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Extract of *Garcinia kola* Seed has Antitussive Effect and Attenuates Hypercholesterolemia in Rodents

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Abstract

Background: The seeds of *Garcinia kola* Heckel (Guttiferrae) are widely eaten in West Africa for cultural and ethnomedicinal reasons including cough suppression and body fat reduction. We prepared a 70% ethanol extract and designed experiments to investigate the antitussive and anti-hypercholesterolemic effects.

Methods: For the antitussive investigations, guinea pigs were exposed to 7.5% citric acid aerosol in a chamber and cough bouts were counted after acute doses of the extract. Mucus expectoration was evaluated in mice after administering the extract for seven days by measuring the concentration of phenol red dye released from mouse trachea. Hypercholesterolemia was induced and doses of the extract were administered for 14 days.

Results: Single doses of 100, 200 and 400 mg/kg (p.o.) significantly ($p<0.01$) and dose-dependently suppressed cough bouts by 67, 78 and 92% respectively when compared cough bouts exhibited before treatment. The dose of 400 mg/kg was slightly but not significantly superior to the standard drug dihydrocodeine (84%) in cough suppression. The extract administered daily for 7 days also significantly ($p<0.05$) reduced concentration of phenol red released from mouse trachea in comparison with control. At 100, 200 and 400 mg/kg/day for 14 days, extract like 5 mg/kg simvastatin significantly ($p<0.0001$) attenuated the increase in plasma cholesterol level but only doses of 200 and 400 mg/kg/day significantly blunted increase in body weight compared to cholesterol treated rats.

Conclusion: The extract possesses antitussive effect and attenuates hypercholesterolemia thereby lending credence to its ethnomedicinal uses.

Keywords: *Garcinia kola* extract; Cough; Plasma cholesterol; Body weight

Introduction

Garcinia kola Heckel (bitter kola) seed is widely eaten in parts of Africa. It is commonly served at traditional ceremonial functions in West Africa. It is believed to possessed medicinal properties and is eaten as a remedy to diverse health problems. The tree is a well-branched evergreen with a compact dense crown found in the subtropical, tropical lowlands and rain forest of West Africa [1].

It is chewed as an aphrodiasiac or as a cough and cold remedy in Ghana [2]. Extracts and constituents of the seeds have been reported to have anti-ulcer [3]; antibacterial [4]; and antitrichomonal [5] properties. They have very high antioxidant [6]; anti-inflammatory [7]; and anti-diabetic [8]. The anti-asthmatic property of the seed extract has been studied in guinea pigs [9]. It is generally believed to be safe having been eaten over the centuries in West Africa. However, the ethanol extract may inhibit reproductive function through low sperm count and reduced sperm motility [10-12]. These adverse effects conflict with the findings by Ralebona *et al.* [13] that it increases testosterone levels which increase sperm count and viability. Constituents may also block ovulation, alter estrous cycle and have teratogenic effects [14].

The seeds contain tannins and flavonoids [15]. Some of the pharmacological properties have been attributed to its flavonoid constituents chief among them being kolaviron, a mixture of bioflavonoids and kolaflavanone [16,17]. It contains linoleic acid, 1,2-benzenedicarboxylic acid and 2,3-dihydro-3,5-dihydroxy-6-methyl ester [4].

Two of its ethnomedicinal uses which seem not to have been reported so far include the treatment of cough and reduction of body fats. We designed experiments to evaluate its antitussive and anti-hypercholesterolemic properties using rodent models.

Materials and Methods

Preparation of seed extract

Fresh seeds of *Garcinia kola* (GK) were purchased from a major market in Benin City, Nigeria. They were peeled, chopped into pieces and sun-dried for three days. The dried pieces were then powdered and afterward extracted with 70% ethanol for 72 h then concentrated at 40°C using Gallenkamp (England) oven for another 72 h (yield=12.86% w/w). The extract was stored in an opaque container at 4°C.

Animals

Adult guinea pigs of either sex weighing 350-500 g were purchased from the Animal House of the College of Medicine, Ambrose Ali University, Ekpoma, Nigeria. Albino rats of either sex with weights of 100-175 g were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City and mice of either sex

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weighing 18-25 g were purchased from a livestock market at Aduwawa, Benin City. They were all kept in standard plastic cages, males separate from females. They were allowed two weeks of acclimatization in the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin City. The room temperature of the animal house was 27 ± 2°C with 12 h light/dark cycle. All the animals had free access to rat pellets and drinking water and were handled in strict compliance with standard ethical procedures for use of animals in the laboratory [18].

