

Evaluation of the protective and ameliorative properties of *Garcinia kola* on histamine-induced bronchoconstriction in guinea pigs

Mina T. Ibulubo, Gerald I. Eze, Raymond I. Ozolua¹, Doroteo Baxter-Grillo, Dickson O. Uwaya¹

Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, ¹Department of Pharmacology and Toxicology, School of Pharmacy, University of Benin, Benin City, Nigeria

Submitted: 05-12-2011

Revised: 23-01-2012

Published: 11-10-2012

ABSTRACT

Background: *Garcinia kola* is popularly used in African traditional medicine for the relief of acute bronchoconstrictive episodes. **Objective:** In this study, we examined the anti-asthmatic and morphological effects of the ethanol extract of *G. kola* in animal model. **Materials and Methods:** Guinea pigs were sensitized with ovalbumin and then given doses of 200 or 400 mg/kg/day for 21 consecutive days. Theophylline (10 mg/kg/day) was used as a standard. At the end of the exposure, the animals were exposed to 0.2% histamine aerosol in a chamber. Lymphocyte count, bronchial histology and morphometry were done. **Results:** Compared with non-sensitized controls, 200 mg/kg/day dose of the extract significantly ($P < 0.05$) increased the time taken for onset of preconvulsive dyspnea while the dose of 400 mg/kg/day significantly ($P < 0.01$) reduced bronchial wall thickness. Lymphocytes counts were not significantly affected but the bronchi of extract-treated animals were histologically clearer of lesions visible in the sensitized. **Conclusion:** These protective and ameliorative properties lend credence to the use of *G. kola* in ethnomedicine.

Key words: Asthma, bronchial histology, bronchial morphometry, *Garcinia kola*

Access this article online

Website:

www.phcogres.com

DOI:

10.4103/0974-8490.102262

Quick Response Code:

INTRODUCTION

Bronchial asthma is a syndrome characterized by increased responsiveness of trachea and bronchi to various stimuli and manifests as acute, recurrent and chronic attacks of widespread narrowing of airways.^[1] It is a global health problem that results from a complex interplay between genetic and environmental factors.^[2] Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome affecting about 7-10% of world population.^[3] The relief offered by the available wide range of orthodox drugs is mainly symptomatic and short-lived. Moreover, these drugs produce side effects. Therefore, there is a dire need to identify more effective and safer remedies for the treatment of bronchial asthma.^[4]

Herbal medicines are being used by about 80% of the world population, primarily in developing countries for

primary health care.^[5] Treatment of respiratory tract diseases with orthodox drugs has taken a new dimension as more and more individuals (both literates and illiterates) have frequently substituted these drugs with traditional herbal remedies.

Garcinia kola (bitter kola) is used in Nigeria as a bronchodilator for the treatment of asthma.^[6,7] It is also known to relax the smooth muscles of the uterus and intestines which may be due to its alkaloid and biflavonoids constituents.^[8] Other traditional African medicinal uses include treatment of cough, constipation, and parasitic and microbial infections.^[9-11] In mice, extracts from *G. kola* have been shown to exhibit dilatory effect on alveolar ducts and sacs, thereby improving respiratory activities, which may be due to its antioxidant properties.^[12]

Despite its acclaimed effect on the respiratory system, there have been no scientific studies on whole animals that include its possible morphological effects on the respiratory smooth muscle. We therefore designed this study to evaluate the anti-asthmatic and morphological effects of ethanol extract of *G. kola* in an animal model.

Address for correspondence:

Dr. R. I. Ozolua, Department of Pharmacology and Toxicology, University of Benin, Benin City-300001, Nigeria.
E-mail: ozolua@uniben.edu

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Fresh nuts of *G. kola* were purchased from a major market in Benin City and identified by Dr H.A. Akinnibosun of the Department of Botany and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. The nuts were peeled, chopped into pieces and sun-dried for 3 days. The dried pieces were later powdered using a mill and then extracted with 70% ethanol for 72 h followed by concentration over a warm water bath. The extract was later dried at 40°C using Gallenkamp (England) oven for another 72 h to give a yield of 54.8% w/w. The extract was stored in an amber-colored bottle at 4°C.

