

Original Research Article

Antidiabetic Activity of *Plumeria Alba* Linn (Apocynaceae) Root Extract and Fractions in Streptozotocin-Induced Diabetic Rats

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Abstract

Purpose: To investigate the effect of total hydroalcohol root extract of *Plumeria alba* (Apocynaceae) and its active fraction on diabetes in rats.

Methods: The effect of total hydroalcohol extract at 250 mg/kg and different fractions was evaluated on oral glucose tolerance test (OGTT) in mice. The effect of the active fractions on OGTT was measured on a model of diabetic rats (fructose-enriched fat diet and streptozotocin-induced).

Results: The results show that the total extract (250 mg/kg), ethyl acetate fraction and supernatant fraction (obtained after centrifugation of total extract) at a dose of 100 mg/kg significantly ($p < 0.01$) reduced hyperglycemia induced by glucose overload in mice. Fructose-enriched fat diet increased blood cholesterol, triglycerides and high density lipoprotein (HDL) levels in hyperlipidemic untreated rats compared to normal control rats. Administration of total extract (250 mg/kg/day) and supernatant fraction of *P. alba* (100 mg/kg/day) during 14 days significantly reduced lipid parameters (total cholesterol, $p < 0.001$; triglycerides, $p < 0.01$; HDL, $p < 0.05$). Analysis of oxidative stress markers shows that the supernatant fraction and total extract significantly increased serum glutathione level ($p < 0.01$) but significantly lowered malondialdehyde (MDA) concentration in liver ($p < 0.05$).

Conclusion: These results suggest that the total extract and fractions of *P. alba* exhibit significant antidiabetic and hypolipidemic properties in streptozotocin-induced diabetic animals. The supernatant fraction (which is free of organic solvent) was the most biologically active

Keywords: Diabetes, Hyperlipidemia, *Plumeria alba*, Fructose-enriched fat diet, Oxidative stress markers, Streptozotocin

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by an absolute or relative lack of/ or resistance to insulin and is characterized by hyperglycemia in the postprandial and/or fasting state [1]. Presently, it is estimated that 220 million people worldwide have diabetes and this number will increase to 300 million on 2025.

Globally, type-2 diabetes (non-insulin dependent diabetes mellitus) accounts more than ninety percent of all cases of diabetes is caused by a combination of resistance to insulin action and impaired insulin secretion, resulting glucose intolerance [2]. This metabolic disorder characterized by chronic hyperglycemia (fasting blood glucose ≥ 7 mmol/l) is known to be the major initiator of micro and macro vascular

complications in diabetics [3,4]. Therefore, control of postprandial plasma glucose levels is important in the early treatment of diabetes and reducing chronic vascular complications.

Impaired glucose tolerance (IGT) is a risk factor for future diabetes and/or adverse outcomes. It is characterized by a moderate hyperglycemia 2 h post-load plasma-glucose that results from muscle insulin resistance and impaired insulin secretion [5]. The loss of early insulin secretion in IGT and type 2 diabetes is the result of malfunctioning of the pancreatic β -cells [6]. In the long-term, high blood glucose levels are toxic to β -cells, leading to further deterioration in β -cell function and worsening blood glucose control [7]. About 20 to 50 % of the people with IGT will, within a period of 10 years develop type 2 diabetes, accompanied by increased risk of cardiovascular disease and micro vascular complications [8].

Elevated blood glucose levels have been shown to increase oxidative stresses which are known to induce β -cell apoptosis [9] and consequently, antioxidant potential of plant secondary metabolites. Hence, compounds with both antihyperglycemic and antioxidative properties would be useful drugs for the control of glycemia and evolution of type 2 diabetes and related cardiovascular complications.

The objective of this study was to develop an alternative non-genetic rat model for type 2 diabetes and to evaluate the anti-diabetic effect of total extract and supernatant fraction of *Plumeria alba* on this model induced by high fructose diet and intraperitoneal administration of streptozotocin. These rats form a model of experimental diabetes with glucose intolerance, hepatic oxidative stress, hyperlipidemia and high risk of coronary heart diseases.

