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ARTICLE

Analgesic anti-inflammatory anti-pyretic activities of Garcinia hydroxybiflavanonol (GB1) from *Garcinia kola*

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Abstract This study was conducted to evaluate the analgesic, anti-inflammatory, and anti-pyretic effects of GB1, a hydroxybiflavanonol of *Garcinia kola.* The analgesic effect was studied using acetic acid-induced writhing and tail withdrawal tests. Its anti-inflammatory effect was studied using carrageenan-induced paw edema and adjuvant-induced arthritis tests. Brewer's yeast-induced pyrexia test was used to evaluate the anti-pyretic effect of GB1. GB1 reduced writhing induced by 0.6 % acetic acid. The number of writhing in 40- and 80-mg/kg GB1-treated groups were significantly lower than writhing in mice treated with distilled water. After 15-min latency period following oral GB1 (40 and 80 mg/kg), there was significant increase in tail withdrawal time compared to control rats. The tail withdrawal time at 30, 45, and 60 min were significantly prolonged in GB1 (20, 40, and 80 mg/kg) treated mice. GB1 (20, 40, and 80 mg/kg) groups had significantly lower paw volumes at 2 and 3 h post carrageenan injection. Also paw volume were significantly lower in GB1 (40 and 80 mg/kg) groups at 18 h post arthritis induction. By day 30 paw volumes of all GB1 groups were significantly lower than paw volume of control group. Rectal temperatures of GB1 (80 mg/kg)-treated group were significantly lower at 1, 2, 3, 4, and 5 h post brewer's yeast injection compared to rectal temperatures of control and 20-mg/kg GB1 groups. These finding shows that GB1 may serve as an alternative to NSAIDs in clinical practice.

Keywords Analgesic · Anti-inflammatory · Anti-pyretic · Garcinia hydroxybiflavanonol (GB1) · *Garcinia kola*

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Introduction

The inflammatory response is typically characterized by heat, swelling, hyperaemia, and pain (Kahn 2002). Fever may also occur due to inflammation (Rao et al. 2002; Roth et al. 2006). Conventionally, to manage these symptoms of inflammation, two major groups of drugs namely the corticosteroids and non-steroidal anti-inflammatory drugs are used (Choi and Hwang 2004). The non-steroidal anti-inflammatory drugs are most widely used (Snow 1981; Kyei et al. 2012). NSAIDs possess anti-pyretic, analgesic, and anti-inflammatory properties (Lascelles et al. 2007; Sparkes et al. 2010). Despite the usefulness of these agents, their prolonged use elicits numerous side effects (Choi and Hwang 2004). For example, due to inhibition of prostaglandin synthesis, the use of NSAIDs may lead to gastrointestinal ulceration, bleeding disorders, and hepatopathy (O'Connor et al. 2003; Lin et al. 2004; Coruzzi et al. 2007). Also the corticosteroids may cause immunosuppression (Kahn 2002). These side effects of steroids and NSAIDs restrict their long-term use. Therefore, herbal remedies with little side effects are often resorted to as alternative therapeutics (Choi and Hwang 2004).

In rural areas of Nigeria, alternative medicines such ad decoctions prepared from aerial parts, roots, and stem barks

of plants are also used in the treatment of inflammation, pain, and fever. These extracts have also been shown to exert anti-inflammatory effects without causing GIT ulceration, a notable side effect of NSAIDs (Muruganandan et al. 2001; Hajare et al. 2001). Based on this finding and those of other empirical studies, these crude drugs are considered safe and efficacious alternatives to conventional agents. These earlier findings have stimulated more interest in screening of more medicinal plant for anti-inflammatory and analgesic activities.

