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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF KOLAVIRON (A *GARCINIA KOLA* SEED EXTRACT)

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Kolaviron is a defatted ethanol extract from the seeds of Garcinia Kola. In the present study, the analgesic and anti-inflammatory properties of Kolaviron is investigated using both thermal and chemical models of pain assessment in mice and rats. Varying doses of Kolaviron were given 30 minutes prior to the induction of abdominal constrictions in mice and the determination of the mean tail immersion duration at water bath temperature of 50.0 ± 1^{0}C in mice. Kolaviron exhibited dose-related anti-nociceptive properties against acetic acid induced abdominal constrictions in mice: at 50mg/kg, it gave 28.92% inhibition (P > 0.05) and at 200mg/kg it gave 55.49% inhibition (P < 0.01). The compound also increased the mean tail immersion duration at water bath temperature of 50.0 ± 1^{0}C in mice.

Keywords: Kolaviron, Garcinia kola, analgesic, anti-inflammatory, rats, mice

Garcinia Kola, Heckel (Gultiferae), a largely cultivated forest tree indigenous to sub-Saharan Africa has been referred to as a 'wonder plant' because almost every part of it has been found to be of medicinal importance (Hutchinson and Dalziel, 1956). The seed (commonly known as bitter kola, male kola or false kola) is a masticatory used in traditional hospitality, cultural and social ceremonies. Extractive of the plant have been traditionally used for ailments such as laryngitis, liver diseases and cough (Ayensu, 1978). The seeds are used to prevent or relieve colic, cure head or chest colds and relieve cough (Iwu, 1993). The seed also has anti-inflammatory, antimicrobial, antidiabetic and antiviral (Iwu, 1986) as well as antiulcer properties (Ibironke et al, 1997).

Kolaviron (Fig. 1) is a defatted ethanol extract from the seeds of *Garcinia Kola* (GK). It is a mixture of three compounds - Garcinia biflavonoid GB_1, GB_2 and Kola flavanone in ratio 2:2:1 (Iwu et al, 1990, Kubanga, 1987). Kolaviron has been extensively studied for its anti-hepatotoxic effects (Akintonwa and Essien, 1990; Farombi et al, 2000,) in various experimental models.

In the present study, we report that Kolaviron may be the active principle for the analgesic and anti-inflammatory activities of *G. Kola*.

MATERIALS AND METHODS

Plant materials

Seeds of *Garcinia Kola* were obtained locally in Ibadan, Nigeria in October 1999 and certified by Prof. Egunyomi in the Department of Botany, University of Ibadan. A voucher specimen is available in the herbarium of the same institution. 7 kg of Peeled seeds were sliced, pulverised with electric blender and dried at 40^{o}C in a Gallenkamp drying oven.

Tested material

Kolaviron was isolated according to Iwu *et al* (1990) as modified by Farombi *et al* (2000). 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 (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. The extract (50g) was suspended in 100ml 0.9% NaCl for oral administration to rats. Appropriate dose dilutions were made with normal saline to provide for a total volume of 0-5ml. 0.5ml of saline was similarly administered orally to rats.

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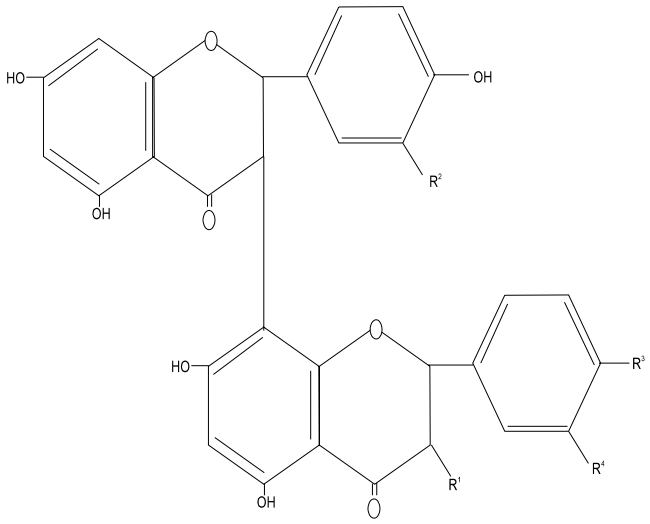
Animals:

Adult male Swiss mice (20-25g) and albino rats (100-120g) obtained from the small animal house, College of medicine, University of Ibadan, Nigeria were used. They were housed in cages at room temperature with free access to mice cubes (Ladokun Feeds Nig. Limited, Ibadan, Ibadan, Nigeria). The body weight changes in the rats before and after administration of the extract was monitored daily.

Analgesic activity

Acetic acid writhing response: Mice were treated orally with the Kolaviron at 50, 100 and 200mg/kg doses. Acetylsalicylic acid (Aspegic^R) was used as a reference analgesic compound at 70mg/kg dose. Control animals received 0.2ml distilled water. 30 minutes later, the animals were given 1.2% acetic acid injection. The number of writhing and stretching within the observation period was recorded. The percentage protection was calculated using the ratio: (control mean – treated mean) X 100/control mean (Baghelikian *et al*, 1997).

Tail-flick method: Mice were treated orally with Kolaviron, reference drug and vehicle 30 minutes before experiment as described above. Water was heated to 50.0 ± 1:0^{0}C in a water bath. By carefully holding the animal, the tail was immersed gently in the hot water bath. The time taken for the animal to flick its tail out of the water was recorded. Each animal served as its own control, as preliminary experiments showed that the procedures involved with injection of the vehicle lone had no significant effect on the response times at 30 minutes.



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Figure 1
Structure of Kolaviron

Anti-inflammatory activity

Edema was induced on the right foot of rats by sublantar injection of 0.05ml of solution of 1.5% carrageenan in 0.9% saline (W/V). The diameter of the injected paws and contralateral paws were measured 1hr, before and 1, 2, 3, 4 and 5 hours after induction of inflammation using cotton thread. The edema was expressed in terms of the difference between the right and left paws. A reference group of animals were treated with Aspirin (150mg/kg).

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM) for the acetic acid writhing tests; statistical comparisons were done using the student's t-test. For the tail-flick test in mice, paired t-test was used to compare pain responses before and after treatment with extract, reference drug or vehicle.

RESULTS

Analgesic activity

Acetic acid writhing response: The effect of Kolaviron on the acetic acid induced abdominal writhing is shown in Table 1. The result shows that the compound exhibited significant and dose –

related anti-nociceptive properties against acetic acid induced abdominal constrictions in mice. A Kolaviron dose of 100mg/kg gave results comparative to 70mg/kg dose of the reference drug, acetylsalicylic acid.

Table 1
Effect of Kolaviron on acetic acid writhing response in mice.

Treatment	No of writhing (Mean ± SEM)	Percentage protection
Vehicle (Distilled water)	30.60 ± 4.5	----
Kolaviron (50mg/kg)	26.52 ± 4.8$^{N.S}$	13.33
Kolaviron (100mg/kg)	21.75 ± 3.11*	28.92
Kolaviron (200mg/kg)	14.20 ± 2.50*	53.59
Aspirin (70mg/kg)	13.62 ± 2.80*	55.49

*N.S= Not significant, *P<0.05 (c.f. vehicle), n= 10.*

Table 2
Thermal pain perception (Tail immersion in 50 ± 1^{0}C hot water) **in the presence or absence of Kolaviron and acetylsalicylic acid.**

