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Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*

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Abstract—Kolaviron, a mixture of C-3/C-8 linked biflavonoids obtained from *Garcinia kola* produces significant hypoglycaemic effects when administered intraperitoneally to normal and alloxan diabetic rabbits at a dose of 100 mg kg^{-1}. The fasting blood sugar in normoglycaemic rabbits was reduced from 115 mg/100 mL to 65 mg/100 mL after 4 h. In alloxan diabetic rabbits, the blood sugar was lowered from 506 mg/100 mL to 285 mg/100 mL at 12 h. The hypoglycaemic effects have been compared with those of tolbutamide. Kolaviron inhibited rat lens aldose reductase (RLAR) activity, with an IC50 value of $5{\cdot}4 \times 10^{-6}$. The significance of these findings in the potential use of kolaviron as an antidiabetic agent is discussed.

Garcinia kola Heckel (Guttiferae) is a cultivated large forest tree, valued in most parts of west and central Africa for its edible nuts (Hutchinson & Dalziel 1956). The seed known as 'bitter kola' or false kola is a masticatory, used as an alternative to true kola nuts (*Cola nitida* and *C. accuminata*). Extracts of various parts of the plant are used extensively in traditional African medicine (Ayensu 1978), especially for the preparation of remedies for the treatment of laryngitis, cough and liver diseases (Iwu 1982).

Chemical investigations of seeds of *G. kola* have shown that they contain a complex mixture of phenolic compounds including GB-type biflavanoids (Geiger & Quinn 1988), xanthones and benzophenones (Waterman & Hussain 1983), as well as cycloartenol, its 24-methylene derivative and related triterpenes (Igboko 1987). Previous biological studies have shown that biflavanones extracted from the seeds of *Garcinia kola* (kolaviron) protected mice from hepatotoxicity due to Amanita toxins, CCl_4 and galactosamine poisoning (Iwu et al 1987). We have investigated the hypoglycaemic properties of the biflavonoids of *G. kola* (kolaviron) on normal rabbits and its effects on alloxan-diabetic rabbits. Kolaviron was also tested for its activity on the enzyme, aldose reductase, which has been shown to be the key factor in initiating the cataractous process in galactosaemic and diabetic animals (Kinoshita 1974).

Materials and methods

Plant material. The seeds of *Garcinia kola* Heckel were collected from cultivated plants in homesteads in Imo State of Nigeria. The identity of the plants was confirmed by the Botany Department of the University of Nigeria and voucher specimens have been deposited at the Pharmacy Herbarium there. The plant material was sliced into cubes and dried at 40°C in a forced draft Gallenkamp drying oven.

Extraction and preparation of kolaviron. The powdered seeds (4·2 kg) were extracted with light petroleum (b.p. 40–60°C) and then acetone (Me_2CO) in a Soxhlet. The petroleum extract was discarded and the Me_2CO extract (5L) was concentrated under reduced pressure to 200 mL and H_2O was added to produce a chalky solution (1L). Extraction with $CHCl_3$ (5 × 200 mL) and concentration gave a yellow solid (180 g), kolaviron, which has been shown to consist of a mixture of *Garcinia* biflavones GB-1 (**1**), GB-2 (**2**) and kolaflavanone (**3**) (Iwu 1985).

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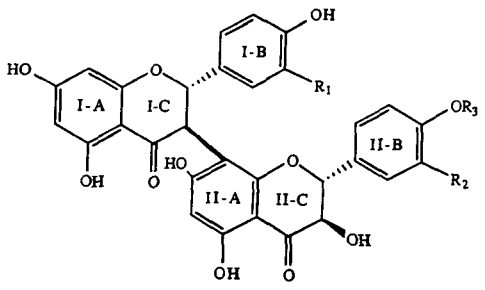

	R_1	R_2	R_3
1	H	H	H
2	H	OH	H
3	H	OH	Me

Preparation of test samples. Kolaviron (50 g) was suspended with 1% Tween 20 in 100 mL of 0·9% NaCl (saline) and appropriate dose dilutions made with saline to provide for a total volume of 5 mL. The vehicle control was prepared by adding 1% Tween 20 in saline and delivering 5 mL i.p. to each control animal.

