

Original Research Article

Effect of *Asparagus africanus* on Glucose Level and Antioxidant Enzymes Activities in Liver of Streptozotocin-induced diabetic Wistar rats: Antidiabetic study

ABSTRACT

Asparagus africanus is a plant used in traditional medicine for the treatment of numerous diseases including diabetes mellitus. The aim of this study is to determine the antidiabetic and antioxidant effect of aqueous root extract of *Asparagus africanus* in streptozotocin induced diabetic Wistar rats. Diabetes was induced physiologically by oral administration of 10 g glucose/ kg body weight and chemically by intraperitoneal administration of 60 mg streptozotocin/kg body weight. The Wistar rats were treated orally with *A. africanus* extract at 100, 200 and 400 mg/kg. Fasting blood glucose levels of the diabetic rats were determined at intervals of 30, 60, 120 and 240 min in glucose loaded rats and on days 4, 7, 10, 14, 18 and 21 in streptozotocin-induced diabetic rats. The effect of the extract on antioxidant enzymes (catalase and reduced glutathione [GSH]) in liver tissues and thiobarbituric acid reactive substance level was determined at the end of the experiment. The results of this study showed that aqueous root extract of *Asparagus africanus* significantly ($P < 0.05$) decreased fasting blood glucose level and increased antioxidant enzymes (catalase and GSH) levels in the liver tissue of 21 days treated diabetic rats when compared with the control (untreated animals). The extracts significantly ($P < 0.05$) decreased lipid peroxidation (thiobarbituric acid reactive substances level was decreased) when compared with the control. *A. africanus* extract at 400 mg/kg exerted more antidiabetic activity and increased antioxidant enzymes levels when compared with 100 mg/kg. In conclusion, the results of this study suggest that *Asparagus africanus* root possesses antidiabetic activity, increased antioxidant enzymes levels in the liver and reduced lipid peroxidation in 21 days treated streptozotocin-induced diabetic Wistar rats.

Key words: Antioxidant; *Asparagus africanus*; diabetes; root, Wistar rats

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that occurs as a result of the inability of the beta cells of the pancreas to produce insulin (type 1 DM) [1]. It also occurs when cells in the body are resistant to the insulin that is being produced by the pancreas (type 2 DM) or as a result of the pancreas not producing enough insulin to promote glucose uptake by body cells [1]. The prevalence of diabetes globally was estimated in 2019 to be 463 million (9.3%) rising to 578 million (10.2%) by 2030 and 700 million (10.9%) in 2034 [2]. The World Health Organization (WHO) reported that the number of people with diabetes has increased substantially between 1980 and 2014, rising from 108 million to current numbers that are approximately four times higher [3]. There are reports that current antidiabetic drugs have side effects which include hypoglycemia, weight loss and weight gain [4]. These recent reports have led to the increase in research in the area of possible antidiabetic effect of medicinal plants with little or no side effects in the last few decades.

Oxidative stress co-exists with a reduction in the anti-oxidant status due to the presence of high levels of free radicals (reactive oxygen species and reactive nitrogen species) in diabetic condition [5]. High level of these radicals may cause lipid peroxidation and damage to body cells. Also, there is a reduction in antioxidant levels due to the presence of high levels of free radicals in people with diabetic condition [6]. Endogenous antioxidants produced in the body and exogenous antioxidants gotten from the diet help neutralize the harmful effect of free radicals. Endogenous antioxidants include catalase, glutathione reductase, superoxide dismutase [7] and exogenous antioxidants received from the diet include vitamin C, vitamin E and flavonoids [8]. Medicinal plants are major sources of antioxidants and some these plants also have antidiabetic effect [9].

Asparagus africanus Lam. is a climbing shrub belonging to the family liliaceae. It is widely spread in tropical Africa [10]. In Nigeria, *Asparagus africanus* is commonly known as “aluki” in Yoruba and “Shekan bera” in Hausa [11,12]. The plant is used in ethno-medicine for the treatment of 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, which are responsible for removal of toxic substances from the cell membrane [18,19]. *Asparagus* genus, such as *Asparagus curillus* and *Asparagus racemosus*, contain a diverse array of bioactive compounds that treat ailments such as dysuria, diabetes, inflammation and dysentery [20].

In this study the antidiabetic effect of aqueous root extract of *Asparagus africanus* was determined in glucose loaded and streptozotocin-induced diabetic Wistar rats. The effect of the root extract on lipid peroxidation and antioxidant enzymes in the liver of Wistar rats was also investigated.

