Effect of Aqueous Seed Extract of Garcinia Kola (Bitter Kola) on Diabetic Hyperlipidemia Profile in Alloxan Induced Diabetic Rats

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Abstract

Diabetes is among the most chronic diseases and it is gaining momentum in developed and developing countries. Hence, this study is aimed at investigating the effect of aqueous seed extracts (200mg/kg and 400mg/kg) of Garcinia Kola (Bitter Kola) on diabetic hyperlipidemia profile in alloxan-induced diabetic rats. A total of 30 albino rats were divided into six groups of five rats each, Group A: (Normal control), with free access to water and animal feed, Group B: (Negative control) with no treatment, Group C: (Positive control I) with oral insulin, Group D: (Positive control II) with oral atorvastatin, Group E: (Test control I) with oral herbal low dose, Group F: (Test control II) with oral herbal high dose. At the end of the experiment, rats were fasted for 24 hours and blood samples were collected under chloroform anaesthesia for the estimation of fasting blood glucose, lipid profile (TG, TC, LDL and HDL) using standard techniques. The results showed that rats treated with aqueous seed extracts of Garcinia Kola and insulin lowered fasting blood glucose levels while rats treated with aqueous seed extracts of Garcinia Kola and atorvastatin significantly lowered TG, TC, LDL concentrations while significantly elevating HDL concentrations when compared to untreated control in a dose dependent fashion. The phytochemical analysis showed the presence of Alkaloids, flavonoids, saponins and tannins. These observations lend support to its folkloric use in the management of diabetes mellitus.

Keywords: Garcinia kola seed, Phytochemicals, Diabetes mellitus and hyperlipidemia.

ABSTRACT

Diabetes is among the most chronic diseases and it is gaining momentum in developed and developing countries. Hence, this study is aimed at investigating the effect of aqueous seed extracts (200mg/kg and 400mg/kg) of Garcinia Kola (Bitter Kola) on diabetic hyperlipidemia profile in alloxan-induced diabetic rats. A total of 30 albino rats were divided into six groups of five rats each, Group A: (normal control), with free access to water and animal feed, Group B: (Negative control) with no treatment, Group C: (Positive control I) with oral insulin, Group D: (Positive control II) with oral atorvastatin, Group E: (Test control I) with oral herbal low dose, Group F: (Test control II) with oral herbal high dose. At the end of the experiment, rats were fasted for 24 hours and blood samples were collected under chloroform anaesthesia for the estimation of fasting blood glucose, lipid profile (TG, TC, LDL and HDL) using standard techniques. The results showed that rats treated with aqueous seed extracts of Garcinia Kola and insulin lowered fasting blood glucose levels while rats treated with aqueous seed extracts of Garcinia Kola and atorvastatin significantly lowered TG, TC, LDL concentrations while significantly elevating HDL concentrations when compared to untreated control in a dose dependent fashion. The phytochemical analysis showed the presence of Alkaloids, flavonoids, saponins and tannins. These observations lend support to its folkloric use in the management of diabetes mellitus.

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Highlights of this paper

- This research was aimed at evaluating the effects of Garcinia kola seed on blood glucose and lipid profile of alloxan-induced diabetic rats.
- Despite the existing pharmacotherapy, it is still difficult to attain adequate glycemic control amongst many diabetic patients due to the progressive decline in β-cell function of the pancreas.
- The need to discover and develop more effective hypoglycaemic and hypolipidemic agents with minimum side effects became essential.

1. INTRODUCTION

Diabetes Mellitus (DM) is among the most common disorder in developed and developing countries [1]. It is a disorder whereby there is a high blood sugar level over a long period of time, due to the inability of pancreas to produce enough insulin or cells of the body not responding properly to the insulin produced. Hyperglycemia in diabetic patient is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels. Nevertheless, the understanding of lipid and lipoprotein metabolism in diabetes is complex, based on the recognition that diabetes is metabolically heterogeneous. The most important defect in insulin deficient subjects appears to be deficiency of lipoprotein lipase, which is responsible for the clearance of the triglyceride rich lipoprotein [2]. Hyperlipidemia is a disorder characterized by elevated Total cholesterol (TC), Triglyceride (TG), low density lipoprotein (LDL) and reduced High Density Lipoprotein (HDL) as the causes may or may not be associated with cholesterol concentrations [3, 4]. Several factors are likely to be responsible for diabetic dyslipidemia which includes insulin effects on liver apoprotein production, regulation of lipoprotein lipase, actions of cholesteryl ester hydrolase and peripheral actions of insulin on adipose and muscle [5].

The use of medicinal plants in managing diabetes and its complications is currently on the increase [6]. Management is directed at improving glycemic control and increasing antioxidant potential of the system thereby normalizing diabetic complications of severe hypertriglyceridemia and the risk of occlusive atherosclerosis [5]. Garcinia kola, otherwise known as bitter cola belongs to the family Guttiferae and are found mainly in the tropical rain forest region of Central and West Africa. It is also well distributed in Asia and Europe [7]. The tree is usually cultivated within villages in southern Nigeria and grows to a height of about 12-14m high. It has been referred to as “Wonder plant”, because almost every part of it has been found to be of medicinal importance. Garcinia kola is used in folklore treatment of ailments because of its biological potentials such as antioxidant, antibacterial, antiviral, antifungal and anti-inflammatory properties [8].

Despite the existing pharmacotherapy, it is still difficult to attain adequate glycemic control amongst many diabetic patients due to the progressive decline in beta-cell function of the pancreas. The need to discover and develop more effective hypoglycaemic and hypolipidemic agents with minimum side effects became apparent. The study therefore sets out to evaluate the effects of Garcinia kola seed on blood glucose and lipid profile of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Materials/Apparatus

Fresh seeds of Garcinia kola, Breaker, conical flasks, crucible, filter papers, pipettes, oven, weighing balance, knife, measuring cylinder, electric grinder, syringes, needles, water bath, sterile sample bottles, desiccator.
2.2. Reagents/Chemical Utilized

Alloxan-monohydrate (St Louis, MD, USA), Cholesterol (Teco Diagnostics, USA), Triglyceride lipo reagent (Teco Diagnostics USA), and HDL cholesterol reagent (Agape Diagnostics, Switzerland), insulin (Novolog USA), Atorvastatin (Unipex USA).

2.3. Plant Sample Collection

Fresh Garcinia Kola seeds were purchased from new market (Ahiaohuru) Aba, Abia State Nigeria. The plant seeds (Garcinia kola) were identified and authenticated by Dr. Ndukwe Okorie, a Botanist of Department of Biology of Abia State Polytechnic, Aba.

2.4. Experimental Animals

A total of 30 healthy albino rats weighing between 80 – 130g were purchase from the animal house of university of Nigeria Nsukka Enugu State and were kept in a well-ventilated animal house of the Department of Biochemistry of the Polytechnic. They were given free access to fresh water and standard animal feed diet for about 7 days before the start of the experiment.

3. METHODOLOGY

3.1. Plant Sample Preparation

The testa of seeds were removed after which the seeds were washed and cut into pieces using a kitchen knife. The resulting pellets were air dried at room temperature for 7 days. Then, the dried seed pellets were grinded into fine powder using a clean electric grinder. The grinded samples were sieved using a 10um mesh sieves, after which it was stored in an airtight glass bottle for further analysis.

3.2. Plant Aqueous Extract Preparation

The grinded Garcinia Kola seed samples (50g) were soaked in 400ml of hot water (50°C – 70°C) for 6 hours. The mixture was filtered into a clean dry white handkerchief with funnel. Fresh filtrate (extract of Garcinia kola) was prepared on daily basis and used to administer orally to the test animals.

3.3. Phytochemical Screening

A small quantity of the Garcinia Kola seed powder was taken for phytochemical screening using the methods described in Ukpabi et al. [9]. The intensity of the coloration determines the abundance of the compounds.

3.4. Induction of Diabetes

A single dose of a freshly prepared alloxan monohydrate in normal saline at a dose of 150mg/kg body weight (10), were injected intraperitoneally into the rats. Blood samples were collected by vein tapping and each monitored for glucose level using glucometer. After 3 days, rats that had blood glucose level above 250 mg/dl were considered diabetic and were selected for the study.

3.5. Experimental Design and Treatment

The 30 albino rats were divided into six groups with five rats in each group, animals in treated groups were orally administered via force feeding as followings:

Group A (Normal control): Clean water and animal feed at free access.
Group B (Negative control): Ip alloxan (150mg/kg) with no treatment.

Group C (Positive control I): Ip alloxan (150mg/kg) and oral insulin (40mg/kg).

Group D (Positive control II): Ip alloxan (150 mg/kg) and oral atorvastatin (30mg/kg).

Group E (Test control I): Ip alloxan (150mg/kg body) and oral herbal low dose (200mg/kg).

Group F (Test control II): Ip alloxan (150mg/kg) and oral herbal high dose (400mg/kg).

Subsequently, treatment regime of hyperglycemia and hyperlipidemia started on the 4th and 8th day of the experiment respectively.

3.6. Collection of Blood Sample

At the end of the experiment, the animals were fasted for 6 hours, anaesthetized with chloroform and sacrificed. Blood sample were collected and centrifuged. The serum triglyceride, total cholesterol, high density lipoprotein, low density lipoprotein and blood glucose level were determined using standard methods.

3.7. Biochemical Analysis

3.7.1. Lipid Profile Assay

The lipid parameters were estimated according to the methods described in Ukpabi, et al. [2]. The Total Cholesterol, Triglyceride, High-Density Lipoprotein were measured using commercial kits and Low-Density Lipoprotein was calculated.

3.8. Glucose Estimation

Blood glucose level was estimated using glucose oxidase method as described in Ukpabi, et al. [2].

3.9. Statistical Analysis

These values were recorded as mean ± standard deviation, students t-test and computer software package SPSS were also used for the analysis P<0.05 was considered statistically significant.

4. RESULTS

4.1. Phytochemical Composition of Garcinia Kola Seed

Results obtained from Table 1 shows that aqueous extract of *Garcinia Kola* seed gave positive reactions for flavonoids, saponins, tannins and alkaloids. Saponin gave a more profound coloration than other phytochemical compounds.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
</tbody>
</table>

(+) present at low level, (++) present at moderate level, (+++) present at high level.

4.2. Serum Glucose Concentrations of Alloxan Induced Albino Rats Following Oral Administration of Aqueous Seed Extract of Garcinia Kola

Result obtained from Table 2 on blood glucose concentration of alloxan induced albino rats showed that the extracts of *Garcinia kola* seed decreased blood glucose level in a dose-dependent fashion. The decrease in glucose
concentration was significant in the groups treated with the extracts when compared to the control group that received animal feed diet and water.

<table>
<thead>
<tr>
<th>Duration Group</th>
<th>Initial BGC</th>
<th>Day 4 BGC</th>
<th>Day 8 BGC</th>
<th>Day 12 BGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>83 ± 2.30</td>
<td>83.40 ± 3.20</td>
<td>84 ± 1.98</td>
<td>85.5 ± 2.91</td>
</tr>
<tr>
<td>B</td>
<td>280.67 ± 1.29</td>
<td>279.62 ± 1.21</td>
<td>281.12 ± 1.10</td>
<td>278.62 ± 1.05</td>
</tr>
<tr>
<td>C</td>
<td>280 ± 1.34</td>
<td>210.25 ± 1.91</td>
<td>160.12 ± 1.53</td>
<td>100.13 ± 1.91</td>
</tr>
<tr>
<td>E</td>
<td>279 ± 2.03</td>
<td>200.43 ± 1.01</td>
<td>151.30 ± 0.90</td>
<td>99.10 ± 2.06</td>
</tr>
<tr>
<td>F</td>
<td>286 ± 0.52</td>
<td>190.51 ± 2.06</td>
<td>120 ± 10.00</td>
<td>87.12 ± 3.01</td>
</tr>
</tbody>
</table>

Table 2. Effect of aqueous seed extract of garcinia kola on blood glucose concentration (mg/dl) of alloxan induced albino rats.

4.3. Serum Lipid Levels in Albino Rats Following the Induction of Alloxan Monohydrate (150mg/Kg)

The induction of albino rats with alloxan monohydrate produced an elevated Total cholesterol, Triglyceride and Low-Density Lipoprotein concentrations which were significantly higher than the normal control and decrease in High Density Lipoprotein as shown in Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80.5 ± 1.34</td>
<td>81.87 ± 1.91</td>
<td>38.64 ± 2.96</td>
<td>28.82 ± 1.33</td>
</tr>
<tr>
<td>B</td>
<td>149.89 ± 3.33</td>
<td>159.21 ± 1.51</td>
<td>23.53 ± 1.59</td>
<td>53.19 ± 2.67</td>
</tr>
</tbody>
</table>

Table 3. Effect of alloxan monohydrate on lipid profile of albino rats.

