

Research Article

Phytochemical Screening and Biochemical Effects of Methanol Extract of Bitter KOLA (*Garcinia Kola*) on Alloxan Induced Diabetes in Male Wistar (Albino) Rats

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Abstract: The phytochemical screening and biochemical effects of methanol extract of bitter kola (*Garcinia kola*) on alloxan induced diabetes was studied using standard methods in order to determine the potential of this seed. Twenty five (25) male Wistar (albino) rats used as the experimental organisms were divided into (5) groups designated A, B, C, D and E with (5) animals per group. Groups A and B which were non-diabetic control and non-diabetic test were treated with normal saline (0.5 mL/kgbody weight) and 50 mg/kg body weight of *Garcinia kola* extract respectively. Groups C, D and E which were made diabetic using alloxan monohydrate had one diabetic untreated group that received normal saline (0.5 mL/kg body weight) and two diabetic treated groups that received reference drug (gliben clamide) (50 mg/kgbody weight) and 50 mg/kgbody weight of *Garcinia kola* extract respectively. After every treatment, blood samples were collected from the test organisms through tail vein puncture and their respective blood glucose concentration was measured with gm glucometer at 0, 2, 4, 6, and 8 h for three (3) days. It was observed that *Garcinia kola* methanol extract treatment significantly reduced blood glucose level. The result of the phytochemical screening which was analyzed using standard analytical method revealed the presence of flavonoids, tannins, saponins and cardiac glycosides while alkaloids were absent. From the finding in this prospective study, *Garcinia kola* extract has useful pharmacological bioactive compounds and as such could be used in ethno-medicine for the treatment of diabetes and other ailments.

Keywords: Blood, *garcinia*, glucometer, intraperitoneally, kola, medicinal

INTRODUCTION

Over few decades, the role of medicinal plants as primary tool in preservation of health and management of diseases has been realized with great concern. This result mainly from the use of synthetic drug molecules that produce harmful side effects, which are comparatively minimal in drugs of plant origin (Ajoy *et al.*, 2001). Current estimates, suggest that in many developing countries, a large proportion of population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although, modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Some of the drugs used today have originated from medicinal plants (Ajoy *et al.*, 2011). The medicinal properties of these plants are attributed mainly to the presence of flavonoids, but they may also

be influenced by other organic and inorganic compounds such as alkaloids, tannins, saponins, phenols etc. (Prabha *et al.*, 2011).

Bitter kola botanically known as "*Garcinia kola*" is a herbaceous perennial medicinal plant grown in tropical rain forest in Central and West Africa (Uko *et al.*, 2001; Okolle *et al.*, 2009) and more predominantly in rainforest belt of Southern Nigeria (Agada and Braide, 2009). The tree is usually cultivated within villages in Southern Nigeria and grows to a height of about 12-14 m high. It has been referred to as a "wonder plant" because almost every part of it has been found to be of medicinal importance. The seed is also known as false fruit unlike kola nut (*Cola nitida*) which is known as "true kola". It is commonly called "Akuilu" in Igbo land, "Namijingoro" in Hausa land and "Orogbo" in Yoruba land of Nigeria. It produces characteristic reddish, yellowish or orange coloured fruit with seeds covered with skimoor husk.

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The tree is probably the most important source of chewing sticks. Unlike the kola nut, it is monocotyledonous plant. It has nothing to do with the tradition of any ethnic group. It can be eaten at anytime and it has no side effect. It is one of those medicines that have no overdose. When bitter kola is taken, one has no chances of contacting or suffers from sour or mouth odour, stomach upset, tooth ache. Bitter kola has a brownish outer cover while the inner part is yellowish in color. It has a juicy substance which is assumed to contain the active ingredients. It does not require any preservative and survives under any atmospheric condition. However, if it is kept in the refrigerator, it remains fresh while if it is left in the open space it dries. In dry condition, it can be revived by soaking it in water for hours or days depending on the intensity of the dryness.

Bitter kola "*Garcinia kola*" has been identified as potent antibiotics which could be effective in the treatment of many diseases such as high fever, jaundice and as purgative (Iwu *et al.*, 1990). The seed is masticatory and is used to relieve and prevent chest cold and cough and can as well be used to treat headache (Ayensu, 1978). Bitter kola "*Garcinia kola*" could serve as raw material for pharmaceutical industries (Iwu *et al.*, 1990). The medicinal use of the plant's leaves and root in the management and treatment of diseases have been an age long practice (Sofowara, 1982). The plant exhibits very potent pharmacological activities such as antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties (Adegboye *et al.*, 2008.)

The seeds of bitter kola are edible and are consumed as adjuvant to the true kola (*Cola nitida*) and for medicinal purposes (Braide, 1989). "*Garcinia kola*" is used in folklore remedies for the treatment of ailments such as liver disorder, diarrhea, laryngitis, bronchitis, gonorrhoea (Adesina *et al.*, 1995), stomach ache and gastritis (Okwu, 2005). Its antifungal (Okwu and Morah, 2007) and antimicrobial effects (Adegboye *et al.*, 2008) have also been reported. Although bitter kola lacks caffeine, its alkaloid and bi-flavonoid fractions are said to relax the smooth muscles (Braide, 1989).