Animals. Local strains of healthy adult rabbits, 1·2–1·8 kg, were kept in the Experimental Animal Room of the Department of Pharmacology and Toxicology (UNN) for 7 days, with free access to food and water, before the beginning of the experiments.

Biological methods. For the fasting blood sugar assay, a group of animals was fasted for 18 h but allowed access to water before and throughout the experiment. At the end of the 18 h fast, time 0 h, blood was withdrawn from the marginal ear vein. Another group of rabbits was used for the alloxan-induced diabetes study. They were injected intravenously with 150 mg kg^{-1} of alloxan monohydrate as a freshly prepared 10% solution in distilled H_2O. Eight days after injection of the alloxan, blood glucose of surviving rabbits was determined. Only rabbits with values above 350 mg/100 mL after the 18 h fast were considered diabetic (Sharma et al 1978) and used for the assay.

Ten animals were used for each treatment group, with two sets of vehicle controls; one for the normal rabbits and the other for the diabetic animals. The treated groups were compared with those given tolbutamide.

Determination of blood glucose. Blood glucose was assayed by the *o*-toluidine method (Bauer et al 1974). Mean blood glucose levels were expressed as mg/100 mL ± s.e.m. in all the experiments and Student's *t*-test was used to check the significance of the difference between treatment and control groups, and between the blood glucose at time zero and at various times after.

Rat lens aldose reductase activity. Aldose reductase activity was spectrophotometrically assayed in the UV-Vis at λ 340 nm using a Guildford 2400-2 automated spectrophotometer as previously described (Kador et al 1981); determination of inhibition of RLAR by kolaviron was by courtesy of Prof. Duane Miller. The

buffer consisted of 50 mL H_2O, 20 mL 0·5 M phosphate buffer, ≈22 mg NADPH (n = 20 eyes).

Results

Effect of kolaviron on blood glucose levels of normal rabbits. Kolaviron showed a significant hypoglycaemic effect at various times when injected i.p. into fasted animals (Fig. 1). These glucose values were compared with those for animals given 1% Tween 20 in saline (vehicle control) and those from animals that received 500 mg kg^{-1} of tolbutamide. The fasting blood glucose was lowered from 115 mg/100 mL at zero h to 65 mg/100 mL after 4 h ($P < 0.05$), with the lowest value of 60 mg/100 mL observed at 8 h. The blood glucose at 12 h was 95 mg/100 mL and after 24 h the blood sugar was the same as that of the vehicle control (105 mg/100 mL). Tolbutamide, caused a significant ($P < 0.05$) lowering of blood sugar at various times from an initial value of 115 mg/100 mL to the lowest value of 53 mg/100 mL after 4 h. The blood glucose returned to near normal values in 12 h and remained unchanged after 24 h. Animals in the control group administered Tween 20 in saline showed no significant differences in the blood glucose at any time (Fig. 1).

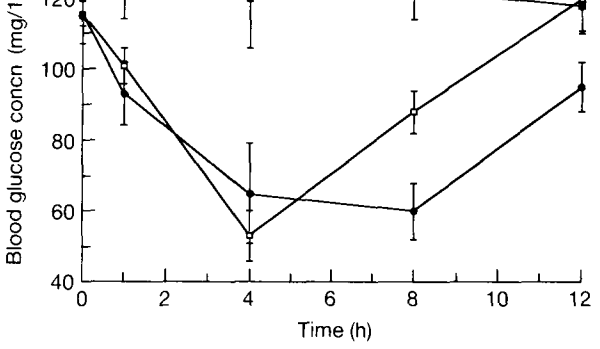

FIG. 1. Hypoglycaemic activity of kolaviron (100 mg kg^{-1}) and tolbutamide (500 mg kg^{-1}) on normal rabbits after 18 h fast. (●) Kolaviron, (□) tolbutamide, (■) vehicle control. Values are mean blood glucose levels expressed as mg/100 mL ± s.e.m. Student's *t*-test was used to check the significance of the difference between the treated groups and vehicle control groups, and between the blood glucose levels at zero h and at various times in each treatment group, n = 10.