Drugs and Chemicals

Histamine dihydrochloride was procured from Sigma-Aldrich (Switzerland). Theophylline (Sigma, UK) was a kind donation by the Department of Pharmacology and Toxicology, University of Benin. Solutions of the two drugs solutions were freshly prepared by dissolving in water. All other chemicals and reagents were of analytical grade and were manufactured by reputable companies.

Animals

Adult guinea pigs weighing 422.3 ± 70.8 g (Mean \pm SD) of either sex were purchased from Aduwawa livestock market, Benin City. They were allowed acclimatization period of two weeks in the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin. The animals were fed throughout with guinea pig pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and elephant grass (*Pennisetumpurpureum*) with free access to drinking water. All experimental procedures followed the recommendations provided in the "Guide for the care and use of laboratory animals" (National Academy Press, 1996). The study was approved by the ethical committee of the School of Basic Medical Sciences, University of Benin.

Animal Grouping and Experimental Protocols

After the period of acclimatization, the guinea pigs were randomly allotted into 5 groups ($n = 5$ per group) and were treated orally for 21 consecutive days. The groups comprised of:

1. Non-ovalbumin sensitized control administered 2 ml/kg/day distilled water
2. Ovalbumin sensitized administered 2 ml/kg/day distilled water
3. Ovalbumin sensitized administered 200 mg/kg/day *G. kola* extract
4. Ovalbumin sensitized administered 400 mg/kg/day *G. kola* extract

5. Ovalbumin sensitized administered 10 mg/kg/day theophylline

Sensitization involved administering 100 mg/kg ovalbumin intraperitoneally on the first day and giving a booster dose of 50 mg/kg intramuscularly on the second day.^[13,14] Treatment of animals was begun on the first day of ovalbumin administration. On the 20th day, they were fasted overnight. The following day, they were treated 6 h before exposure to 0.2% histamine aerosol (Omron® compressor nebulizer, USA) at a rate of 0.4 ml/min with particle size of 5 μ m in a glass chamber (60 \times 36 \times 60 cm).^[13] The time of allergy response (from time of aerosol exposure to onset of preconvulsive dyspnea) caused by 0.2% histamine aerosol was recorded for each animal. After histamine aerosol exposure, blood samples for white blood cell count were collected from the abdominal aorta under chloroform anesthesia and bronchial tissues were collected from the euthanized animals and placed in 10% formaldehyde-in-saline solution. The tissues were subsequently processed and stained with hematoxylin and eosin dyes. Histological slides were examined using Olympus Optical microscope (Japan).

Statistical analysis

Data are expressed as mean \pm standard error of mean (SEM) and n represents the number of experimental animals (Guinea pigs) per group. Data were analyzed using Kruskal-Wallis ANOVA followed by Dunnet's post hoc test (Graph pad prism Software, UK). $P < 0.05$ was taken to denote statistically significant difference in all cases.

RESULTS

Table 1 shows that compared with Groups 1 and 2 (control and OA sensitized) the time taken for guinea pigs to experience preconvulsive dyspnea was significantly increased in the sensitized group given 200 mg/kg of the extract ($P < 0.01$) and the sensitized group given 10 mg/kg theophylline ($P < 0.05$). Compared with control, the group administered the higher dose of 400 mg/kg of the extract did not experience longer time before preconvulsive dyspnea. Table 1 also shows that lymphocyte count was not significantly different among the various groups. However, tracheal wall thickness was significantly ($P < 0.05$) reduced in sensitized group given 400 mg/kg compared with control. The measurements were taken as represented in Figure 1.

