Journal of Science and Practice of Pharmacy
December 2015; 2 (1): 46-50 Available at http://www.jsppharm.org
ISSN: 2449-0458 (print); 2449-0466 (electronic)

Original Research Article

Evaluation of the analgesic property of the ethanolic extract of *Garcinia kola* Heckel (Guttiferae) seeds in mice

Loretta O Iniaghe[1*] and Agatha I Onyemaonyeoru[2]
[1]Department of Pharmacology & Toxicology, University of Benin, Nigeria, [2]Department of Science Laboratory Technology, University of Benin, Nigeria

*For correspondence: *Email:* lo.iniaghe@uniben.edu *Tel:* +2348022113816

Abstract

Purpose: *Garcinia kola* Heckel (Guttiferae) is used in Ethnomedicine for the management of pain, respiratory infections, inflammation and food poisoning. This study was undertaken to evaluate the analgesic property of the ethanolic extract of *Garcinia kola* seeds in mice.
Methods: The oral lethal dose (LD_{50}) of ethanolic extract of *Garcinia kola* seeds was estimated and the extract was screened for analgesic activity orally using the hot plate and acetic acid-induced mouse writhing tests in mice at graded doses of 100, 200 and 400 mg/kg and compared with standard analgesic agents- Acetylsalicylic acid and Morphine.
Results: The LD_{50} of *Garcinia kola* was estimated to be more than 5000 mg/kg. In the acetic acid-induced test, a significant ($p<0.05$) reduction in the number of writhes compared to control animals was observed at all doses of the extract (control, 45.73 ± 3.6; 100mg/kg 24.98 ± 2.48; 200mg/kg 20.08 ± 5.59; 400mg/kg 16.39 ± 4.25 and Acetylsalicylic acid 6.97 ±2.87). Similarly, a significant increase ($p<0.05$) in the latency time spent on the hot plate at different intervals post treatment ranging from 59.62 to 81.67%, 108.37 to 156.26 and 48 to 78.04% respectively for 100, 200 and 400mg/kg dose levels of *Garcinia kola* was recorded in animals used for this study. Latency periods for control groups were not significantly increased (5.83 to 25.77%) while Morphine treated animals gave an increase in latency time of 122.76 to 158.29%.
Conclusion: *Garcinia kola* seeds possess analgesic property which may account for their use in Ethnomedicine.

Keywords: *Garcinia kola*, hot plate, acetic acid, mouse writhing, pain.

Indexing: Index Copernicus, African Index Medicus

Introduction

Acute and chronic pain control is now a major concern especially with the aged population. Pain is an unpleasant sensory and emotional experience associated with actual potential tissue damage, or described in terms of such damage and is a non-specific manifestation of many diseases. Although non-steroidal anti-inflammatory drugs and opiates are used the management of pain, they are associated with adverse drug reactions such as gastrointestinal disturbances, renal toxicity, respiratory depression, and possible dependence [1-4]. The search for analgesic agents with minimal side effects has once again led scientists to Mother Nature - sourcing for compounds from natural sources and medicinal plants. In many countries of the world, herbal and traditional medicines are often employed in the treatment of a myriad of illnesses. The importance of the medicinal plants in drug discovery and development cannot be overemphasized hence the World Health Organization advocates the inclusion of safe and effective herbal medicines in health care programmes of developing countries [5,6].

Garcinia kola commonly called 'Bitter kola' is regarded as 'a wonder plant' as almost every part of the plant is useful. It is found in many countries of tropical rain forest of West Africa such as Benin Republic, Cameroon, Democratic Republic of the Congo (DRC), Cote D'Ivoire Coast, Gabon, Ghana, Liberia, Nigeria, Senegal and Sierra Leone. Garcinia kola is also found in Central African Republic, subtropical or tropical moist lowland forests. Local

names for *Garcinia kola* in Nigeria include *Namijin-goro* (Hausa), *Orogbo* (Yoruba) and *Akilu* (Igbo) [7-11]. The seeds of *Garcinia kola* are used in the treatment of bronchitis, common cold, throat infections, pain, diarrhoea, asthma, dysmenorrhoea, gonorrhoea, colic, hepatitis and as an antidote to food poisoning [12-17]. *Garcinia kola* has been shown to possess antimicrobial, anti-diabetic, antiviral and antiulcer properties [18,19]. Anti-inflammatory, analgesic, antipyretic and central nervous system depressant effects of the stem bark and flavonoids isolated from *Garcinia kola* have also been evaluated [20-24]. Since no documented studies on the analgesic activity of *Garcinia kola* was found in existing literature, we investigated the analgesic activity of the ethanolic extract of *Garcinia kola* in mice.