EXPERIMENTAL

Plant material and extraction

P. alba root were collected from the garden of the Teaching Hospital Sylvanus Olympio of Lomé, Togo. A specimen was identified by the Laboratory of Botany and Plant Ecology (Faculty of Science/University of Lomé) and retained in the department herbarium under number 8035. The roots were washed, dried under air-conditioning and reduced to powder with electric mill (Thomas Scientific™, 3375-E20). 800 g of the powder was cold extracted in ethanol 95% water mixture (80:20) for 72 h. The crude

extracts were filtered with Whatman paper (N° 1) and evaporated under vacuum at 45 °C using a rotary evaporator Büchi R210. The yield of the preparation was 11.34 %.

Fractionation of hydro alcoholic extract

Hydro alcoholic extract of *P. alba* was fractioned using technique of separation by precipitation in cold alcohol. 30 g of total extract were dissolved in 400 ml of 75 % alcohol. The solution was put in a refrigerator at 4 °C for 24 h to allow the precipitate settle at the bottom of the tube. The supernatant was separated from the clot by centrifugation at 2500 g and then evaporated under vacuum at 45 °C. The pellet from the supernatant was dried at ambient air. The fractions obtained are respectively the supernatant fraction (SF) and the Dregs from supernatant fraction (DS).

Ethyl acetate fraction (EA) was obtained by dissolving 30 g of total extract in 5 ml of distilled water and then in 400 ml of ethyl acetate. The mixture was refrigerated at 4 °C for 24 h, treated as above and the supernatant was evaporated to give the ethyl acetate fraction (EA).

These splits have allowed us to have fractions successively named supernatant fraction (SF) with a yield of 73.33 %, dregs from supernatant fraction (DS) yield 23.33 %, an ethyl acetate fraction (EA) 82.20 %.

Animals

Male Sprague Dawley rats (body weight ranging from 150 to 200 g) and ICR mice (body weight ranging from 20 to 30 g) were provided from the Animal House of physiology/pharmacology department of the University of Lomé (Togo). Animals were maintained at the standard environmental conditions (temperature of 25 ± 2 °C and 12/12h of light/dark cycle). They were given standard commercial rat chow and water ad libitum. Principles of laboratory animal care as described in the European Community guidelines were followed [10]. This study has the approval of institute's ethical committee on animal experimentation.

Chemicals

Products used for the pharmacological test were purchased from Sigma (St. Louis, Mo, USA, Richmond, CA, USA). The assay of total cholesterol and triglyceride were made using Labkit Chemelex-SA (Barcelona, Spain).

Oral glucose tolerance test (OGTT) in normal mice

The effect of total extract and fractions were evaluated on oral glucose tolerance test using 42 mice divided into 6 groups of seven animals each: Group I received distilled water (control group); Group II received total extract (ET) at dose of 250 mg/kg; Groups III received supernatant fraction (SF) at dose of 100 mg/kg; Groups IV received ethyl acetate fraction (EA) at dose of 100 mg/kg body weight; Group V received the dregs of supernatant fraction (DS) at dose of 100 mg/kg of body weight; Group VI received glibenclamide (GB) at the dose of 0.6 mg/kg body weight.

Thirty minutes (30 mn) later, D-glucose solution (2.0 g/kg) was given orally to each mouse. Pre-prandial glucose level was measured; 30, 60, 120 and 180 min after the glucose loading (post-prandial glucose level) blood glucose level was measured with a glucometer (One Touch Ultra).

Determination of anti-diabetic activity

Before the administration of streptozotocin (STZ), rats were fed for 11 days with an enriched diet (Bacon-Fructose). The mixture lard-Fructose is prepared as follows: Fructose (6 g) was dissolved in 50 ml distilled water with 1.25 ml of tween 80 %. This solution was mixed to 50 ml of melted lard. After fasting for 16 h, streptozotocin (in a cold freshly prepared solution of 0.1 M citrate buffer, pH 4.5) was administered to rats by intraperitoneal route at 45 mg/kg on day 12 [8]. Animal were maintained under lard-fructose diet for 3 days after STZ injection and Blood glucose level was measured to evaluate the stability. Only rats with blood glucose level greater than 200 mg/dl 72 h after administration of STZ were selected to constitute the group of diabetic animals.

Animal treatment

Five groups of 8 rats were established as follows: Group I as normal control (NC) received distilled water for 14 days; Group II diabetic control (DC) received distilled water for 14 days; Group III, IV and V were treated, respectively, with total extract (250 mg/kg), supernatant fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) for 14 days. Blood glucose was measured every 7 days and body weight of animals was measured every two days. At the end of treatment (day 14), rats were anesthetized under ether and blood was collected by retro - orbital sinus. Blood collected was centrifuged at 3000 rpm for 10 minutes. Serum obtained was used for determination of

blood urea, creatinine, triglycerides, total cholesterol and HDL cholesterol level using commercial kits. Low density lipoprotein (LDL) was calculated by using Friedewald formula [11]. The liver was frozen during 48 h for evaluation of lipid peroxidation.

Total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) assays

The assay is performed by the DCA 2000 HbA1c system. The Ames DCA 2000 HbA1c (Bayer Diagnostics, Elkart, IN, USA) apparatus is a portable analyzer, performing a system based on the inhibition of agglutination of latex particles immunoassay.

Determination of plasma GSH activity

Sedlak and Lindsay [12] method was used with slight modification. 150 μ L of Tris (0.2 M, pH = 8.2) and 10 μ L of 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) (0.01 M) were added to 50 μ L of serum or GSH. To this mixture, 790 μ L of absolute methanol was added to reach 1 ml. All tubes were tightly closed and homogenized for a half hour. Centrifugation of all tubes was done at 3000 rpm for 15 min at room temperature. The absorbance was read at 412 nm after five [5] minutes of incubation.

Lipid peroxidation assay

Solution of 1 -methyl-2-phenylindole at 10 mM mixed with the iron chloride at a concentration of 32 μ M in the proportions of 75: 25 (v/v). Acetonitrile was used as solvent of 1 - methyl - 2 - phenylindole and methanol as solvent of the iron chloride. The liver homogenate was prepared grinding 0.5 g liver in 500 μ L of buffer 0.1 M Tris HCl pH 7.4. The reaction medium contains 650 μ L of 1 -methyl -2- phenylindole activated 250 μ L of MDA liver homogenate, 150 μ L of the HCl solution at a concentration of 12 N and 10 μ L of the solution of BHT (butylated hydroxy toluene) at a concentration of 0.1 M. The incubation of tubes was done at 45 ° C for 1 h. The tubes were then centrifuged at 5000 rpm for 10 min. The absorbance was read at the wavelength of 526 nm. The blank sample was 75 % acetonitrile, 25 % of iron chloride and 200 μ L of Tris solution with a pH equal to 7.4 [13,14].

Statistical analysis

Data are presented as mean \pm SEM (n). One-way ANOVA with Dunett's Multiple Comparison post-test was performed to assess differences between groups (Graph PadPrism 5, San Diego, CA). $P < 0.05$ were considered statistically significant.

RESULTS

Effect of *P. alba* total extract and fractions on oral glucose tolerance (OGT)

Figure 1 (A) shows the variation of blood glucose level. At T_0 , the average blood glucose level in the control like treated group was 91.16 ± 5.75 mg/dl. Thirty minutes (30 min) after administration of glucose, blood glucose level in diabetic control mice reached a maximum average value of 293 ± 17.17 mg/dl against 197.00 ± 1.34 mg/dl, 182.71 ± 2.20 mg/dl, 233.85 ± 4.32 mg/dl; 271.42 ± 2.10 mg/dl and 146.85 ± 2.60 mg/dl respectively for supernatant fraction (100 mg/kg), ethyl acetate fraction (100 mg/kg), total extract (250 mg/kg), Dregs from supernatant fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) in the treated mice. Figure 1(B) showed that AUC of glucose during OGTT of diabetic group was elevated by 60 % ($p < 0,001$) when compared with normal control Group. The AUC of glucose tolerance test in treated groups (total extract, fractions and glibenclamide) was significantly ($p < 0,001$) lower than that of diabetic group showing improved glucose tolerance. This reduction was respectively 32.76 % ($p < 0.001$), 37.64 % ($p < 0.001$), 20.18 % ($p < 0.01$) 7. 36 % ($p < 0.05$) and 49.88 % ($p < 0.001$) of reduction. Area under the curve (AUC) (Fig 1 B) confirmed this reduction.

Effect of *P. alba* total extract and supernatant fraction on blood glucose

Table 1 shows the Levels of blood glucose in diabetic rats during the experiment. On day 0 (start of treatment), blood glucose level in normal control rats was 99.12 ± 1.46 mg/dl against 432.75 mg/dl in diabetic controls (Table 1). After two weeks of treatment, animals treated with total extract, supernatant fraction and glibenclamide showed a significant ($p < 0.01$) reduction of blood glucose level respectively 151.50 ± 3.02 mg/dl, 127.25 ± 2.92 and 110.62 ± 1.48 mg/dl against 268.12 mg/dl in the diabetic controls.

Effect of *P. alba* total extract and supernatant fraction on rat body weight

Table 2 shows rat body weight variation during the experiment. The average body weight of diabetic control (DC) group was 184.14 ± 1.5 g against 168.28 ± 1.67 g in normal control (NC)

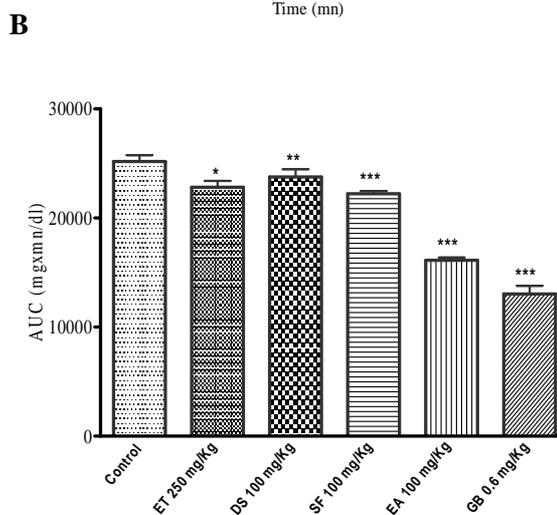
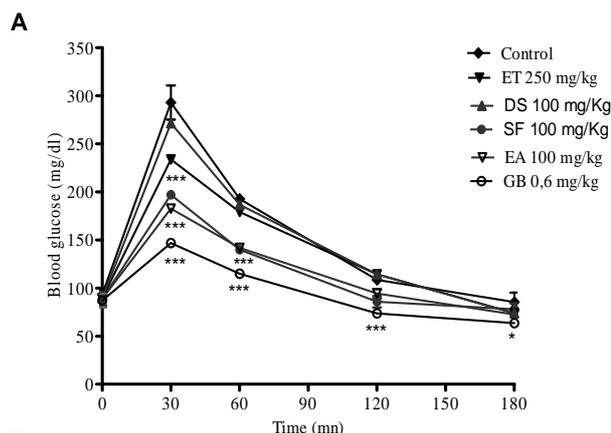