Objectives of the study:

- To investigate qualitatively the phytochemical content of Bitter kola (*Garcinia kola*)
- To investigate the biochemical effects of *Garcinia kola* on diabetic rats.

MATERIALS AND METHODS

Plant material procurement: Fresh *Garcinia kola* seeds were purchased from Eke market, Afikpo, Ebonyi State, Nigeria and was identified and authenticated by Dr M.C. Okafor, the herbarium

curator, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana (AIFPU), Afikpo, Ebonyi State, Nigeria. The voucher samples were kept in the herbarium of Science Laboratory Technology Department for reference purpose.

Preparation of seed samples: The outer testa of each bitter kola (*Garcinia kola*) seeds was removed, washed and air dried for about 24 h. Each seed was cut into small pellets with kitchen knife and the resulting pellets were subsequently dried in an electric oven for 12 h at 40°C. The dried seed pellets were grinded to fine powder using manual grinder and then sieved with 10 mm sieve. The powder was used in the extraction process while the remaining powder aliquots were used for phytochemical screening.

Preparation of *Garcinia kola* extract: *Garcinia kola* extract were prepared by soaking 300 g of the *Garcinia kola* seed powder in 400-450 mL of methanol. The container was well covered and shaken vigorously to mix very well and then allowed to stand for three (3) days with intermittent daily shaken. After the duration, the mixture was then filtered into a conical flask using filter paper and funnel. The filtrate was concentrated by heating in a water bath to allow for the loss of the solvent by evaporation leaving behind the extract of appropriate concentration that was used for the oral administration of the test organisms.

Experimental animals: Twenty-five (25) adult male Wistar (Albino) rats were purchased from the animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. These animals were kept in cages in their respective groups to acclimatize with conditions of the animal housing facility with ambient temperature 26-28°C and adequate ventilation for two weeks and were fed with standard growers mash (vita feeds Nig, Ltd.) and clean water *ad libitum*.

Induction of diabetes mellitus: A single dose freshly prepared alloxan monohydrate (Sigma, St Lois Mo, USA) in normal saline at a dose 150 mg/kg body weight was injected intra-peritoneally into the rats according to the method as outlined in Ebong *et al.* (2008). Blood samples were collected by tail vein puncture after 72 h and were monitored for glucose level using gm glucometer. The rats that had blood glucose level above 200 mg/dL within this period were considered diabetic and hence selected for the study.

EXPERIMENTAL DESIGN

The method according to Ebong *et al.* (2008) was adopted. The twenty five (25) rats were weighed and divided into five groups of (5) five rats per group according to their weights and labeled A, B, C, D and E. Animals in all groups received, by oral administration the following:

Table 1: Phytochemical screening of *Garcinia kola*

Tests	Observation
Alkaloids	-
Tannins	+
Flavonoids	++
Saponins	++
Cardiac glycosides	++

–: Absent, +: Present, ++: Copiously present

Group A: (Non-diabetic control): Normal saline (0.5 mL/kg body weight)

Group B: (Non-diabetic treated group): *Garcinia kola* extract (50 mg/kg body weight)

Group C: (Diabetic control): Normal saline (0.5 mL/kg body weight)

Group D: (Diabetic treated 1): Reference drugs (gliben clamide) (50 mg/kg body weight)

Group E: (Diabetic treated 11): *Garcinia kola* extracts (50 mg/kg body weight)

After every treatment, blood samples were collected from the test organisms through tail vein puncture and their respective blood glucose concentration (level) was measured with gm glucometer at 0, 2, 4, 6 and 8 h for three (3) days. The blood glucose level of all the experimental animals were monitored after each day of oral administration of a single dose of the placebo and the extract. The administration was then continued daily for every 24 h for three (3) days. The blood glucose level were monitored during the three days period and then recorded as the prospective result.

Phytochemical screening: A portion of the *Garcinia kola* seed powder was subjected to phytochemical screening for the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides using the methods as outlined in Harborne(1984). The intensity of the coloration determines the abundance of the bioactive component.

Statistical analysis of data: Analysis of Variance (ANOVA) for the data collected was carried out using Statistical Package for Social Science (SPSS) software for window version 15.1 Chicago, USA and post hoc testing was performed for inter-group comparison using the Least Significant Difference (LSD). All data were expressed as means \pm Standard Error of Mean (SEM). The p-values of <0.05, 0.01 and 0.001 were considered statistically significant.