Experimental

Drugs/Chemicals

Morphine (Sterop, Belgium), Acetylsalicylic Acid (SKG-Pharma Limited), Ethanol (Sigma Aldrich, UK), Acetic acid (Sigma Aldrich, UK) were used in this study.

Plant materials

Garcinia kola seeds purchased from the local herbs market in Benin City were identified and authenticated by Pharm. H.O. Uwumarongie and Mr. S. Nweke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State where a voucher specimen (A009) was deposited for future reference. They were air dried, reduced to fine powder by milling and stored in air tight containers. Four hundred and fifty grams was extracted by maceration method using 70% ethanol. The resulting filtrate which was concentrated by evaporation over a water bath yielded a brown residue. The percentage yield was 4.62%

Animals

Swiss Albino mice weighing 25-35 g procured from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin-City, Edo State, Nigeria were used for the study. They were kept in polypropylene cages at the Animal House of the Department of Pharmacology and Toxicology, University of Benin. The animals were maintained under standard laboratory conditions with access to animal feed and clean water *ad libitum*. Handling of the animals was done according to standard protocols for the use of laboratory animals of the National institute of Health [25].

Acute Toxicity Study in Mice

Determination of oral acute toxicity of *Garcinia kola* seeds was carried out using the method described by Lorke [26] and modified by Builders *et al.* [27]. In the first phase, nine mice randomly distributed into three groups (n=3) were orally administered 10, 100 and 1000 mg/kg of *Garcinia kola* respectively and observed for signs of toxicity and death for 24 hours, systematically at 1, 2, 6, 12 and 24 hours after drug administration. Based on the outcome of this phase, 1600, 2900 and 5000 mg/kg *Garcinia kola* were administered to a fresh batch of animals (n=3) and observed for number of deaths for 24 hours. Other symptoms such as skin changes, restlessness, gnawing and sedation were also recorded. The median lethal (LD_{50}) dose based on mortality was calculated as the geometric mean of the lowest lethal dose at which the animal died and the highest non-lethal dose at the animal survived.

Test for Analgesia

Acetic Acid Induced Mouse Writhing Assay

The method of Koster *et al.,* [28] was used. Twenty five mice were randomly allotted into 5 groups of 5 animals per group. Group I received 1% Tween 80 solution orally administered via an oro-gastric tube while groups II to IV received *Garcinia kola* extract orally at graded doses of 100, 200 and 400 mg/kg respectively. Group V received 100 mg/kg acetylsalicylic acid orally [29]. Thirty minutes post drug administration, acetic acid (0.2 ml of 0.6 %v/v) was administered intraperitoneally. Abdominal writhes were cumulatively counted for 30 minutes following acetic acid injection.

Hot Plate Test

Twenty five mice were divided randomly into 5 groups of 5 animals each. Each animal was trained on the hot plate (Ugo Basile Model no 35100-001 Comerio, Italy) prior to drug administration. The latency time spent on the hot plate prior to drug administration was also determined. The extract (100, 200 and 400 mg/kg), and 1% Tween 80 solution were administered orally while morphine, 2 mg/kg [29] was administered subcutaneously to mice in the test groups. Thirty minutes post treatment (15 min for the morphine group), the animals were dropped gently on the hot plate maintained at 55 ± 0.5 °C, with the cut off time set at 60 seconds. The time (in seconds) taken for the mouse to jump or lick its paw, raise its hind paw or hold the paw tightly against the body was taken as the reaction time. Latency time on the hot plate was measured at an interval of thirty minutes for

a period of ninety minutes. Each animal was used as its own control [30].

Statistical Analysis

Results expressed as mean ± SEM were statistically analyzed using one-way ANOVA followed by the Dunnet's test. A probability value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Graph Pad Instat version 5.0

Results

The oral LD_{50} of *Garcinia kola* seed extract was estimated to be greater than 5000 mg/kg (Table 1).

Table 1: Oral LD_{50} for ethanolic extract of *Garcinia kola* seeds in mice

Groups	N	Dose (mg/kg)	% Mortality
Phase I			
1	3	10	0
2	3	100	0
3	3	1000	0
Phase II			
1	3	1600	0
2	3	2900	0
3	3	5000	0

Treatment with *Garcinia kola* significantly ($p<0.05$) reduced the number of writhes in the acetic acid induced mouse writhing assay in a dose dependent manner with the highest dose level (400 mg/kg) giving the least number of writhes. Acetyl salicylic acid produced a significant decrease in total number of writhes recorded. Data is presented in Table 2.