Effect of kolaviron on blood glucose levels of diabetic rabbits. The effects of kolaviron on the blood glucose of alloxan-diabetic rabbits are shown in Fig. 2. The Figure also shows the response of alloxanated animals treated with tolbutamide. No significant differences were observed in the blood sugar at 0, 1, 8, 12, or 24 h, when only 1% Tween 20 in saline was administered to the diabetic rabbits. Kolaviron-treated animals showed a significant ($P < 0.05$) decrease in the blood glucose at 1, 8, 12, and 24 h when compared with the initial level. The blood glucose of the kolaviron-treated animals decreased progressively over time to give the lowest value of 214 mg/100 mL at 24 h. Tolbutamide administered at 500 mg kg^{-1} caused no significant decrease in the blood sugar levels at the various times when compared with the initial value of 515 mg/100 mL and the vehicle control group that received only saline and Tween 20.

Rat lens aldose reductase activity. From the least square regression lines of the log dose-response curves, the concentration necessary for 50% inhibition of activity (IC50) of kolaviron on rat lens aldose reductase activity was estimated to be 5.4×10^{-6} (based on extrapolation to 100%).

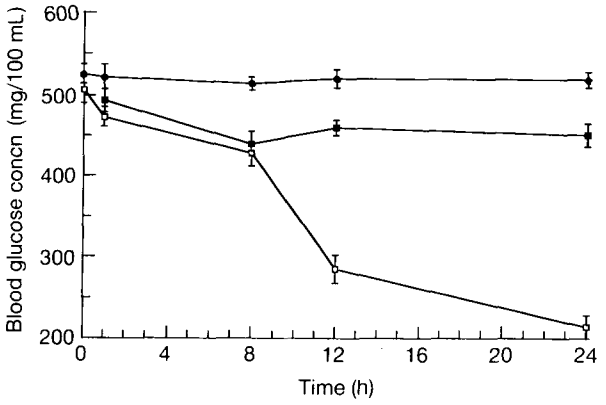

FIG. 2. Effect of kolaviron (100 mg kg^{-1}) and tolbutamide (500 mg kg^{-1}) on alloxan-diabetic rabbits. Blood was drawn from a marginal ear vein 8 days after i.v. injection with 150 mg kg^{-1} alloxan monohydrate; only animals with glucose levels >350 mg/100 mL after 18 h fast were used. (□) Kolaviron, (■) tolbutamide, (●) vehicle control group. Values are mean blood glucose levels expressed as mg/100 mL ± s.e.m. Student's *t*-test was used to check the significance of the difference between the treated groups and vehicle control groups, and between the blood glucose at zero h and at various times in each treatment group, n = 10.

Discussion

Kolaviron, a mixture of the GB-type biflavonoids of *Garcinia kola*, has been shown to produce significant and consistent hypoglycaemic effects on both fasted normoglycaemic and alloxan-diabetic rabbits. Its pronounced antihyperglycaemic effect on alloxan-treated rabbits, in contrast to the activity of tolbutamide, suggests that kolaviron could be a direct hypoglycaemic agent, unlike sulphonylureas that act indirectly by stimulating the pancreatic beta-cells to release more insulin. The significant hypoglycaemic effect on normal rabbits, however, may be indicative that kolaviron exerts its hypoglycaemic activity by *both* direct and indirect mechanisms. If the drug acted only as an indirect hypoglycaemic agent no effect should be observed when kolaviron is given to alloxan-treated rabbits since alloxan causes permanent destruction of the beta-cells. Tolbutamide would appear to be a better hypoglycaemic in normoglycaemic rabbits but failed to reduce the blood sugar significantly in alloxan-diabetic animals. The dose of 100 mg kg^{-1} of kolaviron used for this study was established as the optimum dosage from previous investigations (Iwu 1985).