2. MATERIAL AND METHODS

2.1 Plant collection and identification

Asparagus africanus root was collected from a farm in Bauchi road, Jos, Plateau State. The plant was authenticated at Ife-Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2 Preparation of Aqueous Extract

The root of the plant was washed, air dried, pulverized and macerated in distilled water for 72 hours before undergoing filtration using muslin cloth and cotton wool in funnel. The filtrate was then concentrated into a solid paste in vacuo at 45 °C using a rotary evaporator [21] and was then freeze dried. The dried extract was then stored in a refrigerator at 4 °C prior to use.

2.3 Animals

The animal experiments (PG/Pharmacol/2012/02) were performed according to the approved guidelines of Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. Eight – nine weeks old Wistar rats of both sexes (total of 30 males and 30 females) weighing between 150-200 g were obtained from Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were kept in well ventilated aluminium cages and fed with Vita feed and were given water ad libitum. The rats were allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for two weeks before the start of the experiment.

2.4 Acute toxicity studies (Median lethal dose [LD₅₀] determination)

The (LD₅₀) of the root extract was determined in Wistar rats through oral route (p.o.) [22].

2.5 Glucose loading

Glucose at 10 g/kg body weight was administered orally (p.o.) to Wistar rats that were fasted overnight (for 12 hours). After 30 min of glucose administration, blood was drawn from the vein of the tail [23,24] and the fasting blood glucose level was measured using glucometre [21]. Rats with fasting blood glucose level (FBGL) above 7.0 mmol/l were taken for the study.

2.6 Induction of diabetes using streptozotocin

The animals (rats) were fasted overnight (for 12 hours) and diabetes was induced by a single intraperitoneal injection (i.p.) of freshly prepared solution of streptozotocin (60 mg/kg) in distilled water. After 72 hours, blood was drawn from the vein of the tail [23,24] and the fasting blood glucose level (FBGL) was measured using glucometer. Animals with FBGL above 11.1 mmol/l were selected for the experiment. [25].

2.7 Administration of doses

The Wistar rats were randomly divided into six groups of five rats per group [26,27]. Groups 1 was orally administered 5 ml distilled water/kg body weight (b.wt.) normoglycemic Wistar rats. Groups 2 – 4 were orally administered 100, 200 and 400 mg extract/kg b.wt. diabetic Wistar rats respectively. Group 5 was orally administered 5 mg glibenclamide/kg b.wt. diabetic Wistar rats and Group 6 was administered 5 ml distilled water/kg b.wt. diabetic Wistar rats. The administration was once to glucose loaded rats (15 males and 15 females) and daily for 21 days to streptozotocin-induced diabetic Wistar rats (15 males and 15 females).

2.8 Antidiabetic and antioxidant studies

Blood was drawn from the vein of the Wistar rats tail [23,24] and FBGL was measured at intervals of 30, 60, 120 and 240 min in glucose loaded rats on day 0, 4, 7, 10, 14, 18 and 21 in streptozotocin-induced diabetic Wistar rats [25]. On the 21st day, streptozotocin-induced diabetic Wistar rats were sacrificed and the rats liver were immediately harvested, washed in ice cold normal saline, then in 0.15 M Tris-HCl (pH 7.4), blotted dry and weighed. Liver homogenate (10% w/v) prepared in 0.15 M Tris-HCl, pH 7.4 buffer was used for the estimation of lipid peroxidation by measuring 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

The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparisons tests at 95% ($P < 0.05$) level of significance using **PAleontological Statistics software (version 3.23)** [31]. All results were expressed as mean \pm standard error of mean (SEM).

3. RESULTS AND DISCUSSION

In order to determine the median lethal dose (LD_{50}) of an extract, acute toxicity study 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 (LD_{50}) was ≥ 5000 mg/kg.

Several animal models have been developed for testing anti-diabetic agents over the years. In this study, two models were employed in the induction of hyperglycaemia. These models include oral glucose loading (physiological induction of diabetes mellitus) and streptozotocin-induced diabetes (chemical induction of diabetes mellitus) [32]. In diabetic condition, the fasting blood glucose level is ≥ 11.1 mmol (200 mg/dl/l) [33,34].

In this study, the aqueous extract of *Asparagus africanus* at 100, 200 and 400 mg/kg exerted a significant ($P < 0.05$) decrease in fasting blood glucose level (FBGL) of glucose loaded Wistar rats (Fig. 1) and streptozotocin-induced diabetic Wistar rats (Fig. 2) when compared with the control. The extract also caused a significant ($P < 0.05$) decrease in FBGL of glucose loaded Wistar rats at 0, 30, 60 and 120 minutes when compared with 240 minutes (Fig. 1). There was also a significant ($P < 0.05$) decrease in FBGL in streptozotocin-induced diabetic Wistar on day 0, 4, 7, 10, 14 and 18 when compared with day 21 (Fig. 2). The standard drug (glibenclamide) also exerted a significant a ($P < 0.05$) decrease in FBGL in glucose loaded Wistar rats (Fig. 1) and streptozotocin-induced diabetic Wistar (Fig. 2). Previous in-vitro studies reported that root extract of *Asparagus africanus* promote glucose uptake in hepatic cells [20].

