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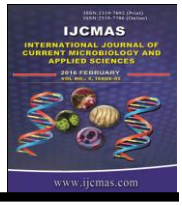

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Anti-diabetic Profile of Extract, Kolaviron, Biflavonoids and Garcinoic acid from *Garcinia kola* seeds

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K e y w o r d s

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A B S T R A C T

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, and neuropathy and causing neurological disorders due to perturbation in utilization ofglucose. In the present study diabetes was induced in albino rat models with alloxan monohydrate. *Garcinia kola* seeds, has beenclaimed to possess antidiabetic properties by many investigators. The present study was undertaken to screen the hypoglycemic activityof ethanol extract, the fraction and compounds of *G. kola* seeds. The results showed thatthe extract, fraction, compounds and the reference drug (glibenclamide) showed different levels of antidiabetic effect. However, GB2 gave the best antidiabetic effect which is an improvement from that of the extract. The effect of GB2 was similar to glibenclamide.

Introduction

Bitter Kola also known as *Garcinia kola* is a tropical flowering plant found in western and central Africa and it produces brown, nut-like seeds. It has been used in African culture for centuries for both traditional and medicinal purposes. It contains dimeric flavonoid, lipase inhibitor which is believed to have many healing benefits. Bitter Kola is a masticatory used in traditional hospitality, cultural and social ceremonies such as naming ceremonies and weddings.

Bitter kola is used in many tropical countries to fight infectious diseases such as AIDS and the Ebola virus. It has shown to possess anti-inflammatory, antimicrobial and antiviral properties. It is often used to treat the symptoms of colds. It is particularly very effective for coughs, nasal congestions and help coagulate phlegm. It is also effective in alleviating sore throat, is sometimes believed to cure impotence. It increases blood flow to the Core area in men who

have hardening of the arteries. *Garcinia kola* has been successfully used to treat patients suffering from knee osteoarthritis. It reduced pain and swelling and improved movement. *Garcinia kola* is known for its anti-inflammatory and antioxidant properties. It is used to prevent infections and viruses, especially of the immune system. Bitter Kola has been known to be a natural hunger suppressant and also increases the urge to drink more water. It is used as a substitute for hops in brewing lager beer. It is especially useful in preventing beer spoilage (Iwu, 2003, Iwu *et al.*,1982). This study was to investigate the antidiabetic property of the crude extract of *Garcinia kola* and the isolates (kolaviron, GB1, GB2 and garcinoic acid).

Materials and Methods

General Experimental Procedures

The UV spectra were obtained with a shimadzu 3101 PC instrument and IR spectra determined with a jasco FT-IR 410 apparatus. ^{1}H (400.6MHz) and ^{13}C (100.13 MHz) nmr spectra were recorded in CDC_{l3} (with its signals at δ 7.25 and 77.0 ppm as reference) TLC was carried out on silica gel 60 GF_{254} pre-coated plates with detection by UV light or by spraying with 50% H_2SO_4 followed by heating at 100°C.

Plant Material, Preparation of Extract, Fractions and Compounds

Garcinia kola seeds were collected within the surrounding of Orba, Nsukka, Enugu State, Nigeria in March 2010, Nigeria, and was identified and authenticated by Mr. Alfred Ozioko of International Centre for Ethnomedicine and Drug Development. The voucher specimen (INTERCEDD 022010) is deposited at the same center.

The air-dried and powdered plant material (5Kg) was macerated in a mixture of CH_2Cl_2-MeOH (1:1) for 48h. Removal of the solvent *in vacuo* in a rotary evaporator provided an organic extract (600g).

