Effect of *Asparagus africanus* on Glucose Level and Enzymatic Antioxidants: Antidiabetic Study

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The plant, *Asparagus africanus* is used for treating diabetes mellitus and other diseases in traditional medicine. This research work was aimed at determining the antidiabetic and antioxidant effect of *Asparagus africanus* root aqueous extract in Wistar rats induced with diabetes using streptozotocin. Oral administration of 10 g glucose/kg body weight was used for physiological induction of diabetes and intaperitoneal administration of 60 mg streptozotocin/kg body weight was used for chemical induction of diabetes. The animals were administered 100, 200 and 400 mg/kg *Asparagus africanus* extract orally. The concentration of glucose in the blood was measured in minutes and days. The concentration of enzymatic antioxidants (catalase and reduced glutathione [GSH]) in the liver tissues and thiobarbituric acid reactive substance (TBARS) level was determined. *Asparagus africanus* root aqueous extract decreased the concentration of blood glucose and increased antioxidant enzymes (catalase and GSH) levels significantly (p < 0.05) in 21 days treated animals when compared to the untreated animals (control). *Asparagus africanus* extract decreased TBARS concentration significantly (p < 0.05) when compared with the control. *A. africanus* extract at 400 mg/kg had a higher antidiabetic and antioxidant activities when compared with 100 mg/kg. This research work suggests that *Asparagus africanus* root posses antidiabetic and antioxidant properties; and it also reduced lipid peroxidation in 21 days treated diabetic rats.

Keywords: Asparagus africanus; root; diabetes; antioxidants, Wistar rats.
1. INTRODUCTION

Diabetes mellitus occurs when the beta cells in the pancreas are unable to produce a hormone known as insulin; this disease condition is known as type 1 diabetes mellitus [1]. Diabetes mellitus also occurs when body cells are resistant to insulin or as a result of the pancreas not producing enough insulin to promote glucose uptake by body cells; this is another type of diabetes known as type 2 [1]. The World Health Organization (WHO) also reported that there is an increase in the number of people with diabetes [2]. Prevalence of diabetes globally was estimated to be 463 million in 2019, 578 million by 2030 and 700 million by 2034 [3]. Current drugs used for the treatment of diabetes causes unfavorable effects such as hypoglycemia and weight gain [4]. In the last few decades more studies are carried out on the use of plants with low or no toxic properties for the treatment of diabetes. In diabetic condition there is a decrease in the concentration of antioxidants because of the increase in free radicals [5,6]. High level of these radicals may cause lipid peroxidation and damage to body cells [6]. Antioxidants such as superoxide dismutase, catalase produced in the body and flavonoids gotten from plants help neutralize the harmful effect of free radicals [7,8]. Plants with antidiabetic properties also have antioxidant effect [9].

Asparagus africanus Lam. is a climbing shrub belonging to the family liliaceae. It is widely spread in tropical Africa [10]. In Nigeria, the common names of Asparagus africanus are Aluki (in Yoruba) and Shekan bera (in Hausa) [11,12]. In traditional medicine, Asparagus africanus is used for treating stomach ache, head ache, haemorrhoids [13], syphilis, malaria and gonorrhoea [10,14]. The root of the plant is used for the treatment of hypertension, epilepsy [15], chronic gout [16] and to ease childbirth [17]. Asparagus africanus contains terpenoids and saponins, that help in cellular detoxification [18,19]. Other genus of Asparagus which include Asparagus racemosus is used for treating diseases such as dysentery and diabetes due to the presence of bioactive compounds [20].

In this study, the fasting blood glucose reduction caused by Asparagus africanus root aqueous extract was determined in Wistar rats induced with diabetes using glucose (physiological induction of diabetes) and streptozotocin (chemical induction of diabetes). Also, the ability of Asparagus africanus root to prevent or reduce lipid peroxidation and to exert antioxidant activity in Wistar rats hepatic tissues was examined.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Asparagus africanus root was collected from a farm in Bauchi road, Jos, Plateau State. The identification of the plant was at the Herbarium, Botany Department, Obafemi Awolowo University (O.A.U.), Ile-Ife, Nigeria.

2.2 Preparation of Aqueous Extract

The root of the plant was cleaned by washing it under a running tap water. It was then dried at room temperature. Thereafter, it was ground to powder and macerated in distilled water for three days. It was then filtered after maceration for three days using a sieve and filter paper (Whatman no. 1). Rotary evaporator (at 45°C) was used for concentrating the filtrate. A freeze dryer was used for removing the water in the filtrate in order to obtain a dried sample (extract) [21]. The dried Asparagus africanus root aqueous extract was then preserved by storing it in a refrigerator before using it for the study.