Figure 2 shows photomicrographs of bronchi tissue taken from the various groups. Ovalbumin caused marked thickening of the bronchial wall in Group 3 (Ovalbumin sensitized only) as evidenced by hypertrophied muscle

bundle, mild transmural edema and presence of luminal mucus plug in its bronchial wall [Figure 2c] when compared with the extract and theophylline treated groups [Figures 2c-e]. Theophylline and *G. kola* ameliorated these changes as there were no signs of hypertrophied

muscle bundle and no luminal mucus plug in the bronchial walls of the animals treated with them. However *G. kola* at 400 mg/kg/day (Group 5) was more effective as evidenced in the thin bronchial walls [Table 1].

DISCUSSION

In this study, ovalbumin sensitization caused bronchial inflammation as evidenced by edema, congestion, increased presence of lymphocytes, eosinophils, neutrophils, and increased mucus secretion into the lumen. The protocol also increased airway hyper-responsiveness as evidenced in faster onset of preconvulsive dyspnea.

In this study, the extract of *G. kola* ameliorated bronchial hyper-responsiveness. Although the specific effect of kolaviron,^[15] a biflavonoid constituent of *G. kola* on the airway, is not known with certainty, flavonoids possess antioxidant property that may underlie their effectiveness in asthma.^[16,17] Flavonoids also impair Ca²⁺ release and utilization mechanisms in smooth muscles.^[18,19] In addition, flavonoids inhibit antigen-induced release of histamine from mast cells, basophils and also inhibit



Figure 1: Measurement of the bronchial wall thickness. BL, bronchial lumen; BWT, bronchial wall thickness; CT, cartilage thickness; MT, mucosal thickness

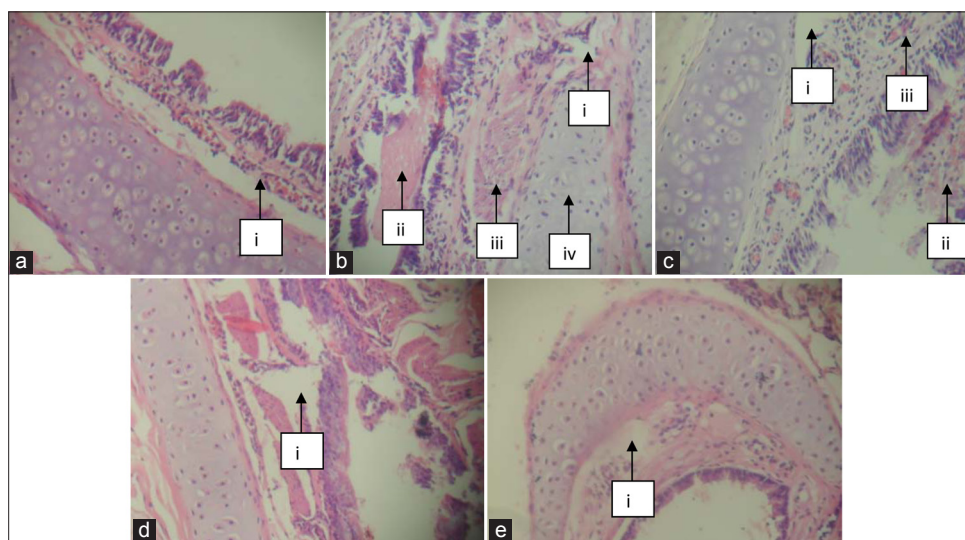


Figure 2: Guinea pig bronchi. (a) Non-sensitized control: (i) transmural edema, (ii) cartilage. (b) Ovalbumin-sensitized: (i) transmural edema, (ii) mucus plug, (iii) hypertrophied muscle, (iv) cartilage. (c) Theophylline-treated: (i) transmural edema, (ii) mucus plug, (iii) submucosal congestion (d) *G. kola* -200: (i) transmural edema (e) *G. kola*-400: (i) transmural edema. (x 100)

Table 1: Response to acute exposure to allergen, lymphocyte count and bronchial wall thickness in guinea pigs treated for 21 consecutive days with 70% ethanol extract of *G. kola*

