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Original Article

Bio-flavonoids and Garcinoic Acid from *Garcinia kola* seeds with Promising Anti-Inflammatory Potentials

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ABSTRACT

Objective: The research was carried out to investigate the anti-inflammatory effect of ethanol extract, fraction (kolaviron) and compounds (garcinoic acid, GB1 and GB2) of *Garcinia kola* seeds. **Materials and Method:** To evaluate the acute anti-inflammatory effect of extract, fraction and compounds of *G. kola* carrageenan-induced edema model in wistar albino rats was used. **Results:** Kolaviron (50 mg/kg), garcinoic acid (50 mg/kg) and the crude extract (50 mg/kg) caused 100, 83 and 74% inhibition of carrageenan-induced paw edema respectively at 6 h post administration. Indomethacin (10 mg/kg), the reference drug induced 100% inhibition of carrageenan-induced paw oedema. While GB1 (50 mg/kg) and GB2 (50 mg/kg) was prominent at 4, 5 and 6 h post administration. **Conclusion:** Results showed that the extract possessed anti-inflammatory activity, which have justified their use in Nigeria traditional medicine to treat inflammation.

Key words: Anti-inflammatory, Carrageenan, *Garcinia kola*, Paw edema, Wistar rats.

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INTRODUCTION

Inflammatory diseases are a major cause of morbidity world-wide. Non-steroidal anti-inflammatory drugs and steroids are the most common drugs used to treat inflammation. Gastrointestinal side effect is a major side effect associated with the currently available non-steroidal anti-inflammatory drugs which limit their application. This may be contributing to the current move by large proportion of world population towards herbal remedies for the treatment of inflammatory diseases.[1] Herbal medicine is still the mainstay of about 75–80% of the whole population, mainly in developing countries, for primary health care because of cultural acceptability, compatibility of herbal medicine and fewer side effects. However, the last few years have seen a major increase in their use in the developed world.[2] Medicinal plants are used in developing countries for the management of a number of disease conditions including pain and inflammatory conditions. The validation of the folkloric claims of these medicinal plants will provide scientific basis for the conservation of tropical medicinal resources, and the use of phytomedicine in the primary health care and the development of precursors compounds in drug design. Therefore, the main purpose of this study is to investigate the anti-inflammatory activity of the ethanolic extract of *Garcinia kola*, kolaviron, GBI, GB2, and garcinoic acid.

MATERIAL AND METHODS

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. ^{1}H (400.6MHz) and ^{13}C (100.13 MHz) nmr spectra were recorded in CDC_{l3} (with its signals at δ 7.25 and 77.0 ppm as reference) TLC was carried out on silica gel 60 GF_{254} pre-coated plates with detection by UV light or by spraying with 50% H_2SO_4 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 (5 Kg) was macerated in a mixture of CH_2Cl_2-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 al.*[3] as modified by Farombi *et al.*[4] Briefly, the powdered seeds were extracted with light petroleum ether (b.pt 40-60°C) in a soxhlet for 24 h. The defatted, dried marc was repacked and extracted with acetone (Me_2CO). 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 CH_2Cl_2/ acetone afforded GB1 and GB2. The fraction obtained with EtOAc/nhex (8:2) was further purified using silica gel as stationary phase and EtOAc/nhex mobile phase yielded garcinoic acid (TGK3).

Identification of GB1, GB2 and TGK3

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

Experimental animals

Thirty five (35) white albino Wistar rats (86-100 g) of either sex were procured from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in stainless steel cages and were fed *ad-libitum* with standard laboratory animal feed (Guinea Feed®). They were also provided with clean tap water. They were maintained in accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). They were allowed two weeks to acclimatize before the start of the experiments.

Brine shrimps lethality test

The effect of the extract on brine shrimps was evaluated using the method of Mclaughlin*et al.*[5] 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 LC_{50} at 95% confidence interval.

Effects on carrageenin-induced paw edema

The anti-inflammatory effect of the extract, fraction compounds of *G. kola* were conducted using carrageenin-induced paw odema in rats.[6] Briefly, 35 rats (86–100 g) of both sexes were randomly divided into 5 groups of six rats each. Group A rats were given distilled water (10 ml/kg), which served as the control, while group B rats were treated with indomethacin (10 mg/kg, p.o.) suspended in 1% carbonated buffer solution. The remaining C-G groups were treated with graded doses 20 mg/kg, b.w. of samples by oral administration. Before the treatment, the volume displacement by the normal paw (Vo) was measured for each rat. Forty five minutes post administration of the extract and indomethacin, 0.5 ml of carrageenin (1%) in normal saline was injected into the sub plantar area of the hind paw. The change in volume due to carrageenin-induced paw swelling (Vt) of the paw was measured at 0, 30 min, 1, 2, 3, 4, 5 and 6 h after, using plethysmographic method. The percent inhibition was calculated using the modified formula[7,8] below:

$$\text{Percent inhibition} = \frac{(Vt\text{-}Vo)\ \text{control} - (Vt\text{-}Vo)\ \text{treated group} \times 100}{(Vt - Vo)\ \text{control}}$$