Figure 1: Effect of *P. alba* total extract and fractions on oral glucose tolerance test in normal rats. **A:** Blood glucose level; **B:** area under the curve (AUC); Control: received distilled water; ET 250 mg/kg: received Total extract at dose of 250 mg/kg body weight; received; SF100 mg / kg: supernatant fraction at dose of 100 mg / kg body weight; EA100 mg/kg received ethyl acetate fraction at dose of 100 mg / kg body weight; DS: received the dregs of supernatant fraction at dose of 100 mg / kg of body weight; GB received glibenclamide at the dose of 0.6 mg / kg body weight. Thirty minutes (30 mn) later, glucose solution (2.0 g/kg b.w.) was given orally to each mouse. 0, 30, 60, 120, 180 min after glucose loading, blood glucose level was measured with a glucometer (One Touch Ultra). Values are expressed as the mean \pm SEM ($n = 7$). * $p < 0.05$; ** $p < 0,01$; *** $p < 0.001$ vs control

group at the start of treatment (D_0). After 2 weeks of treatment (D_{14}), body weight in normal control group was 178.25 ± 154.12 g against 154.14 ± 1.50 g in diabetic control group. Group treated with total extract, supernatant fraction and glibenclamide showed a significant increase ($p < 0.001$) of animals body weight at D_{14} (respectively 165.12 ± 1.39 g, 166.00 ± 0.86 g and 169.14 ± 1.22 g) compared to diabetic control (154.42 ± 1.98 g).

Effect of *P. alba* total extract and supernatant fraction on rat biochemical parameters

Diabetic control showed a significant increase in triglycerides and total cholesterol, compared to normal controls. The administration of *P. alba* total extract significantly reduced serum level of TG while increasing HDL-cholesterol level ($p < 0.01$) compared to diabetic controls.

The supernatant fraction (100 mg/kg/ day) and glibenclamide (0.6 mg /kg/day) administered

orally reduced significantly ($p < 0.01$) serum level of TG and total cholesterol compared to diabetic controls. There was a significant ($p < 0.01$) increase in creatinine level (15.78 ± 0.31) and urea level (47.25 ± 0.85) in untreated diabetic rats compared respectively to normal control rats (10.28 ± 0.25) and (38.33 ± 0.98). Blood creatinine and urea level were significantly ($p < 0.01$) reduced in rats treated with the total extract and compared to diabetic control rats (Table 3).

Table 1: Effect of *P. alba* total extract supernatant fraction and glibenclamide on the evolution of blood glucose level during the treatment

	Blood Glucose (mg/dl)				
	NC	DC	Dose (mg/kg)		
			SF 100	ET 250	GB 0.6
J0					
J3	99.12±1.46	432.75±3.58###	434.75±15.61	453.50±13.54	448.25±17.51
J7	99.62±1.22	423.37±4.35###	398.62±15.97	406.75±18.54	391.00±18.20
J14	100.37±1.33	357.25±8.12###	248.12±11.42***	282.50±9.98***	179.37±4.03***
J14	102.50±0.94	268.12±3.34###	127.25±2.92***	151.50±3.02***	110.62±1.48***

NC: normal control received distilled water for 14 days; DC : diabetic control received distilled water for 14 days; ET250, SF100 and GB0.6 were diabetic animals treated respectively with total extract (250 mg/kg), supernatant fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) for 14 day. Values are expressed as the mean \pm SEM (n = 8). ### $p < 0.05$ vs control; *** $p < 0.001$ vs Diabetic control

Table 2: Effect of total extract and supernatant fraction obtained after centrifugation of total extract of *P. alba* on body weight

	NC	DC	SF 100 mg/Kg	ET 250 mg/Kg	GB 0.6 mg/Kg
D ₀	172.85±1.20	184.14±1.50###	183.14±1.26	181.28±1.40	181.14±1.37
D ₇	175.28±1.40	169.71±1.52	175.14±1.40*	172.85±1.05	176.28±1.06**
D ₁₄	178.28±1.20	154.42±1.98###	166.00±0.86***	165.00±1.39***	169.14±1.22***