Evaluation of antitussive effect

After the acclimatization period, the guinea pigs were exposed to 7.5% citric acid 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) for 10 min. Guinea pigs that had 10 or more bouts of cough were selected for the antitussive experiments.

Twenty four hours (24 h) after the selection, the guinea pigs were randomly allocated into 5 groups (n=5/group): three groups given 100, 200, and 400 mg/kg of the extract respectively; one group given 25 mg/kg dihydrocodeine (DF118) and another group given 2 ml/kg distilled water. Treatment with extract, DF118 and water was *per os* (using an orogastric tube) one hour before they were re-exposed to citric acid aerosol as described above. The number of bouts of cough by each guinea pig within 10 min was recorded.

Evaluation of mucus expectorant effect

After the acclimatization period, the mice were treated orally for seven days (with the exception of mice in group V) and were randomly allocated into six groups (n=5/group): group I was given 2 ml/kg/day of distilled; groups II to IV were given 100, 200 and 400 mg/kg/day GK extract respectively; group V was given 15 mg/kg/day bromhexine; and group IV was given 50 mg/kg sodium cromoglycate [19].

On the 8th day, after an overnight fast, the animals were treated as usual. The animals in group V were given 50 mg/kg (i.p.) sodium cromoglycate (SC). After 30 min, all the animals were treated orally with ammonium chloride (a secretagogue). After another hour, all the animals were 500 mg/kg (i.p.) of phenol red solution. The animals were sacrificed by cervical dislocation 30 min afterwards and each trachea was removed from the thyroid cartilage to the main stem bronchi and immediately placed into 2 ml of normal saline. A volume of 0.1 ml of 1 M NaOH solution was added to the saline and the absorbance of the mixture was read at 460 nm, using a UV-Visible spectrophotometer (T80, PG Instruments Ltd, UK). The concentrations of phenol red were obtained from its standard absorbance/concentration plot.

Evaluation of anti-cholesterolemic effect

Hypercholesterolemia was induced by giving daily doses of 5 mg/kg PTU+200 mg/kg cholesterol (C) in vegetable oil (VO) for 14 days. This is a modification of the method of Hasimun *et al.* [20].

Rats were randomly allotted to 6 groups (n=5 each). Group I rats were given 2 m/kg/day of VO only. Groups II received daily doses of 5 mg/kg PTU+200 mg/kg cholesterol (C) only. Groups III to VI received 100, 200 and 400 mg/kg/day of GK extract, and 5 mg/kg/day simvastatin. All treatments were *per os* by use of an orogastric tube. The animals were weighed daily throughout the period.

Assay of total cholesterol

The rats were sacrificed on the 14th day and blood was drawn from the abdominal aorta into heparinized tubes. The samples were centrifuged for 10 min at 4000 rpm to obtain plasma. Total cholesterol levels were assayed using commercial test kits (Randox®) for total cholesterol based on the principles described by Abel *et al.* [21].

Drugs and chemicals

Citric acid (Sigma-Aldrich, Switzerland), salbutamol tablets (GlaxoSmithkline Nig. Plc), dihydrocodeine tablets and sodium chromoglycate tablets (bought from University of Benin Teaching Hospital, Benin City, Nigeria), bromhexine tablets (Nigerian German Chemicals, Nigeria), propylthiouracil (PTU) tablets (Propycil® by Recordati Ilac Group, Istanbul, Turkey), and simvastatin tablets (TEVA, UK Ltd) were crushed in distilled water to make fine dispersible suspension for oral administration. Cholesterol (Sigma-Aldrich, USA) was dissolved in vegetable oil. All other chemicals and reagents were of analytical grade and were manufactured by reputable companies.

Data presentation and statistical analysis

The results are presented as Mean ± SEM (standard error of mean) and n represents the number of animals used in each experiment. Data analyzed using one-way ANOVA with Turkey post hoc test (Graphpad Prism Version 6 software, UK). $p<0.05$ indicates statistically significant difference.

Results

Effect of GK extract on cough bouts

Figure 1 shows that *GK* extract, administered at doses of 100, 200 and 400 mg/kg, dose dependently significant ($p<0.05$, 0.01 and $p<0.0001$, respectively) reduced the number of cough bouts. Percentage cough suppression was 67%, 78% and 92% respectively, relative to pre-treatment values of cough bouts for the group. The dose of 400 mg/kg of the extract gave a marginally but not statistically significant better suppression compared to DF 118 (84%).

Effect of GK extract on phenol red dye secretion

Figure 2 shows that doses of 100, 200 and 400 mg/kg/day of GK extract significantly ($p<0.05$; $p<0.01$; $p<0.01$, respectively) reduced the concentration of phenol red dye released from tracheae of mice. In effect, the extract prevented expectoration of phenol red dye when compared to control. While bromhexine significantly released phenol red dye in comparison with control, sodium cromoglycate did not.