In the hot plate test, increase in latency time was also evident at all three dose levels compared to base line (pre-treatment) values. Data is shown in Table 3.

Discussion

This study was carried out to evaluate the analgesic potential of the ethanolic extract of *Garcinia kola* seeds in mice. The oral LD_{50} was estimated to be more than 5000 mg/kg showing that the extract is relatively safe for use. The highest dose used in the study was less than 30% of the LD_{50} which is considered safe and recommended for use in Ethnopharmacological assays [31,32].

Table 2: Effect of ethanolic extract of *Garcinia kola* in the acetic induced mouse writhing test

Treatment	Dose (mg/kg)	No of writhes	% Inhibition
Tween 80	-	$45.73 \pm 3.6^{\#}$	-
Garcinia kola	100	$24.98 \pm 2.48^{*\#}$	45.38
	200	$20.08 \pm 5.59^{*}$	56.09
	400	$16.39 \pm 4.25^{*}$	64.59
Acetyl salicylic acid	100	$6.97 \pm 2.87^{*}$	84.76

Data are expressed as mean ± SEM. *$p<0.05$ compared to 1% Tween 80, # $p< 0.05$ compared to acetylsalicylic acid, n=5

Acetic acid induced mouse writhing assay is one of the most commonly used test for assessing compounds which can modulate peripherally mediated pain. Acetic acid is thought to induce the release of prostaglandins E_2 and $F_{2\alpha}$ and lipooxygenase products which sensitize nociceptive neurones in the peritoneum. Non-steroidal anti-inflammatory are able to reduce acetic acid induced pain shown as a reduction in the number of writhes seen due to their inhibitory action on the synthesis of prostaglandins [33-35]. The ability of *Garcinia kola* to reduce number of writhes produced by acetic acid is indicative of peripherally mediated analgesia.

In the hot plate test an increase in latency time was observed for mice treated with all three dose levels of *Garcinia kola*. Compounds which increase reaction time have been demonstrated to be centrally acting analgesic agents via elevation of the pain threshold of animals towards heat and pressure [36,37]. From the results of the hot plate and mouse writhing tests, the mechanism of action of *Garcinia kola* in ameliorating pain may be a combination of both peripheral (inhibition of prostaglandin synthesis) and central (elevation of pain threshold to heat) mechanisms.

The results from this study and those of Olaleye *et al.,* [20] Kagbo [21] and Ibironke and Fasanmade, [23] lend credence to the analgesic property of *Garcinia kola.*

Table 3: Effect of ethanolic extract of *Garcinia kola* in the hot plate test

Treatment	Pre-treatment t_0 (secs)	30 minutes post treatment (secs)	60 minutes post treatment (secs)	90 minutes post treatment (secs)
Tween 80	9.94 ± 2.28	12.90 ± 2.21	10.52 ± 1.76	11.33 ± 2.17
Garcinia kola 100 mg/kg	9.06 ± 1.87	$15.73 \pm 2.33^{*}$	$14.46 \pm 2.67^{*}$	$16.46 \pm 2.15^{*}$
Garcinia kola 200 mg/kg	8.60 ± 1.22	$17.92 \pm 2.04^{*}$	$22.10 \pm 2.50^{*}$	$19.48 \pm 1.38^{*}$
Garcinia kola 400 mg/kg	11.84 ± 1.09	$21.08 \pm 1.17^{*}$	$17.53 \pm 1.47^{*}$	$18.69 \pm 2.50^{*}$
Morphine 2 mg/kg	7.6 ± 1.10	$19.63 \pm 0.87\#^{*}$	$17.86 \pm 0.76^{*}$	$16.93 \pm 0.80^{*}$

Data are expressed as mean ± SEM, *$p<0.05$ compared to pre-treatment (t_0), n=5, #15 minutes post treatment

Conclusion

The results obtained from this study indicate that the ethanolic extract of *Garcinia kola* seeds possesses analgesic activity which maybe mediated via central and peripheral mechanisms may account for its in Ethnomedicine as an analgesic agent.

Declarations

Acknowledgement

The authors are grateful to Mr. I. Ibeh who took care of the animals during the course of the study.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. LOI designed the study; AIO sourced for the plant and carried out the extraction. Pharmacological experiments were carried out by LOI and AIO. Manuscript was approved by all the authors.

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