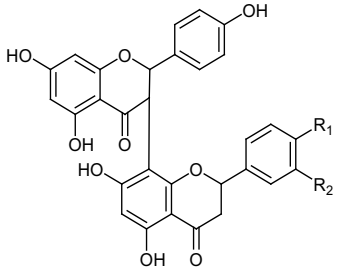
Table 1: Effects of the crude and fractions of *Garcinia cola* on Carrageenan- induced Paw Oedema (Anti-inflammatory test)

		Paw Oedema inhibition %						
Drug/Fraciton	**Dose (mg/kg)**	**30 min**	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**
Indomethacin	10	14.5	24.8	56.1	10	68.4	100	100
Crude	50	0	0	0	38.8	29.9	68.8	74.4
TGA2	50	28.6	57.1	42.9	14.3	57.1	85.7	100
TGK3	50	21.7	11.7	5.0	28.3	21.7	66.7	83.4
GB1	50	-14.3	-5.7	28.6	38.6	20.0	-4.3	-10
GB2	50	-33.3	8.3	-30.8	-14.2	-16.7	0	2.5

RESULTS AND DISCUSSION

Fractions TGA2, TGK3 and the crude extract caused 100, 83 and 74% inhibition of carrageenan-induced paw edema respectively at 6h post administration.

Indomethacin (10 mg/kg), the reference drug induced 100% inhibition of carrageenan-induced paw edema. For the fractions and the drug, the effect was prominent at 4, 5 and 6h post administratio

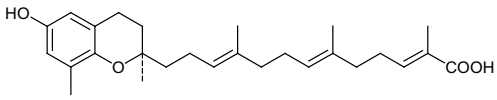


GB1, R1= OH, R2= H

GB2, R1=R2= OH

TGA2= Kolaviron

Crude = Ethanolic extract of *Garcinia kola* seeds



TGK3 = Garcinoic acid

In the present study, the anti-inflammatory effects of the ethanolic extract of *G. kola* seeds, fraction (kolaviron) compounds (garcinoic acid, GB1 and GB2) were demonstrated in an *in vitro* animal model, which focused on the inhibitory effect of the extract, fraction, and compound on anti-inflammatory activity. Inflammation can be defined as the response of living tissues to injury which involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair.[9] The anti-inflammatory effects may be elicited by a variety of chemicals agent and that there is no remarkable correlation between their pharmacological activity and chemical structure.[10]

The acute anti-inflammatory of extract, fraction and compounds of *G. kola* was evaluated carrageenin-induced edema model (Table 1). Kolaviron (50 mg/kg), garcinoic acid (50 mg/kg) and the crude extract (50 mg/kg) caused 100, 83 and 74% inhibition of carrageenan-induced paw edema respectively at 6h post administration. Indomethacin (10 mg/kg), the reference drug induced 100% inhibition of carrageenan-induced paw edema. While GB1 (50 mg/kg) and GB2 (50 mg/kg) was prominent at 4, 5 and 6h post administration. This model, presents three phases with production of various mediators. The first phase (0-2h) is due to the release of serotonin and histamine; the second phase (3-4h) is predominantly due to kinins and the third phase (>4h) is due to the release of prostaglandine.[11,12] Kolaviron is a mixture of three bioflavonoids GB1, GB2 and kolaflavanone[2] flavonoids and polyphenolic compound have been found in other natural products with anti-inflammatory property. Therefore, the anti-inflammatory activity of ethanolic extract of *G. kola* seeds may due to the presence of bioflavonoids. Further we noticed that kolaviron extract caused 100% inhibition of carrageenan-induced paw edema at 6h post administration while GB1 and GB2 caused-10% and 2.5% at 6h. This result may allow us to conclude that the biflavonoid found in kolaviron have synergism effect on inflammatory. This finding support the fact that *G. kola* is used to treat inflammation in traditional medicine.[5]

CONCLUSION

From the result obtained from the experiment it is concluded that kolaviron, garcinoic acid and the crude extract caused 100, 83 and 74% inhibition of carrageenan-induced paw edema respectively at 6h post administration at the concentration of 50 mg/kg. This results support the traditional use of this plant in inflammatory.

ACKNOWLEDGEMENT

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ABBREVIATION USED

CH_2Cl_2: Dichloromethane, **MeOH:** Methanol, **Me_2CO:** Acetone

GB1: Garcinia biflavonoid 1, **GB2:** Garcinia biflavonoid 2, **TLC:** Thin Layer Chromatography.

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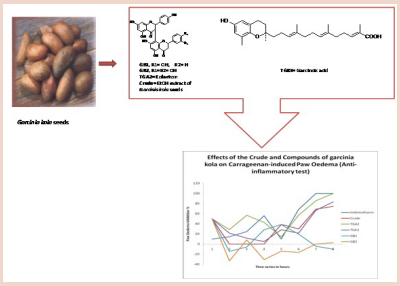
PICTORIAL ABSTRACT



SUMMARY

- Extract, Compounds, Anti-Inflammatory

ABOUT AUTHOR

Tchimene Kenne Michel: Obtained his Ph. D. degree in 2006 from University of Yaounde 1 Cameroon. His research focused on the evaluation of anti-inflammatory, antioxidant, antimicrobial, antidiabetic activity and cytotoxicity of natural products. His currently working with International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria.