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Evaluation of antioxidant potential and anti-diabetic effect of ethanol seed extract of *Garcinia kola* (Bitter Kola) in albino rat

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ABSTRACT

The medicinal properties of plants have been investigated in the recent scientific world because of their potent antioxidant activities, mild side effects and economic viability. This study evaluated the antioxidant property and antidiabetic effect of ethanol seed extract of *G. kola* (bitter kola) on alloxan induced diabetic albino rats. The *in-vitro* antioxidant assay was done using standard methods. Thirty (36) albino rats were used for the *in-vivo* study and fasted for 16-18hours and thereafter induced diabetes with 150mg/ kg b.w. of alloxan monohydrate via intraperitoneal injection. The animals were divided into six groups of six (6) rats each. Group A was the normal control, group B was the positive control (treated with 5mg/kg b.w of glabeclamide), Group C was the negative control (untreated) and groups D through F were administered 500mg/kg, 250mg/kg and 125mg/kg of the extract respectively. The results showed 49.70% reduction in blood glucose level of the 500mg/kg extract treated group compared to the positive control (45.03 %) on the day 7. The antioxidant results showed a significant ($p<0.05$) dose dependent increase in its ability to scavenge free radicals. The results of this study suggested the use of *G. kola* seed for the treatment of free radical mediated diseases and management of diabetes.

KEYWORDS: Diabetes, *Garcinia kola*, antioxidant, medicinal plant, effect

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorders in which there are high blood sugar levels over a prolonged period [1,2]. The prevalence of diabetes is 8.5% among adults and serious long-term complications of this disease include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes [3,4,5,6].

Plant-derived substances have recently attracted great research interest owing to their versatile applications [7]. Specifically, medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [7]. This is due to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance. *Garcinia kola* is widely consumed habitually and used as traditional remedy for various diseases in Nigeria.

Garcinia kola commonly called bitter kola belongs to the family Guttiferae and found mainly in the tropical rain forest region of Central and West Africa [8]. It is predominant in rainforest belt of southern Nigeria [9]. The seeds are edible and are consumed as adjuvant to the true kola (*Cola nitida*) and for medicinal purposes [10]. *Garcinia kola* plant is a wonder plant because every part of it has been found to be of medicinal importance. *Garcinia kola* is used in folklore remedies for the treatment of ailments such as liver disorders, diarrhoea, laryngitis, bronchitis and gonorrhoea [11]. The seed is masticatory and used to prevent and relieve chest colds and cough and can as well be used to treat headache [12]. It is also used in treatment of jaundice, high fever and purgative [13]; stomach ache and gastritis [14]; cirrhosis and hepatitis [15]. Its antifungal [16] and antimicrobial effects has also been established. Moreover, continuous use of the synthetic anti-diabetic drugs causes side effects and toxicity [17]. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the

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dietary antioxidants vitamin A, vitamin C, and vitamin E [18]. Free radicals produced in the body during normal metabolic functions or introduced from the environment are highly reactive species [19,20]. Free radicals contribute to more than 100 disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer. The body possesses defense mechanisms like enzymes and antioxidant nutrients, which arrest the damaging properties of reactive oxygen species [21, 22]. The continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body, beyond its capacity to control them, and can cause irreversible oxidative damage [23], that is why exogenous antioxidant especially from plants are very crucial. This present study is designed to evaluate the antioxidant properties and antidiabetic effect of ethanol extract of *Garcinia kola*.

MATERIAL AND METHODS

Plant Material

Fresh fruits of *Garcinia kola* were bought from Ndolu market in umudike and identified at the plant science and biotechnology department in Micheal Okpara University of Agriculture. The seeds were peeled, chopped and air dried so as to reduce the moisture content. The dried pulp was blended and the powdered samples were stored in polythene bags and placed at room temperature until they were used.

Preparation of plant extract

Five grams (5g) of powdered samples were weighed in separate extraction bottle and 100ml of solvent (ethanol) was added to the bottle containing the powdered sample and left to stand for 48hours to allow for extraction at room temperature. Thereafter, each solution was filtered using a Whatman filter paper (No.1). The filtrates was concentrated over a water bath (45°C) and the extract was stored in an airtight container in a refrigerator until required for use [24].

ANIMALS

Thirty six (36) adult albino rats (110-145g) were obtained from the Laboratory Animal House of the department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. The animals were maintained in a well-ventilated room under 12 h light: dark cycle and were acclimatized for 2weeks before the start of the experiment. Animals were given commercial vital pelleted feed and water *ad libitum*. All the experimental procedures used in this study were performed in accordance with Animal welfare and the Institutional Animal Ethics.

INDUCTION OF EXPERIMENTAL DIABETES MELLITUS

Diabetes mellitus was induced using the method of Lenzen [25].

EXPERIMENTAL DESIGN

The albino rats that recorded an elevated blood glucose concentration above 240 g/dL were randomly assigned into six groups (A-F) of six rats (n=6) each as follows:

Group A: received distilled water and served as normal control group,

Group B: Alloxan induced diabetic rats which received 5 mg/kg Gilbenclamide and served as positive control group,

Group C: Alloxan induced diabetic rats (untreated) and served as the negative control group.

Groups D: Alloxan induced diabetic rats treated with 500mg/kg of the *Garcinia kola* extract

Group E: Alloxan induced diabetic rats treated with 250mg/kg of the *Garcinia kola* extract

Group F: Alloxan induced diabetic rats treated with 125mg/kg of the *Garcinia kola* extract

The study lasted for a period of seven days.

DETERMINATION OF BLOOD GLUCOSE LEVELS

The modified method of Beach and Turner, [26] based on the glucose oxidase principle was adopted in the determination of blood glucose concentration of the experimental rats. The blood samples were collected by cutting the tip of the tail artery of the rats, and a drop allowed to touch the sensor part of one touch glucose strips in Accuk chek glucometer. The values obtained were recorded in mg/dl. The blood glucose concentration was sampled at intervals of before induction, day 1, day 3 and day 7 of treatment, respectively.

DETERMINATION OF ANTIOXIDANTS

Assay of DPPH Scavenging Activity

2,2-diphenyl-1-purythdrazine (DPPH) scavenging activity was quantified in the presence of stable DPPH radical using a method used by Singh et al. [27].

Assay of Lipid Peroxidation Scavenging Activity

The determination of anti-lipid peroxidation activity was done by using the ethanol extract of *Garcinia kola* at different concentrations (12.5mg, 25mg, 50mg, 100mg) individually as described by Ohkwa et al. [28].

Nitric Oxide Inhibition Activity

The nitric oxide scavenging activity was conducted based on the Greiss assay method.

STATISTICAL ANALYSIS

All the data are expressed as mean \pm SEM. Statistical comparisons were performed by one-way analysis of variance (ANOVA) using SPSS vision 20.0 followed by Duncan's multiple range tests to separate the mean. The results were considered statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

The results showed that the treatment groups were significantly ($p < 0.05$) reduced in a dose dependent manner compared to the negative control group. There was significant ($p < 0.05$) increase in the negative control compared to the normal.

The results above showed significant ($p < 0.05$) increase in percentage reduction of increased blood glucose in the treated group compared to the negative control.

DISCUSSION

This study evaluated the folkloric claim that *G. kola* seed can be used to reduce the blood glucose level of diabetic patients. Diabetes mellitus is among the most common disorder in developed and developing countries [29]. The disease is increasing rapidly in most parts of the world [30]. Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels [31]. Despite the presence of known anti diabetic medicines in the pharmaceutical market, screening for new anti-diabetic sources from natural plants remains the best alternative because they contain substances which are safer for use on diabetes mellitus [9].

In this study, Diabetes was induced in rats by a single intraperitoneal injection of alloxanmonhydrate at 150 mg/kg body weight. Alloxan is a cytotoxic agent known to induce diabetes in a wide variety of animal species by damaging insulin secreting β -cell, resulting in decrease of endogenous insulin release, which paved the way for the decrease utilization of glucose by the tissues which leads to increases blood glucose level [25]. The findings of this study indicate that administration of seed extract at the graded dosage used, on alloxan-induced diabetic rats caused a significant ($P < 0.05$) reduction of the elevated glucose level (anti-hyperglycemic effect). The extract from the seed of *Garcinia kola* caused a significant maximum decrease of about 50.32 % at the extract dose of 500 mg/kg in blood glucose concentration as against

51.96 % for Gilbenclamide after 3 days' treatment and 49.70% and 45.03% on day 7 for 500mg/kg extract and Gilbenclamide (5mg/kg) respectively (Table 1). Also, it was observed that the extract at the various doses studied caused a significant reduction of the blood glucose concentration in a dose dependent manner (Table 2) and this anti-hyperglycemic effect was more pronounced after 3 days' treatment. The propensity of the extract of *Garcinia kola* to significantly decrease elevated blood glucose levels to almost normal levels is an essential trigger for the liver to return to its normal homeostasis in experimental diabetic rats. This could mean that the anti-hyperglycemic activity of this extract may be partly due to insulin release from the existing cells of the pancreas, stimulation of insulin secretion and release, regeneration of beta cells of Langerhans islets, and/or activation of enzymes responsible for glucose utilization [32]. This finding is not in isolation as it is in agreement with other studies reported by various researchers such as Richards [33]; Adebisi [34]; Srinivasan [35]; Lenzen [25], who evaluated the anti-hyperglycemic potentials of other parts of this medicinal plant.