Groups	Allergy response (min)	Lymphocyte count (x 10 ³ /μl)	Bronchial wall thickness (cm)#
Non-OA Sensitized	1.91 ± 0.21	5.22 ± 0.40	13.00 ± 0.70
OA Sensitized	1.40 ± 0.05	4.60 ± 0.56	14.30 ± 1.01
OA Sensitized + 200 mg/kg/day extract	3.42 ± 0.36**	8.62 ± 1.30	11.90 ± 1.27
OA Sensitized + 400 mg/kg/day extract	2.51 ± 0.36	6.66 ± 0.92	9.40 ± 1.11*
OA Sensitized + 10 mg/kg/day Theo	3.11 ± 0.40*	8.88 ± 2.47	12.50 ± 0.55

*P < 0.05, **P < 0.01 compared with non-OA sensitized. OA, ovalbumin; Theo, theophylline. # H and E x100. n = 5 per group

contractions induced by histamine, acetylcholine and phosphodiesterase.^[20] Flavonoids have also been shown to inhibit phospholipid metabolism through the 5-lipoxygenase (5-LO) pathway, thereby inhibiting the products of 5-LO that mediate constriction of airway smooth muscles.^[21,22] These may be the reasons behind the morphological changes in the extract-treated groups. Theophylline on the other hand causes bronchodilatation by accumulation of cyclic adenosine monophosphate (cAMP) through phosphodiesterase enzyme inhibition.^[23] *G. kola* has been reported to exhibit dilatatory effect in alveolar ducts, alveolar sacs and alveoli, thereby improving respiratory activities in experimental animals.^[12] The physiological mechanisms underlying the use of *G. kola* in the treatment of asthma have been studied and results suggest its potentials in the management of asthma.^[24] Our histological and bronchial wall morphometry findings agree with these previous reports.

The role of histamine in asthma is well established.^[25] The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. Although there are various models of asthma, guinea pig airways react to histamine, acetylcholine, leukotrienes and other bronchoconstrictors in a manner similar to that seen in humans.^[1] Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β -adrenergic antagonists.^[26] Thus the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release.

Our results show the effectiveness of *G. kola* in ameliorating symptoms of an animal model of bronchial asthma through inhibition of bronchoconstriction and reduction in morphological changes that may occur in the disease. While these effects may not be dose-dependent, they lend credence to the use of *G. kola* in ethnomedicine.

ACKNOWLEDGMENT

The authors are grateful to the laboratory staff of the Departments of Anatomy, and Pharmacology and Toxicology for their technical assistance. The study was part of the M. Sc thesis of ITM.