Garcinia kola (Bitter kola) is referred to as a "wonder plant" because almost every part of it has medicinal effect (Ijomone et al. 2012). In traditional medicine, it is used in the treatment of liver disorders, gastritis, headache, jaundice, fever, and stomach ache (Madubunyi 1995; Ijomone et al. 2012). Several researches have authenticated some of these acclaimed medicinal effects of *G. kola.* Kolaviron, a bioflavonoids from *G. kola* exhibited hepatoprotective (Farombi et al. 2005), hypocholesterolemic (Nwaneri et al. 2010), nephroprotective (Adaramoye 2009), and neuroprotective (Ijomone et al. 2012) activities. Garcinia hydroxybiflavanonol (GB1) has been reported to possess wound healing property (Nwaehujor et al. 2013). To the best of our knowledge, the anti-inflammatory effect of GB1 have not been ascertained, thus the need for this study.

Materials and method

Source of plant material and identification

Mature seeds of *G. Kola* were purchased from a local Market in Calabar. The seeds were identified by Dr. Michael Ekpo of the Department of Botany, University of Calabar, Nigeria. A voucher specimen was deposited in the herbarium of the Department of Biochemistry, University of Calabar (BCM/UNICAL/887/2011).

Extraction and fractionation of crude extract

The fresh seeds of *G. kola* were dried at room temperature and reduced to coarse powder by grinding. 1 kg of the pulverized plant material was defatted with 3 L of n-hexane in a Soxhlet apparatus (Büchi, Switzerland) for 24 h. The n-hexane was distilled off to afford 15.7 g of a yellowish-brown oily sample. The fat-free plant material was then extracted with 80 % methanol for 72 h. This was concentrated with a rotary evaporator to afford 140.2 g of methanol extract (brown sticky gum). 100 g of the methanol extract was suspended in distilled water and subjected to liquid–liquid partitioning with ethyl acetate (EA) to give 17 g of the EA fraction.

Isolation of Garcinia hydroxybiflavanonol (GB1)

This was done according to the methods of Nwaehujor et al. (2013). Briefly, 12 g of the EA fraction was subjected to column chromatography over silica gel 60F with $CHCl_3$: MeOH: H_2O (14:9:4), $CHCl_3$: MeOH: H_2O (64:50:10), and MeOH as solvent systems to give three major fractions coded fractions A (17 mg), B (270 mg), and C (36 mg). Fraction B was subjected to a combination of thin layer chromatography over silica gel 60 F_{254} and column chromatography using $CHCl_3$:$(CH_3)_2CO$:HCOOH (9:2:1) as solvent system to afford nine compounds which were further purified by washing in 1 % HCl and concentrated *in vacuo* for 72 h over P_2O_5/KOH to obtain compounds coded 2.2 mg B1, 70 mg B2, 0.2 mg B2a, 1.2 mg B2b, 0.5 mg B2c, 220 mg B3, 7.8 mg B4, 3.0 mg B5, and 1.6 mg B6. These compounds were allowed to dry into crystals using a freeze drier.

Experimental

All experiments with animals were approved by the Ethical Committee of the University of Calabar, Nigeria prior to the commencement of the various tests.

Animals

Male Swiss albino mice (23–34 g), albino Wistar rats of both sexes (125–170 g), and white rabbits of both sexes (1.35–2.1 kg) were used. They were housed in standard environmental conditions and fed with standard rodent diet and water ad libitum.

Anti-nociceptive activity

Writhing test

Writhing was induced in mice ($n = 5$) by a single intraperitoneal injection (10 ml/kg) of 0.6 % acetic acid. The numbers of writhing were counted over a 20-min period as described by Koster et al. (1959). Animals were treated orally 30 min before injection of acetic acid with Garcinia hydroxybiflavanonol, GB1 (20, 40, and 80 mg/kg) and acetylsalicylic acid (200 mg/kg) all dissolved in 1 % suspension of Tween 20. Control group received only distilled water (3 ml/kg) of 1 % suspension of Tween 20.

Tail immersion test

Six mice per group were administered orally with Garcinia hydroxybiflavanonol, GB1 (20, 40, and 80 mg/kg) dissolved

in 1 % Tween 20, pentazocine (30 mg/kg), and distilled water (3 ml/kg) in 1 % Tween 20, respectively. The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0 °C. The time taken to withdraw the tail was noted as reaction time (Dash et al. 2004). A cut-off time of 10 s was maintained at 55 °C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45, and 60 min after treatment, respectively.

Anti-inflammatory studies

Carrageenan-induced rat paw edema

Six rats of both sexes per group were treated orally with either Garcinia hydroxybiflavanonol, GB1 (20, 40, and 80 mg/kg) dissolved in 1 % Tween 20, Diclofenac (12.5 mg/kg), or distilled water (3 ml/kg) suspension in 1 % Tween 20, 60 min prior to an injection of 1 % carrageenan (Ramchandran et al. 2002) into the plantar tissue of the right hind paw. The contra-lateral hind paws were injected with 0.1 ml of normal saline as control. Paw edema volume was measured using a plethysmometer at 0, 1, 2, and 3 h after carrageenan injection.

Adjuvant-induced polyarthritis

The arthritic syndrome was induced in rats by an injection of 0.1 ml of Freund's complete adjuvant into the sub-plantar region of the right hind paw according to methods of Ramchandran et al. (2002). Animals were orally treated with either Garcinia hydroxybiflavanonol, GB1 (20, 40, and 80 mg/kg) dissolved in 1 % Tween 20, Diclofenac (12.5 mg/kg), or distilled water (3 ml/kg) for 30 days. Plethysmographic determination of paw volume was performed on both injected and contra-lateral foot. Paw volume after 18 h of adjuvant injection was taken as sub-acute phase of inflammation and that of the 30th day was observed as an index of chronic inflammation.

Anti-pyretic activity

The test was performed as described by to Lu et al. (2004) on rabbits of both sexes. An aliquot of 3 ml/kg of 10 % yeast suspension was subcutaneously injected into the rabbits' back. After 5 h, animals showing at least an increase 1 °C of rectal temperature were selected for the experiment. The test animals were administered with either Garcinia hydroxybiflavanonol, GB1 (20, 40, and 80 mg/kg) dissolved in 1 % Tween 20, acetylsalicylic acid (200 mg/kg), or distilled water (3 ml/kg) orally. Rectal temperature was measured at 1, 2, 3, 4, and 5 h after treatment using a digital thermometer.

Statistical analysis

The results were analyzed statistically using one way ANOVA followed by Dunnett's *t* test. Probability less than 0.05 were considered significant. SPSS version 16 software was used for the analysis.

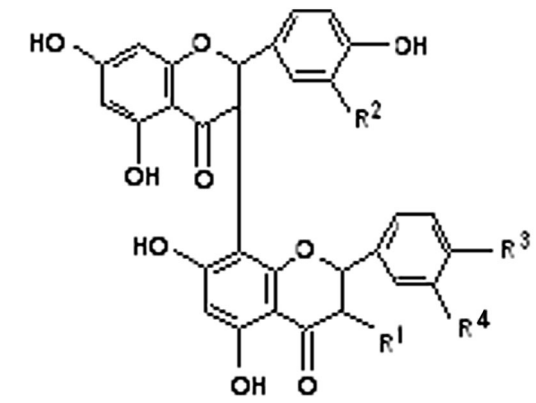
Results

Identification of the isolate

Garcinia hydroxybiflavanonol, GB1 was identified as previously described by Madubunyi (1983) and Sonnenbichler et al. (1986), as shown in Fig. 1.

Anti-nociceptive studies

GB1 reduced writhing induced by 0.6 % acetic acid. The number of writhing in 40- and 80-mg/kg GB1-treated groups were significantly lower than writhing in mice treated with distilled water. After 15-min latency period following oral GB1 (40 and 80 mg/kg), there was significant increase in tail withdrawal time compared to control rats (Table 1). The tail withdrawal time at 30, 45, and 60 min were significantly prolonged in GB1 (20, 40, and 80 mg/kg)-treated mice (Table 2).



	R1	R2	R3	R4
GB1	OH	H	OH	H

Fig. 1 Structure of Garcinia hydroxybiflavanonol (GB1)

Table 1 Effect of Garcinia hydroxybiflavanonol, GB1 on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	Number of writhings
GB1	20	34.2 ± 2.4
	40	29.4 ± 3.0*
	80	22.31 ± 2.2*
Acetylsalicylic acid	200	24.4 ± 2.3*
Distilled water	–	52.7 ± 4.0

Values are expressed as mean ± S.E.M.; $n = 5$

* $p < 0.01$ compared with negative control

Anti-inflammatory studies

GB1 (20, 40, and 80 mg/kg) groups had significantly lower paw volumes at 2 and 3 h post carrageenan injection (Table 3). Also paw volume were significantly lower in GB1 (40- and 80-mg/kg groups at 18 h post arthritis induction. By day 30 paw volumes of all GB1 groups were significantly lower than paw volume of control group (Table 4).

Anti-pyretic study

Rectal temperatures of GB1 (80 mg/kg)-treated group were significantly ($p < 0.05$, $p < 0.01$) lower at 1, 2, 3, 4, and 5 h post brewer's yeast injection compared to rectal temperatures of control and 20-mg/kg GB1 groups (Table 5).

Discussion

The acetic acid-induced writhing and tail withdrawal tests were performed to investigate peripheral and central analgesic activities of GB1. This study indicated that GB1 has both peripheral and central analgesic properties. In the writhing test, acetic acid causes inflammatory pain by increasing capillary permeability and eliciting release of endogenous pain mediators (Couture et al. 2001; Choi and Hwang 2004). In the tail immersion test, thermal stimulus is applied and the reaction time to thermal stimulus is considered an important parameter for evaluating central anti-nociceptive effect (Gill et al. 2011). In the acetic acid-induced writhing test, comparison between GB1 and Acetylsalicylic acid showed that GB1 (80 mg/kg) was as potent as ASA (200 mg/kg), while in the tail immersion test, GB1 was as potent as pentazocine, an agonist–antagonist opioid. Thus, it might be assumed that like acetylsalicylic acid and pentazocine, GB1 inhibited peripheral and central pain by blocking the effect of prostaglandin and spinal reflex, respectively.

Screening of GB1 for anti-inflammatory effect using the carrageenan-induced paw edema test and adjuvant-induced arthritis showed that GB1 (80 mg/kg) possessed anti-inflammatory activity. This compound by inhibiting carrageenan-induced inflammation might have inhibited histamine, serotonin, and prostaglandins release in paw tissues (Sini et al. 2010). Diclofenac, an NSAID, exhibited similar

Table 2 Effect of Garcinia hydroxybiflavanonol, GB1 on tail immersion in mice

Treatment	Dose (mg/kg)	Average tail-withdrawing time (min)				
		0 min	15 min	30 min	45 min	60 min
GB1	20	3.64 ± 0.44	4.47 ± 0.11	5.21 ± 0.24*	6.22 ± 0.36**	5.85 ± 0.27*
	40	3.78 ± 0.30	5.25 ± 0.38**	6.88 ± 0.37**	7.48 ± 0.36**	7.71 ± 0.37**
	80	3.81 ± 0.18	5.63 ± 0.41**	7.63 ± 0.31**	7.84 ± 0.17**	8.18 ± 0.11**
Pentazocine	30	3.75 ± 0.15	6.02 ± 0.30	7.88 ± 0.38**	8.04 ± 0.30**	8.47 ± 0.41**
Distilled water	–	3.83 ± 0.11	3.88 ± 0.11	3.89 ± 0.13	3.91 ± 0.12	3.95 ± 0.14

Values are expressed as mean ± S.E.M.; $n = 5$

* $p < 0.05$; ** $p < 0.01$ compared with negative control

Table 3 Effect of Garcinia hydroxybiflavanonol, GB1 on carrageenan-induced rat paw edema