NC: normal control received distilled water for 14 days; DC : diabetic control received distilled water for 14 days; ET250, SF100 and GB0.6 were diabetic animals treated respectively with total extract (250 mg / kg), supernatant fraction (100 mg / kg) and glibenclamide (0.6 mg / kg) for 14 day. Values are expressed as the mean \pm SEM (n = 8). ### $p < 0.05$ vs control; *** $p < 0.001$ vs Diabetic control

Table 3: Effect of *P. alba* total extract and supernatant fraction and glibenclamide on biochemical parameters

Parameter	NC	DC	ET250	SF 100	GB 0,6
TG (mg/dl)	29.83±1.90	74.17±4.01###	54.67±5.60*	47.00±6.48**	36.70±20***
Ch (mg/dl)	59.67 \pm 3.28	68.33 \pm 3.62##	57.95 \pm 3.20	63.6 \pm 3.40*	64.86 \pm 4.62*
HDL-C (mg/dl)	42.50 \pm 1.67	31.20 \pm 2.2##	49.33 \pm 4.60*	51.67 \pm 3.36**	54.56 \pm 3.09**
LDL-C (mg/dl)	11.20±1.10	22.29±3.40##	2.31±1.60***	2.53±1.50***	2.96±1.40***
Urée(mg/dl)	38.33± 0.98	47.25±0.85##	30.33± 0.98*	29.16± 0.70*	27.00 \pm 0.51**
Créat (mg/dl)	10.28 \pm 0.25	15.78±0.31##	9.28 \pm 0.25**	7.80 \pm 0.19***	8.46± 0.26**

NC: normal control received distilled water for 14 days; DC : diabetic control received distilled water for 14 days; ET250, SF100 and GB0.6 were diabetic animals treated respectively with total extract (250 mg/kg), supernatant fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) for 14 day. Values are expressed as the mean \pm SEM (n = 8). ### $p < 0.05$ vs control; *** $p < 0.001$ vs. Diabetic control

Effect of *P. alba* total extract and supernatant fraction obtained after centrifugation of total extract on total hemoglobin and HbA1c of diabetic rats

Total hemoglobin in normal control group was 12.80 ± 0.40 mg/dl against 9.53 ± 0.14 mg/dl in diabetic control. In animals treated with total extract, supernatant and glibenclamide, HbA1c values were respectively 10.65 ± 0.04 mg/dl, 10.60 ± 0.04 mg/dl and 11.61 ± 0.26 mg/dl. There was a significant reduction ($p < 0.01$) of the glycated hemoglobin, to 12.33 ± 0.23 % for diabetic control against 10.20 ± 0.02 %, 10.84 ± 0.08 % and 7.18 ± 0.25 % respectively for extract, the supernatant and glibenclamide treated group (Table 4).

Effect of *P. alba* total extract and supernatant fraction on hepatic lipid peroxidation and serum GSH levels

The concentration of MDA in diabetic control (1.53 ± 0.01 nM/mg) was significantly ($p < 0.01$) higher than that of normal control group (0.96 ± 0.02 nM/mg). Administration of total extract (250 mg/kg/day) and the supernatant (100 mg/kg) daily for 14 days reduced the MDA concentration respectively to 1.4 ± 0.01 nM/mg and 1.21 ± 0.09 nM/mg ($p < 0.05$). Administration of total extract (250 mg/kg) or supernatant (100 mg/kg) induce significant increase ($p < 0.001$) of GSH level in serum compared to untreated diabetic animals (Table 5).