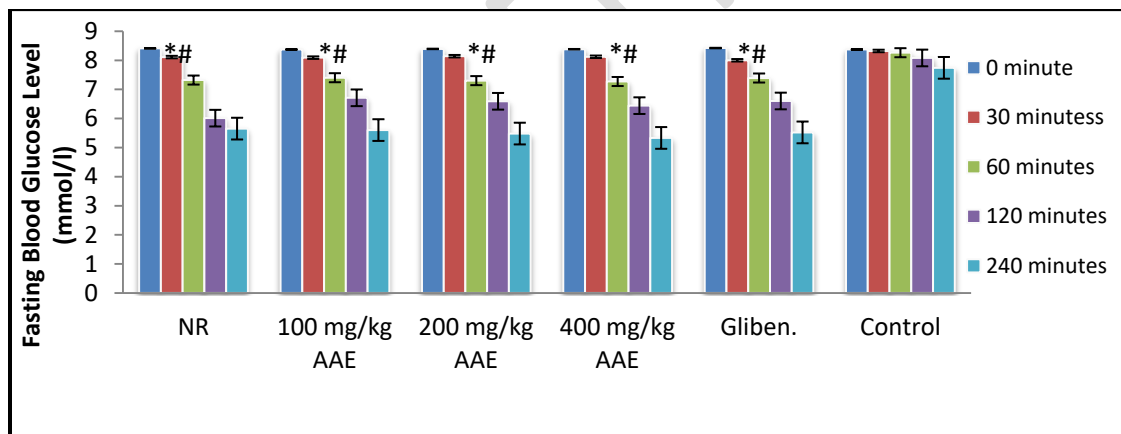


Fig. 1: Effect of *Asparagus africanus* extract on fasting blood glucose levels (mmol/L) in glucose loaded Wistar rats

Values are given as Mean \pm SEM; $n = 5$; AAE: *Asparagus africanus* root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide. * $P < 0.05$ compared to the untreated diabetic rats (control); # $P < 0.05$ compared to fasting blood glucose level at 240 minutes.

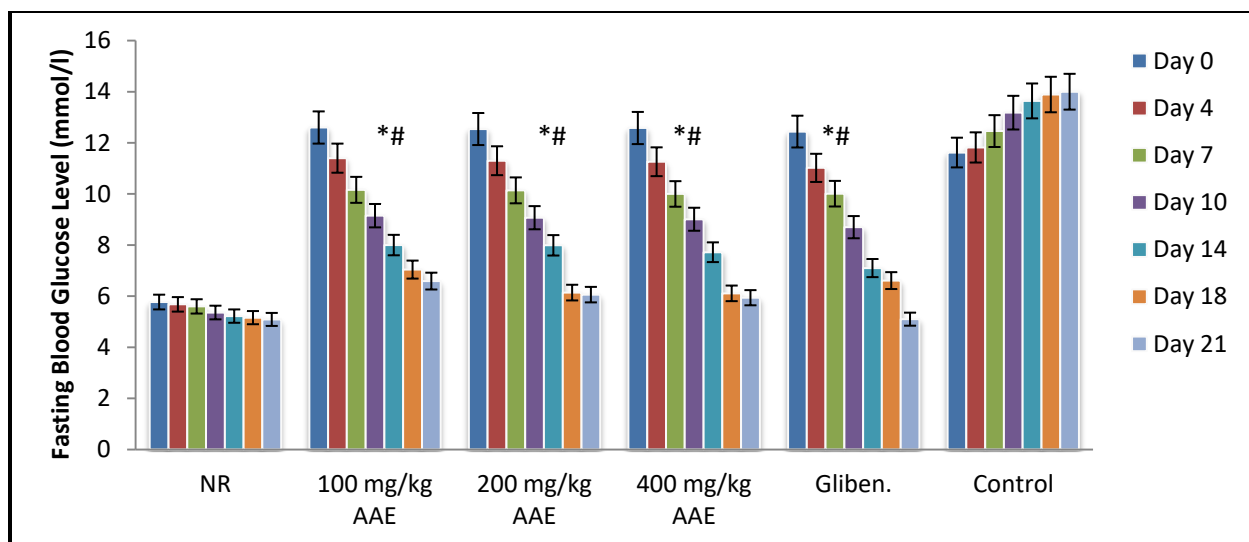


Fig. 2: Effect of *Asparagus africanus* extract on fasting blood glucose levels (mmol/L) of streptozotocin-induced diabetic Wistar rats.

Values are given as Mean \pm SEM; n = 5; AAE: *Asparagus africanus* root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide. * $P < 0.05$ compared to the control; # $P < 0.05$ compared to fasting blood glucose level in day 21.