Kolaviron was isolated according to Iwu et al. 1990as modified by Farombi et al. 2000. Briefly, the powdered seeds were extracted with light petroleum ether (b.pt 40-60 o C) in a soxhlet for 24h. The defatted, dried marc was repacked and extracted with acetone (Me_2CO). The extract was concentrated and diluted twice its volume with water and extracted with ethyl acetate. The concentrated ethyl acetate fraction gave a yellow solid known as Kolaviron (TGA)

Further purification of TGA (203.5g) using silica gel as stationary phase and mixture of CH_2Cl_2/ actone afforded GB1 (68,1g) and GB2 (101,6g). The fraction obtained with EtOAc/nhex (8:2) was further purified using silica gel as stationary phase and EtOAc/nhex mobile phase yielded garcinoic acid (TGK3, 107,3g).

Identification of GB1, GB2 and TGK3

The know compounds GB1, GB2 and garcinoic acid were identified by comparison of NMR data with published data (Kenji *et al.,* 1997).

Experimental Animals

Thirty five (35) white albino Wistar rats (86 - 100 g) of either sex were procured from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in stainless steel cages and were fed *ad-libitum* with standard laboratory animal feed (Guinea Feed®), except in situations, where fasting was required. They were also provided with clean tap water. They were maintained in

accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). They were allowed 2 weeks to acclimatize before the start of the experiments.

Brine Shrimps Lethality Test

The effect of the extract on brine shrimps was evaluated using the method of Mclaughlin *et al* 1991. Briefly, brine shrimp eggs were hatched in culture tank containing sea water under bright light for 48 h. Ten nauplii were counted into bijou bottles in triplicates and were incubated with graded concentrations of the extract (10, 100 and 1000 ppm) at room temperature for 24 h. The mean surviving nauplii was determined for each concentration of the extract and compared with that of the control. The result was analyzed using probit analysis (minitab for windows release 12.21) to determine the LC_{50} at 95% confidence interval.

Chemicals

Alloxan monohydrate, a most widely used chemical diabetogen was procured from Loba chemie, Mumbai, India and other reagents used in the experiment were of analytical grade. Chemically alloxan is 2, 4, 5, 6 tetra oxo hexahydro pyrimidine. Glibenclamide, a standard antidiabetic agent was purchased from Sigma, Jos, Nigeria.

Antihyperglycaemic Studies Induction of Diabetes

Hyperglycaemia was induced in overnight fasted adult Wistar strain albino rats weighing 180-240 g by a single intraperitoneal injection of freshly prepared alloxan monohydrate in normal saline (200 mg/kg body weight) in a volume 1 ml/kg body weight (Kastumata *et al.,* 1999). Hyperglycaemia was confirmed by the elevated glucose level in plasma, determined at 48 h after injection (Mandal *et al.,* 1997).The rats found hyperglycaemic were screened for the antihyperglycaemic study.

Experimental Design

Animals were divided into six groups (A-F) of five rats each. Test groups were administered samples (crude extract, kolaviron, GB1, GB2 and garcinoic acid) at dose of 50mg/kg body weight by oral route. Standard and control animals were treated with standard drug glibenclamide at an oral dose of 5mg/kg body weight and distilled water respectively. All doses were started 48 h after alloxan injection. Fasting blood glucose levels were estimated on Hour 0, 1, 3, and 6 h post treatment.

Statistical Analysis

Data were statistically calculated by utilizing one-way ANOVA and expressed as mean ± S.E.M. followed by Dunnett's *t* -test using computerized GraphPad InStat version 3.05, Graph pad software, U.S.A.

Results and Discussion

Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucoseconcentration in the blood and in response to elevated blood glucose, insulin is secreted[9]. Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas(Prince *et al.*,2000; Jelodar *et al*. 2003).

Alloxan causes a massive reduction in insulin release bythe destruction of b-cells of the islets of langerhans, thereby inducing hyperglycaemia (Grover *et al*. 2000) Insulin deficiencyleads to various metabolic alterations in the animals viz increased

blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases (Shammugasundraram *et al.* 1983; Begum *et al*. 1978).

