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

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## 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.

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## 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.

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## 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 hto 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 ± 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 orallyfor 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 μm in a glass chamber (60 × 36 × 60 cm).[3] 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 ± 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].

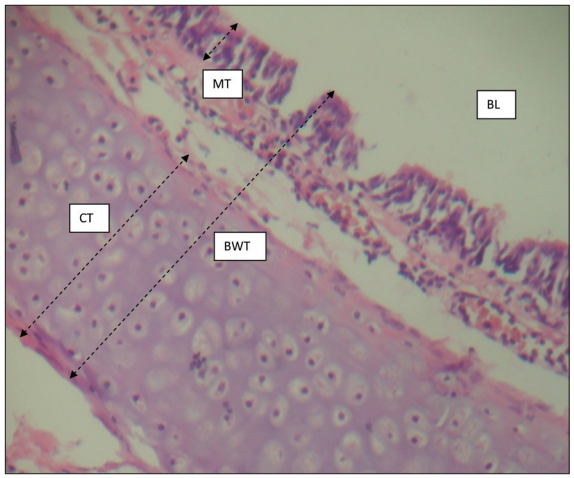


**Figure 1:** Measurement of the bronchial wall thickness. BL, bronchial lumen; BWT, bronchial wall thickness; CT, cartilage thickness; MT, mucosal thickness

## 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 $Ca^{2+}$ 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

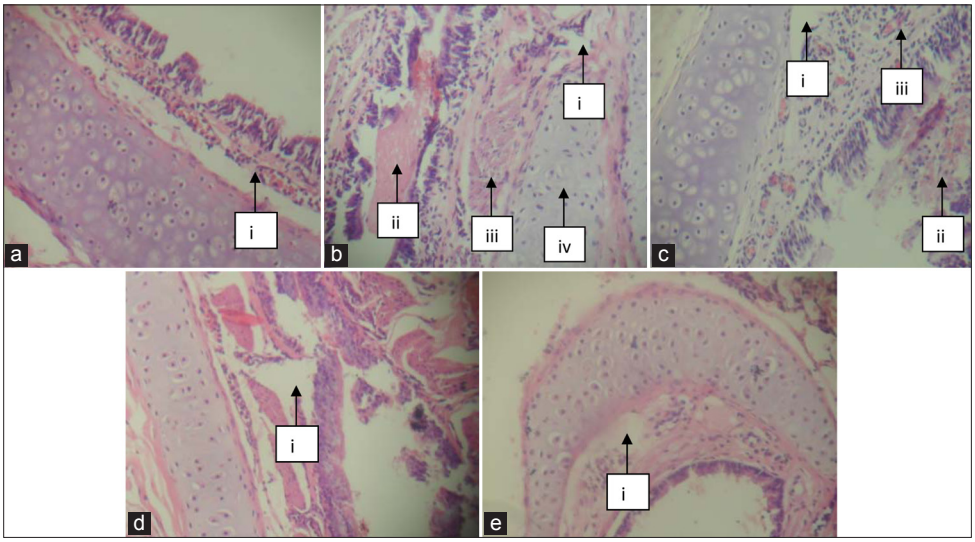


**Figure 2:** Guinea pig bronchi. (a) Non-sensitized control: (i) transmural edema, (ii) cartilage. (b) Ovalbumin-sensitized: (i) transmural edema, (ii) mucus plug, (iii) hypertrophied muscle, (iv) cartilage. (c) Theophylline-treated: (i) transmural edema, (ii) mucus plug, (iii) submucusal congestion (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 dilatory 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.

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