The level of thiobarbituric acid reactive substances in liver homogenate helps in determining lipid peroxidation [35,36]. In this study, the aqueous root extract of *Asparagus africanus* significantly ($P < 0.05$) decreased the level of thiobarbituric acid reactive substance (TBARS) in the liver of streptozotocin-induced diabetic Wistar rats in a dose dependent manner when compared to the control (Fig. 3). The extract at 400 mg/kg caused a significant decrease in the level of TBARS when compared to 100 mg/kg (Fig. 3). Glibenclamide also caused a significant ($P < 0.05$) decrease in the level of thiobarbituric acid reactive substance (TBARS) when compared to the control (Fig. 3). The increase in TBARS level in untreated streptozotocin-induced diabetic Wistar rats suggests enhanced lipid peroxidation.

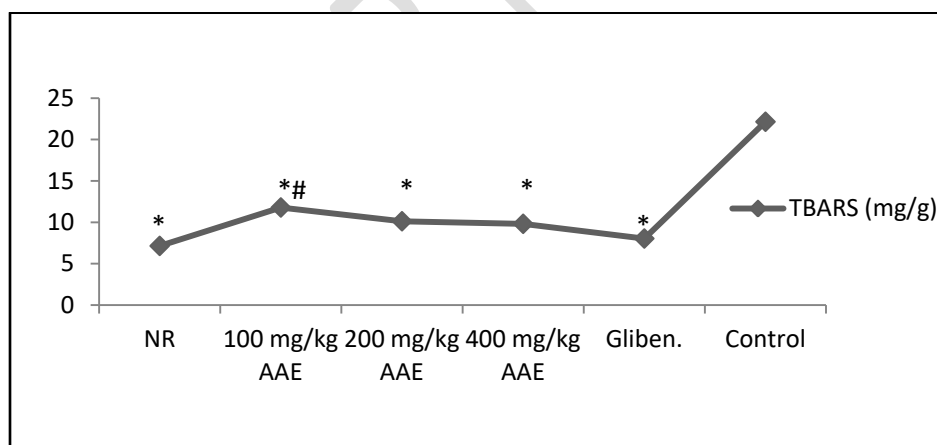


Fig. 3: Effect of *A. africanus* extract on lipid peroxidation in streptozotocin-induced diabetic Wistar rats.

Values are given as Mean \pm SEM; n = 5; AAE: *Asparagus africanus* root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide. * $P < 0.05$ compared to the control; # $P < 0.05$ compared to 400 mg/kg AAE.

There are reports from previous studies that presence of antioxidants in different parts of plants including the leaves, root, stems and fruits 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 the scavenging of free radicals and prevention of lipid peroxidation [36,38]. The aqueous root extract of *Asparagus africanus* significantly ($P < 0.05$) increased catalase and GSH levels in the liver of streptozotocin-induced diabetic Wistar when compared to the control (Table 1). The extract at 400 mg/kg caused a more significant increase in catalase and GSH levels when compared to 100 mg/kg (Table 1). Glibenclamide also caused a significant ($P < 0.05$) increase in GSH and catalase levels when compared to the control (Table 1). These results suggest that *Asparagus africanus* extract has antioxidant property.

Table 1: Effect of *Asparagus africanus* extract on antioxidant enzymes in the liver of streptozotocin-induced diabetic Wistar rats

Sample concentration (mg/kg)	Reduced Glutathione ($\mu\text{g/mg}$)	Catalase ($\mu\text{mol/min/mg}$)
Normoglycemic rats	19.34 \pm 0.07*	27.93 \pm 0.23*
100 mg/kg AAE	14.67 \pm 0.13*#	22.00 \pm 0.09*#
200 mg/kg AAE	15.50 \pm 0.10*	23.53 \pm 0.29*
400 mg/kg AAE	16.28 \pm 0.12*	24.69 \pm 0.24*
5 mg/kg Glibenclamide	18.15 \pm 0.03*	26.97 \pm 0.28*
Control (untreated diabetic rats)	10.39 \pm 0.16	13.73 \pm 0.25

Values are given as Mean \pm SEM; n = 5; AAE: *Asparagus africanus* root aqueous extract; NR: Normoglycemic rats; Gliben.: 5 mg/kg glibenclamide. * $P < 0.05$ compared to the control; # $P < 0.05$ compared to 400 mg/kg AAE.

4. CONCLUSION

The results of the study suggest that aqueous root extract of *Asparagus africanus* possesses antidiabetic effect. The extract also increased antioxidant enzymes levels in the liver and reduced lipid peroxidation in 21 days treated streptozotocin-induced diabetic Wistar rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist. Also, the research was funded by personal efforts of the authors.

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