Radical scavenging activity is very crucial to the survival of living organisms, due to the deleterious role of free radicals in foods and biological systems. There are many methods for evaluating the antioxidant activity of both natural and artificial compounds. Routinely, antioxidant potencies of plants are assessed by their ability to scavenge DPPH, an unstable compound which turns to a stable diamagnetic molecule when protonated. This stability is visually noticeable as a discoloration from purple to golden yellow. The free radical scavenging activity of ethanolic extract of *G. kola* and also that of ascorbic acid was evaluated through its ability to quench the synthetic DPPH radical. The DPPH assay constitutes a rapid and low cost method that has frequently been used for evaluation of the antioxidative potential of various natural products [36]. The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reaction of DPPH with *G. kola* was also fast but slower compared to that with ascorbic acid. Nitric oxide is a free radical produced in mammalian cells involved in regulation of various physiological processes. However, excess production of nitric oxide is associated with several diseases [37]. The development of substances to prevent the overproduction of nitric oxide has become a new research target for treating chronic inflammatory diseases [38,39]. There is a significant ($p < 0.05$) dose dependant increase in percentage Nitric oxide inhibition of the plant extract as shown in Table 3. The antilipid peroxidation also showed significant ($p < 0.05$) dose dependant increase.

Table 1: Percentage reduction on blood glucose concentration (mg/dl) of albino rats

Treatment (mg/kg)	Percentage protection after Day 1 (%)	Percentage protection after Day 3 (%)	Percentage protection after day 7 (%)
Normal control	0.43 \pm 0.73 ^c	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^c
Positive control	7.05 \pm 0.73 ^b	51.96 \pm 11.07 ^a	45.03 \pm 0.74 ^{ab}
Negative control	0.00 \pm 0.00 ^d	0.00 \pm 0.00 ^c	0.00 \pm 0.00 ^d
500 mg/kg <i>G. kola</i> seed extract	15.73 \pm 0.92 ^a	50.32 \pm 3.58 ^a	49.70 \pm 0.61 ^a
250 mg/kg <i>G. kola</i> seed extract	7.74 \pm 0.83 ^b	43.41 \pm 3.08 ^b	43.80 \pm 2.79 ^b
125 mg/kg <i>G. kola</i> seed extract	7.43 \pm 0.65 ^b	35.52 \pm 6.67 ^b	40.52 \pm 3.31 ^b

Values are expressed as means \pm SEM in (mg/dL). Mean value with the same superscript along the same column are not significantly different ($P < 0.05$)

Table 2: The effect of ethanol extract of *Garcinia kola* seed on blood glucose concentration (mg/dl) of albino rats

Treatment (mg/kg)	Glucose level (g/dL) before induction	Glucose level (g/dL) Day 0	Glucose level (g/dL) Day 1	Glucose level (g/dL) Day 3	Glucose level (g/dL) Day 7
Normal control	77.33 ± 0.88	79.00 ± 1.23	78.66 ± 0.90	79.00 ± 1.00	80.12 ± 0.73
Positive control	77.33 ± 0.88	267.00 ± 25.23	249.66 ± 24.90	163.66 ± 6.00	90.00 ± 4.04
Negative control	75.66 ± 2.84	262.00 ± 22.53	379.33 ± 38.77	500.33 ± 13.34	560.66 ± 9.59
500 mg/kg <i>G. kola</i> seed extract	76.33 ± 0.33	289.00 ± 6.92	249.66 ± 4.48	145.33 ± 10.65	83.66 ± 3.28
250 mg/kg <i>G. kola</i> seed extract	78.33 ± 0.33	281.00 ± 13.65	261.00 ± 14.36	182.00 ± 9.45	86.66 ± 2.18
125 mg/kg <i>G. kola</i> seed extract	77.66 ± 0.88	284.33 ± 9.56	264.66 ± 8.98	196.66 ± 14.62	116.00 ± 2.64

Values are expressed as means ± SEM in (mg/dL) with significant level (P<0.05)

Table 3: Antioxidant activity of *Garcinia kola*

Concentration (mg/ml)	% DPPH Radical scavenging activity	% Anti-lipid peroxidation	% Nitric oxide inhibition
Vit. C 100	76.13 ± 3.59 ^a	85.53 ± 9.24 ^a	65.67 ± 3.95 ^e
100	58.43 ± 5.03 ^b	80.80 ± 3.30 ^a	50.00 ± 2.86 ^d
50	47.57 ± 5.10 ^c	65.13 ± 5.95 ^b	36.57 ± 4.86 ^c
25	33.30 ± 4.70 ^d	51.10 ± 5.53 ^c	26.50 ± 8.34 ^b
12.5	14.90 ± 4.83 ^e	26.43 ± 9.51 ^d	11.10 ± 1.93 ^a

Values are expressed as means ± SEM in (mg/dL). Mean value with the same superscript along the same column are not significantly different (P<0.05). There is a significant (p< 0.05) dose dependant increase in all the assay

CONCLUSION

G. kola seed extract has anti-diabetic activity which is comparable to the standard drug (Glibenclamide) in alloxan induced diabetic rats and hence maybe a good alternative in the treatment of diabetes. *G. kola* seed also possess antioxidant properties and could serve as free radical inhibitors or scavengers.

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