4.4. Serum Lipid Levels in Diabetic Rats Following Oral Administration of the Aqueous Seed Extract of Garcinia Kola

The results from Table 4 showed that total cholesterol, triglyceride, and low-density lipoprotein concentrations in alloxan monohydrate intoxicated rats decreased when compared to normal control after the administration of the aqueous extracts of Garcinia kola seed (200mg/kg and 400mg/kg). Atorvastatin treated rats showed greater efficiency though not significant. Similarly, the decrease in HDL concentrations were reversed by the treatment with the extracts.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>13</td>
<td>119.88 ± 3.12</td>
<td>121.77 ± 3.38</td>
<td>28.99 ± 1.59</td>
<td>46.60 ± 0.95</td>
</tr>
<tr>
<td>E</td>
<td>13</td>
<td>136.99 ± 2.84</td>
<td>148.68 ± 2.59</td>
<td>27.93 ± 2.20</td>
<td>49.93 ± 2.20</td>
</tr>
<tr>
<td>F</td>
<td>13</td>
<td>107.88 ± 1.97</td>
<td>109.99 ± 2.62</td>
<td>33.19 ± 0.64</td>
<td>39.73 ± 4.01</td>
</tr>
</tbody>
</table>

Table 4. Effects of atorvastatin and aqueous seed extracts treatments of Garcinia kola on diabetic albino rats.

5. DISCUSSION

Naturally occurring compounds, known as phytochemicals are thought to be largely responsible for the protective health benefits of plant-based foods and beverages [10]. These phytochemicals which are part of a large and varied group of chemical compounds are responsible for the color, flavor, bitter and odour of plant foods. Research suggests that phytochemical-rich foods may directly decrease the risk of type 2 diabetes, most likely by reducing inflammatory and improving insulin sensitivity and indirectly by preventing weight gain [10]. Positive effects on fasting blood glucose levels and insulin sensitivity have been found specifically with the consumption of polyphenols in laboratory animal studies [11]. Dietary phytoconstituents may inhibit carbohydrate digestion and
glucose adsorption in the intestine stimulates glucose release from the liver, activates insulin receptors and glucose uptake in insulin-sensitive tissue and modulate intracellular signaling pathways and gene expression [11].

In this present study, the qualitative photochemical screening of *Garcinia Kola* seed was found to contain the following; flavonoids, saponins, tannins and alkaloids as shown in Table 1. These findings agree with an earlier report of Adegboye, et al. [8] who recorded similar result for the aqueous extract of *Garcinia kola*. They further reported the antibacterial, antiviral, antifungal and anti-inflammatory properties maybe attributed to the presence of these bioactive compounds [12].

Alloxan induced diabetes by partially destroying the insulin-secreting cells of the pancreas islets cells in experimental animals leading to hyperglycemia and hence diabetes [8]. Results from Table 2 shows that oral administration of different doses of *Garcinia kola* seed extracts (400mg/kg and 200mg/kg), significantly reduced the increased blood glucose levels in a dose-dependent fashion in diabetic animals following treatment compared to positive control. This observation is in agreement with the reports of Iwu, et al. [13] and Adeneye and Olagunju [14]. This could be attributed to the presence of flavonoids which promotes the entry of glucose into the cells, stimulating glycolytic enzyme or inhibiting glucose-6-phosphate in the liver and thus reducing the release of glucose in the blood. Apart from hyperglycemia, the diabetic animals developed hypercholesterolemia and hyperlipidemia after some days.

The hyperlipidemia associated with diabetes mellitus may be as a result of accelerated de novo hepatic biosynthesis and release of VLDL-C (Very Low-Density Lipoprotein) without a corresponding increase in the rate of clearance from the blood by lipoprotein lipase whose activity is dependent on insulin-glucagon ratio [15]. The induction of alloxan monohydrate showed an increase in total cholesterol, Triglyceride, and low-density lipoprotein while high density lipoprotein decreased. Treatment with the doses of aqueous *Garcinia kola* seed extracts reversed the effect of the intoxication. Similarly, Atorvastatin treated rats (positive group II) normalized the intoxication effect with greater efficiency, though not significant (p>0.05).

Lowering of serum cholesterol levels decreases the risk of coronary heart disease [17]. A number of drugs called statins are potent competitive inhibitors of HMG-CoA Reductase, (3-hydroxy-3methylglutarly-coenzyme A reductase) and can be given to diabetic patients to raise HDL levels [5]. HMG-CoA reductase is an interconvertible enzyme that plays a principal role in the synthesis and regulation of cholesterol [15]. Treatment with the extracts and Atorvastatin had lipid lowering potentials, as hypertriglyceridemia did not persist which is commonly due to an increase production of VLDL in the liver [16]. This observation that dyslipidemia in alloxan induced diabetic rats were ameliorated using aqueous extracts of *Garcinia kola* seed could be attributed to the increase in insulin level via the regeneration of the Beta cells [17].

**6. CONCLUSION**

Moderate consumption of *Garcinia Kola* seed could be helpful in the reduction of hyperglycemia and complication associated with diabetes mellitus especially hyperlipidemia.

**REFERENCES**