Treatment	Reaction times (seconds) (Mean ± SEM)		
	Pre-treatment	. Post-treatment	
		30min	60min
Vehicle (Distilled water)	7.35 ± 0.13	7.95 ± 2.19$^{N.S}$	8.06 ± 2.15
Kolaviron (50mg/kg)	7.40 ± 0.18	9.63 ± 0.50* (21.13	10.12 ± 1.10 (25.56)
Kolaviron (100mg/kg)	7.85 ± 0.13	11.52 ± 1.66** (44.91)	10.97 ± 1.36 (36.10)
Kolaviron (200mg/kg)	7.55 ± 0.17	16.95 ± 1.32** (113.21)	16.85 ± 0.93 (109.06)
Aspirin (70mg/kg)	7.95 ± 0.26	18.72 ± 1.65** (135.47)	20.32 ± 1.15 (152.11

**P<0.05, **P<0.001, N.S= Not Significant, (c.f. Vehicle, paired t-test, n=15*
Values in parenthesis represent percentage protection

Latency of tail immersion in mice: The mean tail immersion in hot water bath (55 ± 1^{0}C) one hour before and 30 minutes after oral administration of varying doses of Kolaviron are shown in Table 2. Treatment with the vehicle did not have any significant effect on the latency of tail immersion. Kolaviron, at all doses tested, showed significant and dose-related increases in tail immersion duration.

Anti-inflammatory effect
The anti-inflammatory potencies of acetyl salicylic acid and Kolaviron are compared in figure 1. Kolaviron showed relatively good anti-inflammatory activity when compared with aspirin. The maximum inhibition of edema attained in the rats pre-treated with 100mg/kg kolaviron (59.52% ± 4.65) is not significantly different from that given by 150mg/kg Aspirin. (62.05% ± 3.75). The inhibition produced by Kolaviron dose of 150mg/kg (72.40% ± 3.35) was significantly higher than that of aspirin.

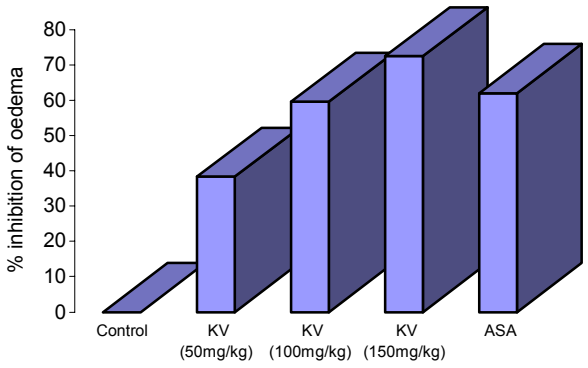


Figure 2.
Maximum inhibition of oedema induced by 1.5% carrageenan. (KV = Kolaviron, ASA = Aspirin

DISCUSSION

In this study, the analgesic and anti-inflammatory properties of Kolaviron, a defatted seed extract of *Garcinia kola* (bitter kola), was investigated in mice. We have shown here that Kolaviron exhibited a weak analgesic but very strong anti-inflammatory activities when compared to a standard reference drug, acetyl salicylic acid. There appears to be a fair degree of agreement between the thermo- and chemonociceptive assays used in the present study. There is no generally accepted paradigm for pain assessment in either human or animal experiments. It is therefore essential to employ two or more of this method in a single study before a definite conclusion can be made on the action of any agent affecting pain responses.

The activity of Kolaviron may not be unrelated to the presence of the biflavonoid group. The biflavanones of *Garcinia kola* are pharmacologically active with several pharmakokinetic advantages over simple monomeric flavonoids. For instance, the biflavonoids have been shown to survive first pass metabolism which inactivates most flavonoids and they have been proved to possess very high therapeutic potentials (Iwu, 1986). Furthermore, many plants containing flavonoids have been shown to have diuretic, laxative, antispasmodic, anti-hypertensive and anti-flammatory actions (Okuda, 1962). The traditional use of G. kola in the traditional management of inflammatory conditions in hepatic and respiratory systems is thus justified.

Further studies aimed at identifying the component of Kolaviron responsible for the observed anti-inflammatory activity is in progress.

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