia at 10mg/ml, which was similar to the effect of Xylocaine (0.66mg/kg).

It was noted that the solution of the extract and GB1 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^{19,20,11}. We therefore suggest that the absence of inflammation after injection of this solution may be due to its anti-inflammatory and anti-oxidant properties²¹. 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^{22,23}. ROS play an important role in the pathogenesis of local and systemic inflammatory disorders^{22,23}. For this reason, agents that can effectively inhibit prostaglandins, cytokines and the oxidative burst of activated leukocytes contribute to the prevention of inflammation²⁴.

G. kola Heckel (Clusiaceae), commonly known as bitter kola possesses hepatoprotective, anti-inflammatory, antioxidant, analgesic, hypoglycemic, viral activities²⁵. Also flavonoids are been reported to exhibited anti-inflammatory and antioxidant properties²⁵. The local anesthetic activity of *G. kola* seed reported here for the first time may be due the presence of garcinia biflavonoid (GB1) in the plant.

CONCLUSION

The ethanol extract of *G. kola* seeds and the isolated GB1 caused 69.4 and 92% local anesthesia respectively at 10mg/ml, the effect of GB1 was similar to Xylocaine (0.66mg/kg). The results of this study have shown that *the extract and GB1 of G. kola* seeds has promising local anesthesia properties; thus, the plant can be exploited in the development of phytomedicines or as a source of lead compounds for local anesthesia drug development.

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