Treatment	Dose (mg/kg)	Paw volume (ml)			
		0 h	1 h	2 h	3 h
GB1	20	0.34 ± 0.01	0.48 ± 0.02	0.43 ± 0.03*	0.40 ± 0.02*
	40	0.31 ± 0.02	0.44 ± 0.01	0.40 ± 0.01*	0.35 ± 0.01*
	80	0.33 ± 0.01	0.44 ± 0.03*	0.35 ± 0.02*	0.32 ± 0.03*
Diclofenac	12.5	0.35 ± 0.01	0.41 ± 0.02*	0.33 ± 0.02*	0.26 ± 0.01*
Distilled water	–	0.33 ± 0.01	0.53 ± 0.01	0.55 ± 0.02	0.58 ± 0.02

Values are expressed as mean ± S.E.M.; $n = 5$

* $p < 0.01$ compared with negative control

Table 4 Effect of Garcinia hydroxybiflavanonol, GB1 on Adjuvant-induced paw arthritis in rats

Treatment	Dose (mg/kg)	Paw volume (ml)		
		0 h	18 h	Day 30
GB1	20	0.32 ± 0.02	0.77 ± 0.01	0.60 ± 0.02**
	40	0.33 ± 0.01	0.67 ± 0.02*	0.54 ± 0.01**
	80	0.31 ± 0.02	0.58 ± 0.01**	0.46 ± 0.02**
Diclofenac	12.5	0.33 ± 0.01	0.54 ± 0.03**	0.43 ± 0.01**
Distilled water	–	0.33 ± 0.03	0.82 ± 0.02	0.77 ± 0.02

Values are expressed as mean ± S.E.M.; $n = 5$

* $p < 0.05$; ** $p < 0.01$ compared with negative control

Table 5 Effect of Garcinia hydroxybiflavanonol, GB1 on yeast-induced pyrexia in rabbits

Treatment	Dose (mg/kg)	Average rectal temperature (°C)					
		0 h	1 h	2 h	3 h	4 h	5 h
GB1	20	41.7 ± 0.1	41.2 ± 0.1	39.3 ± 0.2	38.5 ± 0.2	39.2 ± 0.1	38.3 ± 0.2
	40	42.2 ± 0.2	40.5 ± 0.1	37.6 ± 0.3	37.2 ± 0.3	37.9 ± 0.2	37.9 ± 0.3*
	80	41.6 ± 0.2	39.1 ± 0.2*	34.8 ± 0.1**	35.1 ± 0.2**	34.9 ± 0.1*	35.5 ± 0.2**
Acetylsalicylic acid	200	41.9 ± 0.1	36.7 ± 0.3**	34.7 ± 0.2**	34.6 ± 0.3**	34.4 ± 0.2**	34.8 ± 0.2**
Distilled water	–	42.1 ± 0.3	42.4 ± 0.2	41.6 ± 0.2	40.7 ± 0.1	40.8 ± 0.1	41.0 ± 0.3

Values are expressed as mean ± S.E.M.; $n = 5$

* $p < 0.05$; ** $p < 0.01$ compared with negative control

activity showing that both compounds might have the same mechanism of action.

Fever occurs due to the body's response to infection, tissue damage, or inflammation (Romanovsky et al. 2005; Roth et al. 2006). Prostaglandins sensitize the skin to painful stimuli and also function as the final fever mediators (Li et al. 2008). Thus, to increase prostaglandin synthesis, brewer's yeast is injected subcutaneously (Moltz 1993; Muhammad et al. 2012). NSAIDS such as Diclofenac and acetylsalicylic acid inhibit cyclo-oxygenase expression in peripheral tissues thereby interfering with prostaglandin synthesis, thus are used as anti-pyretic agents (Hinz et al. 2008; Lawal et al. 2010). It was interesting to observe that GB1 exhibited analgesic, anti-inflammatory and anti-pyretic activity, therapeutic effects which are only exhibited by NSAIDS. This finding provides further evidence showing that GB1 inhibited prostaglandin synthesis.

The results obtained validate the traditional use of *Garcinia kola* in treating fever and pain resulting from headache, stomach ache, as well as other inflammatory disorders such as gastritis and laryngitis. These findings show that GB1 may serve as an alternative to NSAIDs in clinical practice.

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