REFERENCES

- Agrawal DK, Bergren DR, Byorth PJ, Townley RG. Platelet-activating factor induces non-specific desensitization to bronchodilators in Guinea pigs. *J Pharmacol Exp Ther* 2002;257:1-7.
- Phillip F. Gene therapy for asthma. *Mol Ther* 2003;7:148-52.
- Sachin P, Amit G, Navin S. Evaluation of antiasthmatic activity of a polyherbal formulation containing four plant extracts. *J Curr Pharm Res* 2010;2:40-4.
- Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. *J Ethnopharmacol* 1999;66:205-10.
- Kamboj VP. Herbal medicine. *Curr Sci* 2000;78:35-9.
- Orie NN, Ekon EU. The bronchodilator effects of *Garcinia kola*. *East Afr Med J* 1993;70:143-5.
- Ebomoyi MI, Iyawe VI. Effects of *Garcinia conrauana* ingestion on airway resistance in a population of healthy adult Nigerians. *J Med Biomed Res* 2003;2:22-7.
- Braide VB. Antispasmodic extracts from seeds of *Garcinia kola*. *Fitoterapia* 1989;60:123-9.
- Adefule-Ositelu AO, Adefule AK, Oosa BO, Onyene PC. Antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in Lagos. *Nig Q J Hosp Med* 2004;14:112-4.
- Madubuyi II. Antimicrobial activities of the constituents of *Garcinia kola* seeds. *Internet J Pharmacogn* 1995;33:232-7.
- Okunji CO, Iwu MM. Molluscicidal activity of *Garcinia kola* biflavonones. *Fitoterapia* 1991;67:74-6.
- Ofusori DA, Ayoka AO, Adelakun AE, Falana BA, Adeeyo OA, Ajeigbe KO, et al. Microanatomical Effect of ethanolic extract of *Garcinia kola* on the lung of Swiss albino mice. *Internet J Pulm Med* 2008;10. DOI: 10.5580/297c
- Salari H, Yeung M, Howard S, Schellenberg RR. Increased contraction and inositol phosphate formation of tracheal smooth muscle from hyperresponsive guinea pigs. *J Allergy Clin Immunol* 1992;9:405-10.
- Bramley MA, Langlands JM, Jones KA, Burgoyne LD, Ya Li, Andersen JR, et al. Effects of izp-94005 (contignasterol) on antigen-induced bronchial responsiveness in ovalbumin-induced guinea pigs. *Br J Pharmacol* 1995;115:1433-8.
- Adaramoye OA, Medeiros IA. Endothelium-independent vasodilation induced by kolaviron, a biflavonoid complex from *Garcinia kola* seeds, in rat superior mesenteric arteries. *J Smooth Muscle Res* 2009;45:39-53.
- Tapiero H, Tew KD, Ba GN, Mathé G. Polyphenols: Do they play a role in the prevention of human pathologies? *Biomed Pharmacother* 2002;56:200-7.
- Raviv S, Smith LJ. Diet and asthma. *Curr Opin Pulm Med* 2010;16:71-6.
- Gordon E, Lazarus SC. Effects of hispidulin, a flavone isolated from *Inula viscosa* on isolated guinea-pig smooth muscle. *Gen Pharmacol* 1988;19:559-63.
- Ghayur MN, Gilani AH. Studies on cardio-suppressant, vasodilator and tracheal relaxant effects of *Sarcococca saligna*. *Arch Pharm Res* 2006;29:990-7.
- Simoies CM. Pharmacological investigations on *Achyrocline satureioides*. *J Ethnopharmacol* 1988;22:281-93.
- Dahren SE, Bjork J, Hedqvist P, Hammarstorm S, Samuel B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;288:484-6.
- Pichurko BM, Ingram RH Jr, Sperting RI, Lafleur JE, Corey EJ, Austen KF, et al. Localization of the site the bronchoconstrictors effects of leukotriene C4 compared with that of histamine in asthmatic subjects. *Am Rev Respir Dis* 1989;140:334-9.
- Homer AB, Bertram GK. Drugs used in Asthma. In: Bertram GK, editor. *Basic and Clinical pharmacology*. New York: McGraw-Hill; 2004. p. 319-21.
- Okojie A, Ebomoyi M, Ekhatior C, Emeri C, Okosun J, Onyesu G,

et al. Review Of Physiological Mechanisms underlying the use of *Garcinia Kola* In The Treatment Of Asthma. Internet J Pulm Med 2009;11. DOI: 10.5580/2909

25. Nelson HS. Prospects of antihistamines in the treatment of asthma. J Allergy Clin Immunol 2003;112:S96-100.
26. Matsumoto T, Ashida Y, Tsukuda R. Pharmacological modulation of immediate and late airway response and leukocyte infiltration

in guinea pigs. J Pharmacol Exp Ther 1994;269:1236-44.

Cite this article as: Ibulubo MT, Eze GI, Ozolua RI, Baxter-Grillo D, Uwaya DO. Evaluation of the protective and ameliorative properties of *Garcinia kola* on histamine-induced bronchoconstriction in guinea pigs. Phcog Res 2012;4:203-7.

Source of Support: Nil, **Conflict of Interest:** No.