Table 4: Effect of *P. alba* total extract supernatant fraction and glibenclamide on haemoglobin (Hb) and glycosylated haemoglobin (HbA1c)

Parameter	NC	DC	ET 250	SF 100	GB 0.6
Hb (g/dl)	12.80 ± 0.40	9.53 ± 0.14	$10.60 \pm 0.04^*$	$10.65 \pm 0.04^*$	$11.61 \pm 0.26^{**}$
HbA1c (%Hb)	5.80 ± 0.32	$12.33 \pm 0.23^{###}$	$10.20 \pm 0.02^{**}$	$10.84 \pm 0.08^*$	$7.18 \pm 0.25^{***}$

NC: normal control received distilled water for 14 days; DC : diabetic control received distilled water for 14 days; ET250, SF100 and GB0.6 were diabetic animals treated respectively with total extract (250 mg/kg), supernatant fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) for 14 day. Values are expressed as the mean \pm SEM (n = 8). $^{###} p < 0.05$ vs control; $^{***} p < 0.001$ vs. Diabetic control

Table 5: Effect of *P. alba* total extract supernatant fraction and glibenclamide on MDA and liver glutathione in streptozotocin-induced diabetic rats

Parameter	NC	DC	ET 250	SF100	GB 0.6
MDA (nM/mg)	0.96 ± 0.02	$1.53 \pm 0.01^{###}$	$1.4 \pm 0.01^*$	$1.21 \pm 0.09^{**}$	$0.97 \pm 0.01^{**}$
GSH (μ mol/mg.prot)	34.50 ± 0.73	$19.87 \pm 1.002^{###}$	$26.87 \pm 0.76^{**}$	$31.5 \pm 0.70^{***}$	$36.12 \pm 0.895^{***}$

NC: normal control received distilled water for 14 days; DC : diabetic control received distilled water for 14 days; ET250, SF100 and GB0.6 were diabetic animals treated respectively with total extract (250 mg / kg), supernatant fraction (100 mg / kg) and glibenclamide (0.6 mg / kg) for 14 day. Values are expressed as the mean \pm SEM (n = 8). $^{###} p < 0.05$ vs control; $^{***} p < 0.001$ vs. Diabetic control

DISCUSSION

The present study focuses on the antidiabetic activity of *P. alba*. The effect of hydroalcohol total extract and fractions on disturbances metabolic related diabetic condition such as: dyslipidemia dominated by hypertriglyceridemia, depressed HDL-cholesterol; insulin resistance characterized by accumulation of intra-abdominal fat and glucose intolerance.

The hypoglycemic effect of the total extract, supernatant fractions (SF), ethyl acetate (EA), and the dregs of supernatant was evaluated on normal mouse hyperglycemia caused by OGTT. The results of this test showed that the total extract, supernatant and ethyl acetate fraction

have significantly reduced hyperglycemia in 30 min. A comparison of different fractions activities showed that mice treated with ethyl acetate fractions and supernatant fraction at a dose of 100 mg/kg had the highest hypoglycemic effect. Our previous studies performed on the hydroalcoholic total extract showed a decrease in basal glucose levels in normal rats [14]. This indicates that hypoglycemic activity of the plant is mediated by increased peripheral glucose metabolism or by a direct or indirect stimulation of insulin secretion. This hypoglycemic activity would be comparable to that of the reference drug used (glibenclamide 0.6 mg/kg). It is an oral antidiabetic agent which acts sulfonamide stimulating insulin secretion by binding a specific receptor Sulfonyl Urea Receptor (ON) present on

the membrane of pancreatic beta cells. Sulfonamides thus stimulate the release of insulin already synthesized.

In this study, STZ was used to induce diabetes. It selectively destroys the insulin secreting pancreatic cells, leaving the less active cells and thus resulting in diabetic state [15]. STZ-induced diabetes is characterized by severe weight loss which was observed in the present study. The reduction in body weight may be attributed to insulin depletion provoking a loss of adipose tissues. The loss of weight in diabetic rats might also be the result of degradation of structural proteins due to unavailability of carbohydrate as energy source [16]. For this reason, weight reduction is being used as a marker of diabetes mellitus induced by STZ [17]. In the present study, supernatant fraction (SF) was chosen among all active fractions for STZ induced diabetes because of his similarity to traditional preparation with no toxic solvent. Then, oral administration of total extract and supernatant of *P. alba* to STZ-induced diabetic rats caused significant improvement in the body weight gain. This may be due to the effect of total extract and supernatant fraction of *P. alba* on glucose metabolism.