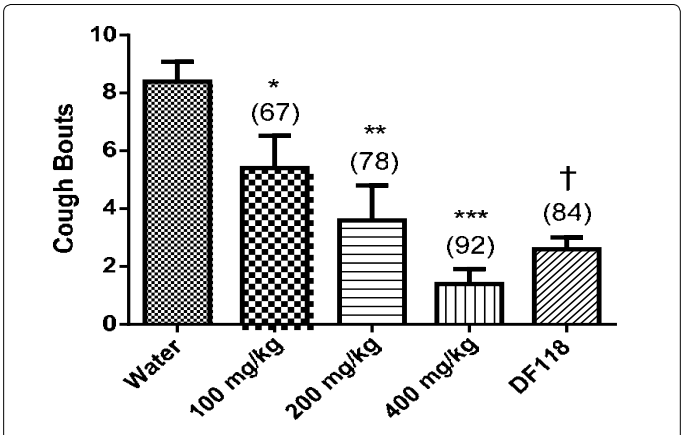

Figure 1: Suppressive effect of *G. kola* extract on citric acid induced cough in rats. Values in parentheses represent mean percentage cough suppression pre versus post treatment in each group. *p<0.05, **p<0.01, ***p<0.0001, †p<0.001 versus water (control) (n=5 per group).

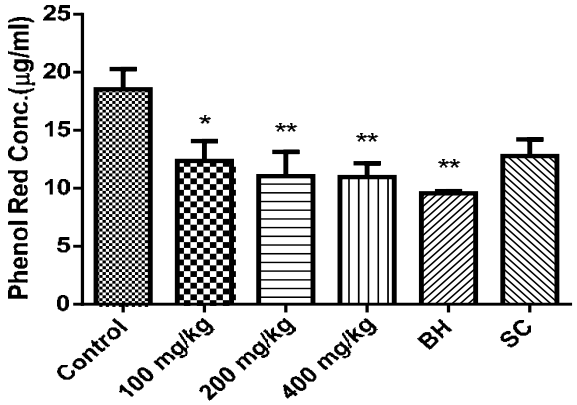

Figure 2: Effect of *G. kola* extract on phenol red dye secretion in mice. *p<0.05, **p<0.01 versus control group. BH: Bromhexine; SC: Sodium chromoglycate (n=5 per group).

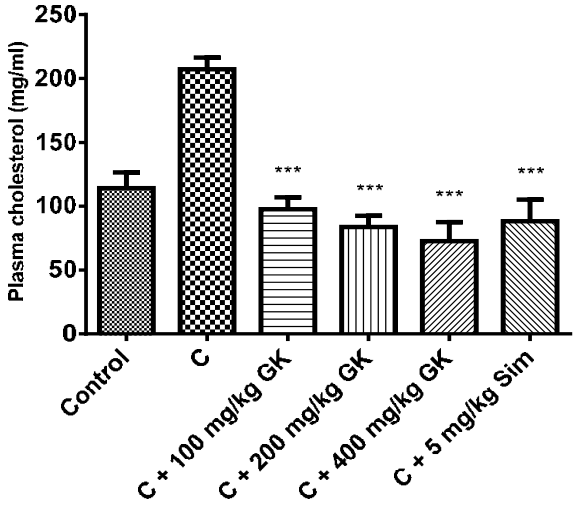

Figure 3: Attenuating effect of *G. kola* (GK) extract on plasma total cholesterol levels following 14 days treatment. **p<0.0001 versus control. C: Cholesterol; Sim: Simvastatin (n=5 per group).

Effect of GK extract on plasma total cholesterol level

Changes in plasma total cholesterol levels are shown in Figure 3. There was a significant ($p<0.0001$) difference in all the extract and simvaststin-treated groups compared to the group in which there was no intervention (cholesterol only). There was no significant difference between the extract treated groups and the simvastatin treated group. Also, doses of extract and simvastatin succeeded in attenuating the increase in cholesterol level and bringing them to values comparable to those of control (vegetable oil only).

Effects of GK extract on weight

Figures 3 and 4 shows weight variations among the different experimental groups. There was a significant difference in the groups given 200 mg/kg and 400 mg/kg GK extracts compared to the control but no significant difference compared to the standard (simvastatin).