The ability of kolaviron to inhibit rat lens aldose reductase activity is considered important for its evaluation as an antidiabetic agent since the enzyme aldose reductase (aldictol: NADP oxido-reductase, EC 1.1.1.21), found in the lens and other tissues has been implicated in many diabetic complications such as neuropathy and retinopathy (Gabay et al 1979; Kinoshita et al 1979). The enzyme is also believed to be the initiating agent in the formation of cataracts. Although the RLAR inhibitory activity of kolaviron may not compare favourably with those of strong inhibitors of the enzyme such as alrestatin (Kador et al 1981), kolaviron has the advantage of combining antidiabetic activity with RLAR inhibition. Kolaviron, which is obtained from an edible plant, is an excellent potential antidiabetic agent.

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Insulin absorption from conjunctiva studied in normal and diabetic dogs

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Abstract—The dynamics of insulin absorption from the ocular conjunctiva of anaesthetized normal and pancreatectomized dogs have been examined. A porcine insulin preparation of 1000 units mL^{-1} (pH 8·0) was administered as either 1 or 10 units kg^{-1} to the upper conjunctival sacs of recumbent dogs following an overnight fast. Plasma insulin concentrations increased significantly at 5 min after the insulin administration. Plasma glucose concentrations decreased significantly, in both normal (given 10 units kg^{-1}) and diabetic dogs (given 1 unit kg^{-1} or 10 units kg^{-1}). There was a dose-dependent increase in plasma insulin concentration following conjunctival administration. Estimated absorption was significantly higher in diabetic than in normal dogs.

Various sites have been examined for possible insulin absorption (Moses & Flier 1987), including intestinal (Kawamori & Shichiri 1982), rectal (Kawamori & Shichiri 1982; Yagi et al 1983), nasal (Hirai et al 1978), pulmonary (Wigley et al 1971; Kohlert et al 1984), and buccal mucosal membranes (Nagai 1985). However, there have been no reports of insulin absorption from the ocular conjunctiva.

We have investigated insulin absorption from conjunctiva in anaesthetized normal and diabetic dogs and have tried to evaluate conjunctiva as a potential route for insulin administration to control blood glucose concentration.

Materials and methods

Animals. Five mongrel dogs (11 ± 2 kg, mean ± s.e.) were used as their own controls. As undosed controls, sterile 0·9% NaCl (saline) solution was applied to upper conjunctival sacs. Animals were then pancreatectomized.

Insulin preparations. 30 000 units (1·172 g) of porcine crystalline insulin (Sigma, St. Louis, MO) was dissolved in 22·9 mL of 0·04 M HCl, and the pH of the solution adjusted to 8·0 with 0·5 M NaOH: Distilled water was added to make up to 30 mL (1000 units mL^{-1}).

Methods. Following an overnight fast, dogs were anaesthetized with pentobarbitone, then laid in the recumbent position to minimize loss of insulin solution and its possible absorption through nasal membranes.

The insulin preparation, at either 1 unit kg^{-1} or 10 units kg^{-1}, was administered to the upper conjunctival sac in a random order. There was no leakage of insulin into the nasal cavity through naso-lacrimal ducts. All experiments were carried out in the morning.

Blood samples were collected through an indwelling catheter placed in the femoral vein. Plasma glucose concentrations (mmol L^{-1}) were measured by the Glucose Analyzer (Beckman Instruments, Fullerton, CA) using a glucose oxidase method. Plasma insulin concentrations (m units L^{-1}) were measured by radioimmunoassay, as immunoreactive insulin (IRI).

To evaluate the efficiencies of insulin absorption through conjunctiva, the area under the curve of insulin concentrations (AUC.IRI) was calculated and compared with that after i.m. insulin (0·2 units kg^{-1}) and bioavailability was determined according to the equation:

$$\text{Bioavailability} = \frac{(\text{AUC.IRI})}{(\text{AUC.IRI i.m.})} \times \frac{0{\cdot}2}{\text{Dose}} \times 100\%.$$

All the data were expressed as mean ± s.e. and statistical analysis was carried out by a paired *t*-test.

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