Table 2: Effects of *Garcinia kola* extract on blood glucose concentration (level) of diabetic and non- diabetic rats

Sample (g)	WER \pm SD (mg/dl)	GLBI \pm SD (mg/dl)	GLAI \pm SD (mg/dl)	Day(1)GLAT \pm SD (mg/dl)	Day(2)GLAT \pm SD (mg/dl)	Day(3)GLAT \pm SD (mg/dl)
Group A	230.10 \pm 11.9 ^a	78.40 \pm 17.9 ^c	78.18 \pm 17.9 ^b	79.20 \pm 25.7 ^c	78.28 \pm 12.7 ^c	77.15 \pm 24.7 ^a
Group B	247.25 \pm 12.1 ^a	79.82 \pm 17.9 ^c	78.14 \pm 17.4 ^c	77.02 \pm 19.2 ^b	70.24 \pm 15.2 ^a	62. 17 \pm 17.9 ^c
Group C	201.23 \pm 30.2 ^b	76.38 \pm 19.2 ^c	523.00 \pm 17.5 ^b	520.22 \pm 15.4 ^b	518.18 \pm 15.3 ^a	520.18 \pm 16.4 ^a
Group D	182.18 \pm 28.2 ^c	76.20 \pm 18.2 ^a	589.00 \pm 17.4 ^a	525.40 \pm 14.4 ^a	496.20 \pm 16.2 ^b	437.16 \pm 17.9 ^a
Group E	178.26 \pm 14.5 ^c	78.42 \pm 12.8 ^a	600.20 \pm 12.6 ^a	513.60 \pm 13.2 ^a	437.20 \pm 15.5 ^{ab}	331.14 \pm 14.2 ^b

WER: Weight of Rat, GLBI: Glucose Level Before Induction, GLAI: Glucose Level After Induction, GLAT: Glucose Level After Treatment, p<0.001 significantly different from non-diabetic control; ^ap<0.05, ^bp<0.01, ^cp<0.001 significantly different from diabetic control

RESULTS

Phytochemical screening of *Garcinia kola*: The result of the phytochemical screening of *Garcinia kola* is shown in Table 1. The result revealed the presence of tannins, flavonoids, saponins and cardiac glycosides while alkaloids were absent.

Biochemical effects of *Garcinia kola*: The result of the effects of *Garcinia kola* extract on blood glucose level of both diabetic and non-diabetic rats is shown in Table 2. It could be observed from the result that in group B which is non diabetic rats treated with the extract, that the Glucose Level after Treatment (GLAT) for three days decreased mildly showing that *Garcinia kola* extract has the potential of reducing the blood glucose level to some extent in both diabetic and non-diabetic rats. On the other hand, it could also be observed from the result that there is no much disparity in the blood glucose level after treatment for three days with 0.5 m L/kg normal saline in diabetic group C indicating that normal saline does not have the potential of reducing blood glucose level in the experimental organisms. The result also revealed that the blood glucose level decreased drastically in group E which received the *Garcinia kola* extract, when compared with the other diabetic treated groups. Although, treatment with the reference drug (gliben clamide), also recorded an appreciable decrease in blood glucose level (that is Group D). It can therefore be deduced from these findings that the *Garcinia kola* extract is even more potent in reducing blood glucose level in the test organisms than the reference drug.

DISCUSSION

Phytochemical analysis is very useful in the evaluation of some bioactive and biochemical components of seeds and other parts of plants. This present study assessed the phytochemical and biochemical potentials of methanol extract of *Garcinia kola*.

The result of the phytochemical screening revealed the presence of flavonoids, tannins, saponins, cardiac glycosides while alkaloids were found to be absent (Table 1). These findings agreed with an earlier report

of Adegboye *et al.* (2008) who recorded similar results for the aqueous extract of *Garcinia kola*. They further reported that the invaluable pharmacological potential of *Garcinia kola* such as antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties may be attributed to the presence of these bioactive compounds (Adegboye *et al.*, 2008). Epidemiological studies have shown that flavonoids intake, is inversely related to mortality from coronary heart diseases and other incidences of heart attacks (Shills and Young, 1992).

It was observed from the result (Table 2) that blood glucose level examined at 0, 2, 4, 6 and 8 h of alloxan diabetic untreated rats was significantly higher than in non-diabetic rats. This observed increase in glucose levels has been reported in diabetic untreated rats (Iwu *et al.*, 1990; Ebong *et al.*, 2008; Mohammed *et al.*, 2009).

Alloxan induced diabetics by causing damage and death of the insulin-secreting cells of the pancreas islets cells in experimental animal models leading to hyperglycaemia and hence diabetes (Lenzen, 2008). The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Then highly reactive hydroxyl radicals are formed by Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B-cells, hence precipitating experimental diabetes mellitus (Lenzen, 2008).

The result of our study (Table 2) shows that oral administration of different doses of *Garcinia kolamethanol* extract significantly reduced the increased blood glucose level in diabetic animals following treatment. This observation is in agreement with the earlier report of Iwu *et al.* (1990) and Adaramoye and Adeyemi (2006), who reported the hypoglycaemic effect of ethanol extract of *Garcinia kola* in normoglycaemic rats as observed in this study.

CONCLUSION

From the finding in this prospective study, *Garcinia kola* extract has useful pharmacological bioactive compounds and as such could be used in ethno-medicine for the treatment of diabetes and other ailment. Phytochemical screening shows that the sample contains useful bioactive components (alkaloids, saponins, flavonoids, tannins) which are responsible for its antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory effects and hence supports the acclaimed efficacy of the seed as possessing very high medicinal value.

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