2.3 Animals

The animal experiment (PG/Pharmcol/2012/02) was carried out using the guidelines approved by Pharmacology Department, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria.

Eight – nine weeks old Wistar rats (150-200g) of both sexes (total of 30 males and 30 females) gotten from Animal House, Pharmacology Department, were kept in cages that are ventilated. Broilers mash was used for feeding the animals and they had free access to water. The Wistar rats were allowed to get used to the environment for two weeks prior to commencing the research work.

2.4 Acute Toxicity Studies (Median Lethal dose [LD50] Determination)

The median lethal dose of Asparagus africanus root aqueous extract in Wistar rats via oral route was ascertained by carrying out acute toxicity studies [22].
2.5 Glucose Loading

After twelve hours fasting of the animals, 10/g/kg glucose was administered orally to the animals. Blood was taken from the vein of the animal tail after thirty minutes of administering glucose [23,24]. The fasting blood glucose concentration was measured with glucometer [21]. Animals with ≥ 7.0 mmol/l fasting blood glucose concentration were used for the experiment.

2.6 Induction of Diabetes Using Streptozotocin

Streptozotocin at 60 mg/kg prepared in distilled water was used to induce diabetes in Wistar rats (via intraperitoneal route) after twelve hours fasting of the animals. After seventy-two hours, blood was taken from the vein of the animal tail [23,24] and the concentration of the fasting blood glucose was determined. Animals with concentration of the fasting blood glucose > 11.1 mmol/l were taken for the experiment.

2.7 Administration of Doses

There was six groups with five Wistar rats in each group [26,27]. Groups 1 was orally administered distilled water (5 mL/kg) to normoglycemic Wistar rats. Asparagus africanus root aqueous extract at 100, 200 and 400 mg/kg was orally administered to diabetic Wistar rats in groups 2, 3 and 4 respectively. The diabetic animals in group 5 were orally administered glibenclamide (5 mg/kg). Group 6 (control group) was orally administered distilled water (5 mL/kg) to diabetic Wistar rats. In glucose loaded rats (15 males and 15 females) the administration was once. Whereas to Wistar rats (15 males and 15 females) that were induced diabetes with streptozotocin, administration was daily for twenty-one days.

2.8 Antidiabetic and Antioxidant Studies

The concentration of the fasting blood glucose in the blood taken from the vein in the tail of the animals was determined in minutes and days [25]. On the 21st day, Wistar rats that were induced diabetes with streptozotocin were sacrificed. The liver of the animals harvested were washed using cold normal saline and thereafter with 0.15 M Tris-HCl (pH 7.4). The homogenate of liver (10% w/v) was used for the determination of the concentration of thiobarbituric acid reactive substance level (absorbance was read at 532 nm) [28], reduced glutathione (absorbance was read at 412 nm) [29] and catalase (absorbance was read at 570 nm) [30].

2.9 Statistical Analysis

One-way analysis of variance was used for analyzing the results. Tukey’s pairwise comparisons tests was used after the One-way analysis of variance at \( P < 0.05 \) with PAleontological Statistics software (3.23) [31].

3. RESULTS AND DISCUSSION

In order to determine the doses of Asparagus africanus root aqueous extract that will be used for the study, acute toxicity study was first carried out because it is the first step in pharmacological studies [22]. In this study, the aqueous extract of Asparagus africanus caused no mortality after oral administration. The median lethal dose (LD50) was ≥ 5000 mg/kg.

The animal models used in this research work for inducing hyperglycemia in Wistar rats are glucose loading via oral rout and interperitoneal injection of streptozotocin which are physiological and chemical methods respectively [32]. In diabetic condition, the concentration of blood glucose after fasting is ≥ 200 mg/dl /l (11.1 mmol) [33,34].

