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 Methods:** 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 edema. 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. 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. 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, GB1, 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 PT-IR 410 apparatus. ¹H (400.6MHz) and ¹³C (100.13 MHz) nmr spectra were recorded in CDC₅₀ (with its signals at δ 7.25 and 77.0 ppm as reference) TLC was carried out on silica gel 60 GF₆₉ 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 (INTER-CEDD 022010) is deposited at the same center.

The air-dried and powdered plant material (5 Kg) 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 al.* as modified by Farombi *et al.* 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 (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/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. 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.

Effects on carrageenin-induced paw edema

The anti-inflammatory effect of the extract, fraction compounds of G. kola were conducted using carrageenin-induced paw edema in rats. 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 in 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 the release 12.21) to determine the LC50 at 95% confidence interval.

Percent inhibition = (Vt - Vo) control – (Vt - Vo) treated group x 100

(Vt - Vo) control

<table>
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<th>Drug/Fraciton</th>
<th>Dose (mg/kg)</th>
<th>30 min</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
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<td>Crude</td>
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<td>0</td>
<td>38.8</td>
<td>29.9</td>
<td>68.8</td>
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<td>57.1</td>
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<tr>
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<td>11.7</td>
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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 administration.

CONCLUSION

From the result obtained from the experiment it is concluded that kolaflavanone, 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

CHCl3: Dichloromethane, MeOH: Methanol, MeCO: Acetone
GB1: Garcinia biflavonoid 1, GB2: Garcinia biflavonoid 2, TLC: Thin Layer Chromatography.
REFERENCES