In the present study, an increase of blood glucose level in diabetic rats confirmed the induction of diabetes by STZ. The fundamental mechanism underlying hyperglycemia in diabetes involves the overproduction of glucose by excessive hepatic glycogenolysis, gluconeogenesis and decreased utilization by the tissues [18]. A significant decrease of blood glucose level in total extract and supernatant of *P. alba* treated diabetic rats compared to diabetic control rats was observed. This could be due to the regeneration of existing pancreatic beta cells and enhanced transport of glucose to the peripheral tissues by hydro alcoholic extract and supernatant of *P. alba*.

A significant decrease in the level of total hemoglobin and significant increase in the level of glycosylated hemoglobin observed in STZ induced diabetic rats might be due to the increased formation of glycosylated hemoglobin. Glycosylated hemoglobin was found to increase in uncontrolled diabetes and the increase is directly proportional to the fasting blood glucose level for about 3 months [19]. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin [20]. Therefore, total hemoglobin level is decreased in diabetic rats. The rate of glycation is directly proportional to the concentration of blood glucose [20]. The level of glycosylated

hemoglobin has been shown to provide an index of blood glucose concentration [22]. The decreased level of glycosylated hemoglobin and increased level of total hemoglobin observed in hydro alcoholic extract and supernatant of *P. alba* administered diabetic rats may be due to the reduction of blood glucose level.

Lipid profile is of crucial importance in the diagnosis and treatment of several cardiovascular diseases and control of diabetic patients [23]. Studies have reported that cardiovascular complications associated with diabetes are due to disturbances of lipid metabolism [24]. The results of this study showed significant increase of lipid parameters in STZ induced diabetic animal such as: total cholesterol, TG and LDL-cholesterol, all this combined with a decrease in HDL-cholesterol. The administration of total extract and the supernatant fraction of *P. alba* led to a significant improvement in lipid profile. A low concentration of HDL-cholesterol can be regarded as an additional risk factor and then a high concentration of HDL-cholesterol is a protective factor. It was proposed to use the ratios (total cholesterol/HDL-cholesterol) or (LDL-cholesterol/HDL-cholesterol) as an indicator of cardiovascular risk. Administration of total extract and supernatant of *P. alba* increased significantly HDL-cholesterol. *P. alba* is endowed with hypoglycemic and hypolipidemic properties and can therefore be used in the treatment of cardiovascular complications associated with diabetes.

Chronic hyperglycemia in diabetes leads to oxidative stress characterized by an imbalance between the production of free radical species, peroxides and their removal by enzymatic antioxidant defenses and nonenzymatic cellular. It is reported that the production of ROS and lipid peroxidation are increased in diabetic patients and that oxidative stress is responsible for the pathophysiology of diabetes [23], especially, that associated with antioxidant treatment showed significant improvement and prevention against cardiovascular complications [25].

In this study, lipid peroxidation was evaluated in the liver by measuring the level of MDA (malondialdehyde) marker of peroxidation regarding the evaluation of antioxidant defense mechanisms by the glutathione peroxidase (GSH) in serum. The results showed a significant high MDA levels in STZ diabetic animals with reduction of antioxidant defenses (glutathione decreased). Our results agree with those reported by some authors [9]. The increase of MDA rate indicates increased lipid peroxidation

and the degree of damage to these organs. GSH depletion may mean oxidation by free radicals oxidized GSSG. Treatment of the diabetic rats with *P. alba* extract not only reduced the formation of MDA in the liver but also positively changed GSH.

CONCLUSION

Findings from this study reveal that the total extract and supernatant fraction of the total extract possess antidiabetic and hypolipidemic activities which can potentially be developed for the management of diabetes. The findings also lend credence for the use of this plant in the traditional treatment of diabetes mellitus in Togo.

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