In this study, the aqueous extract of Asparagus africanus root at 100, 200 and 400 mg/kg caused a reduction (significantly at \( p < 0.05 \)) in the concentration of glucose in the blood of the Wistar rats that were induced diabetes using glucose loading (Fig. 1) and streptozotocin (Fig. 2) when compared with the diabetic animals that were not treated (control group). Asparagus africanus root reduced (significantly at \( p < 0.05 \)) the concentration of glucose in the blood of glucose loaded Wistar rats at 0, 30, 60 and 120 minutes when compared with 240 minutes (Fig. 1). In animals induced diabetes using streptozotocin, Asparagus africanus root also caused a reduction (significantly at \( p < 0.05 \)) in the concentration of glucose in the blood of the animals on the twenty-first day of the study when compared to earlier days (Fig. 2). The standard drug (glibenclamide) also caused a reduction (significant at \( p < 0.05 \)) in the concentration of glucose in the blood of Wistar rats that were induced diabetes using glucose loading (Fig. 1) and streptozotocin (Fig. 2). Previous in-vitro studies reported that root extract of Asparagus africanus promote glucose uptake in hepatic cells [20].
Lipid peroxidation is determined by measuring the concentration of thiobarbituric acid reactive substances concentration in the homogenate of the Wistar rats liver [35,36]. In this study, *Asparagus africanus* root aqueous extract reduced (significant at p < 0.05) thiobarbituric acid reactive substances concentration in the liver of Wistar rats that were induced with diabetes using streptozotocin when compared to the control (Fig. 3). Highest dose of *Asparagus africanus* root aqueous extract used in this study reduced (significant at p < 0.05) thiobarbituric acid reactive substances concentration at 400 mg/kg significantly (p < 0.05) when compared with the lowest dose (Fig. 3). Glibenclamide also reduced (significant at p < 0.05) thiobarbituric acid reactive substances concentration when compared to the diabetic Wistar rats that were not treated with *Asparagus africanus* root aqueous extract (Fig. 3). The increase in thiobarbituric acid reactive substances concentration in the diabetic Wistar rats that were not treated with *Asparagus africanus* root aqueous extract may be due to increase in lipid peroxidation.
Effect of *A. africanus* extract on thiobarbituric acid reactive substances concentration in Wistar rats induced with diabetes using streptozotocin

![Graph showing effect of *A. africanus* extract on TBARS concentration](image)

**Fig. 3. Effect of *A. africanus* extract on thiobarbituric acid reactive substances concentration in Wistar rats induced with diabetes using streptozotocin**

*Data: Mean ± SEM; Number of animals in a group: 5; AAE: Asparagus africanus root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide; "a" Significant (p < 0.05): control; "b" Significant (p < 0.05): 400 mg/kg AAE.

**Table 1. Effect of *Asparagus africanus* extract on enzymatic antioxidant present in the liver of Wistar rats induced with diabetes using streptozotocin**

<table>
<thead>
<tr>
<th>Sample concentration (mg/kg)</th>
<th>Reduced Glutathione (µg/mg)</th>
<th>Catalase (µmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemic rats</td>
<td>19.34 ± 0.07</td>
<td>27.93 ± 0.23</td>
</tr>
<tr>
<td>AAE at 100 (mg/kg)</td>
<td>14.67 ± 0.13 ab</td>
<td>22.00 ± 0.09 ab</td>
</tr>
<tr>
<td>AAE at 200 (mg/kg)</td>
<td>15.50 ± 0.10 a</td>
<td>23.53 ± 0.29</td>
</tr>
<tr>
<td>AAE at 400 (mg/kg)</td>
<td>16.28 ± 0.12 a</td>
<td>24.69 ± 0.24</td>
</tr>
<tr>
<td>Glibenclamide (5 mg/kg)</td>
<td>18.15 ± 0.03 a</td>
<td>26.97 ± 0.28</td>
</tr>
<tr>
<td>Control (untreated diabetic rats)</td>
<td>10.39 ± 0.16</td>
<td>13.73 ± 0.25</td>
</tr>
</tbody>
</table>

*Data: Mean ± SEM; Number of animals in a group: 5; AAE: Asparagus africanus root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide; "a" Significant (p < 0.05): control; "b" Significant (p < 0.05): 400 mg/kg AAE.

There are reports from previous studies that antioxidants found in the leaves, root, stems and fruits of plants might have a correlation with the antidiabetic effect of some medicinal plants [37]. Catalase and reduced glutathione (GSH) in the liver, are endogenous antioxidants that function in scavenging of free radicals [36,38]. *Asparagus africanus* root aqueous extract significantly (p < 0.05) increased catalase and GSH levels in Wistar rats induced with diabetes using streptozotocin (Table 1). The highest dose of *Asparagus africanus* root aqueous extract used in this study exerted a higher increase (significantly at p < 0.05) in the concentration of catalase and GSH than the lowest dose of the extract (Table 1). Glibenclamide also significantly (p < 0.05) increased GSH concentration and catalase concentration (Table 1). The results gotten from this study shows that *Asparagus africanus* root aqueous extract might posses antioxidant property.

4. CONCLUSION

In conclusion, the results suggest that *Asparagus africanus* root aqueous extract posses antidiabetic effect. The extract also increased the concentration of enzymatic antioxidant and reduced lipid peroxidation in Wistar rats that were induced diabetes using streptozotocin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal experiment (PG/Pharmcol/2012/02) was carried out using the guidelines approved by Pharmacology Department, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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