Discussion

The results from this study show that 70% ethanol extract of *G. kola* at doses of 100, 200 and 400 mg/kg reduce the number of cough bouts. This is an acute cough model because the animals were not treated for many days before exposure to the irritant. Citric acid induces cough possibly by activating C-fibers in the airways or through activation of rapidly adapting receptors by tachykinins released from activated C-fibres [22,23]. Tachykinin receptor antagonists have antitussive effect [24,25]. Although their mechanisms are not completely understood, opiods such as codeine and dextromethorphan are the main medications for cough and act by stimulating mu and kappa receptors in the cough centre [26]. Whether *G. kola* seed extract possesses opiod-like properties or anti-tachykinin effects is not certain but the extract has been shown to possess anti-inflammatory effect [7] which may be through anti-tachykinin action.

The extract did not exhibit expectorant property since it decreased the release of phenol red dye from tracheae of mice. Ability to cause release of phenol red is indicative of expectorant property [27] because the seeds are rich in tannins (Adegboye *et al.*). Tannins are known for their astringent properties [28,29] with capacity to inhibit mucus secretion and soothe the airways. *Achillea millefolium* L. (Asteraceae) with high contents of tannins is also used in ethnomedicine as an astringent and cough [30,31]. The absence of mucus in the airway reduces the triggering of the coughing reflex [32].

Rat models for the induction of hypercholesterolemia are often a challenge because most employ the feeding of the animals with high fat diet for at least two months and even so, success depends on animal species [33,34]. In the present study, we have used a modification of protocol by Hasimun *et al.* [20] to induce the condition. Propylthiouracil is an antithyroid drug that promotes the hypothyroid state and enhances cholesterol absorption [35] and decreased expression of LDL receptors [36]. The success of our protocol can be seen in the significant difference in cholesterol levels between the group given vegetable oil (vehicle for administering cholesterol) and the group given cholesterol. It is a notable observation that the extract attenuates hypercholesterolemia. Although the mechanism involved is not clear, reduced absorption and increased fecal excretion, increased hepatic storage, and modulation of key enzymes involved in cholesterol metabolism are possibilities [37]. Taken together with weight reduction seen in the groups that received 200 and 400 mg/kg/day of the extract, it seems that reduced absorption and increased fecal loss of cholesterol is a most likely mechanism. The flavonoid contents of the extract possess functional groups (e.g., C=O,

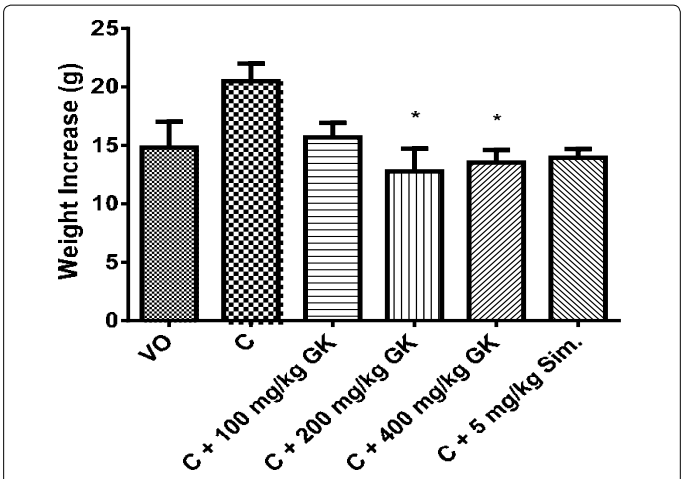

Figure 4: Increase in weight of rats following 14-day treatment with doses of *G. kola* extract (GK) and simvaststin (Sim). C: Cholesterol. *p<0.05 compared to cholesterol group (n=5 per group).

OH) with which they can form non-absorbable complexes [38]. The astringent property of the tannin constituent of the extract may be involved in reduced cholesterol absorption since similar hypolipidemic effects have been associated with extract containing tannins [39,40]. *G. kola* extract reduces the intestinal absorption of ofloxacin [38]. These mechanisms are similar to those of plant-based sterols and cholestyramine in reducing cholesterol absorption [41,42]. Generally, substances with astringent action reduce gastrointestinal absorption of drugs [38].

Conclusion

The 70% ethanol extract of *G. kola* seeds has produced antitussive effect in guinea pigs but lacks expectorant action. The extract also attenuates increase in plasma cholesterol levels. Our findings lend credence to the ethnomedicinal popularity of the seeds for the treatment of cough and reduction of body fat.

Competing Interests

The authors have no competing interests in the conduct of the study.

Authors' Contributions

RIO conceptualized the study, provided general supervision and drafted the manuscript; AOS, NA, SOO and DOU manuscript were all physically involved in the experimentations. All authors read and approved the manuscript before its submission.

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