The results of the present study indicate that the ethanol extract of *G. kola* seeds, kolaviron, garcinoic acid, GB1, GB2 and the reference drug (glibenclamide) showed different levels of antidiabetic effect in aminals made diabetic with alloxan. However, GB2 gave the best antidiabetic effect which is an improvement from that of the crude extract. The effect of GB2 was similar to glibenclamide (table 1).

Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage.

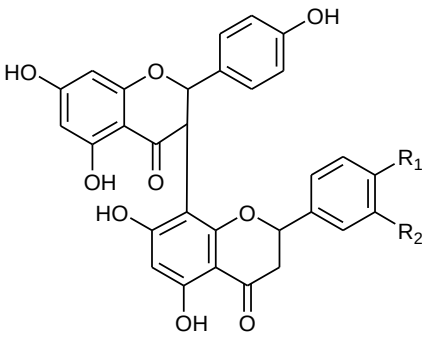
The activity of the extract and the kolaviron is related to presence of bioflavonoids in G. kola seed. Bi-flavonoids comprise a group of phenolic secondary plant metabolites that are widespread in nature. Major flavonoids that have well categorized structure and well defined structure function relationships are: flavans, flavanones, flavones, flavonols, flavanonols, cetechins anthocyanidins and isoflavones. Bio-flavonoids are well known for their multi-directional biological activities including antidiabetic efficacy (Brahmachari, 1978; 2008) Numerous studies have been carried out to explore their potential role in the treatment of diabetes (Jung *et al*,, 2008; Matsui *et al*.,2006; Qi *et al*, 2010). A good number of studies have already demonstrated the hypoglycemic effects of flavonoids using different experimental models and treatments.

In conclusion, from this study, we can state that the Ethanolic extract, kolaviron and GB1 of *G. kola* have beneficial effects on blood glucose levels as well as improving hyperlipidemia and other metabolic aberrations. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research.

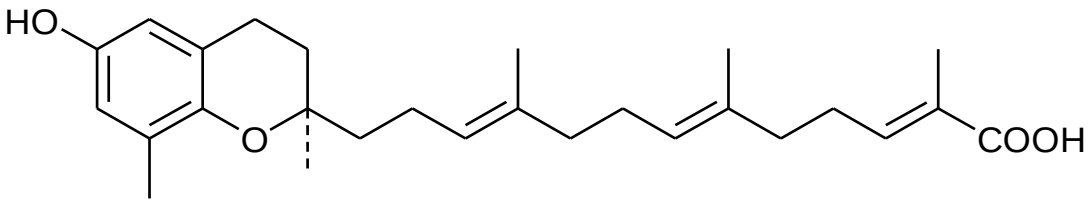
Table.1 Effects of the Crude and Fractions of *Garcinia cola* on Alloxan-induced hyperglycaemia (Antidiabetic Assay)

		Mean Blood glucose level ± SEM (mmol/L) at time interval (h)				
Drug/Fractions	**Dose(mg/kg)**	**FBS b4 induction**	0	1	3	6
Glibenclamide	5	2.8± 0.27	14.23± 2.11	8.74± 3.21	4.44± 0.78	2.27± 0.54
Crude	50	3.1±0.17	13.97± 2.61	6.03± 2.26	5.27± 1.74	3.77± 1.20
TGA2	50	1.8±0.11	14.6± 0.98	9.87± 4.45	8.3± 3.31	8.5± 3.02
TGK3A	50	3.4±0.31	12.93± 1.64	7.93± 1.31	5.3± 0.22	5.8± 0.33
GB1	50	3.2±0.25	14.1± 3.01	10.03± 5.21	9.37± 5.35	6.33± 3.52
GB2	50	3.5±0.11	13.5± 2.83	7.1± 2.96	7.57± 2.96	2.6± 0.37

Figure.1 Structures



GB1, R1= OH, R2= H
GB2, R1=R2= OH
TGA2= Kolaviron
Crude = Ethanolic extract of *Garcinia kola* seeds



TGK3= Garcinoic acid

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