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Hypoglycemic effect and antioxidant potential of *Pterocarpus erinaceus* Poir. stem bark and *Amaranthus spinosus* L. roots extracts

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Abstract

Pterocarpus erinaceus and *Amaranthus spinosus* are medicinal plants belonging respectively to Fabaceae and Amaranthaceae family, used traditionally by Togolese to treat diabetes mellitus. The aim of this study was to evaluate the hypoglycemic and antioxidant activities of both plants. The hydroethanolic extracts of *P. erinaceus* steam bark and *A. spinosus* roots were evaluated in ICR mice oral glucose tolerance and basal blood glucose. The antioxidant activities of extracts were performed *in vitro* by the total antioxidant capacity, DPPH free radical scavenging and FRAP tests. The phytochemicals were sought in the extracts by standard staining tests in chemistry, then quantified in spectrophotometer. Glucose adsorption was determined as equivalent of glucose. After 30 min of glucose overload (4 g/kg) in mice, extracts 125; 250 and 500 mg/kg b.w of both plants showed a significant ($p < 0.001$) decrease in blood glucose levels. Extracts also decreased significant ($p < 0.001$) in basal blood glucose. For antioxidant power, extracts acted by dose-dependent. Alkaloids, phenolic compounds, flavonoids, reducing compounds, polysaccharides, saponosides and terpenes were present in both extracts. Tannins were present only in *P. erinaceus* extract. Some of this phytochemical compound could be responsible for hypoglycemic and antioxidant activities obtained. One of mechanism of action of extracts in blood glucose decreased was glucose adsorption, by complexing with glucose. These results demonstrate that *P. erinaceus* 250 and *A. spinosus* 250 or 500 mg/kg b.w can be used to control mild to moderately severe type II diabetes mellitus and its complications related to oxidative stress.

Keywords: *P. erinaceus*, *A. spinosus*, hypoglycemic, antioxidant, phytochemical, glucose absorption.

INTRODUCTION

Diabetes is a metabolic disease that affects people of all ages in all geographic regions of the world and whose prevalence is increasing. The prevalence of the disease was 9.3% in 2019 and estimated to be 10.2% in 2030 and 10.9% by 2045 if efforts are neglected. According to these estimates, about 1 person dies of diabetes every 8 seconds in 2019 and 15.8% of live births are affected by hyperglycaemia in pregnancy [1]. Most of them occur in low- and middle-income countries (79.4%). In 2019, 3.9% of adults (or 4.7% when adjusted with age) had diabetes in Africa, approximatively the half of the global prevalence [1].

Indeed, diabetes occurs when the fasting blood glucose is ≥ 126 mg/dL (7 mmol/L) or ≥ 200 mg/dL (11.1 mmol/L) after oral glucose tolerance test or at any time of the day [2]. The main types of diabetes are type 1 diabetes mellitus (T1DM: ~3%), common among to children and youth, type 2 diabetes mellitus (T2DM: ~90%), usually seen in adults, and gestational diabetes that affects about 37.0% of pregnant women (45-49 year old) and is manifested by an increase in blood glucose levels due to the resistance of cells to insulin action likely after 24 weeks of pregnancy [1]. The two main types of diabetes result in a progressive loss of pancreatic β-cells by an autoimmune process leading to insulin deficiency (T1DM) and a decrease in insulin receptor susceptibility to insulin and dysfunction of pancreatic β-cells leading to insulin resistance followed by progressive insulinopenia (T2DM) [3]. Regardless of the type of diabetes, hyperglycemia results from decreased in insulin secretion and/or resistance to insulin action; untreated leads to complications such as metabolic disorder, retinopathy, nephropathy, neuropathy, cardiovascular diseases and ulceration [4].

The conventional oral antidiabetic drugs and insulin have been proven efficiency in managing the disease, but their side effects and cost have led some patients to alternative solutions. Thus, traditional medicine that uses among other plants to heal is appropriate. Many Laboratories looking for new active compound have turned to this traditional medicine. The plants used in traditional medicine are very effective, have lower side effects, are accessible and affordable with low cost, but need to be studied scientifically to prove their traditional used [5]. Thus, the ethnobotanical survey of traditional healers in Togo showed that *P. erinaceus* and *A. spinosus* are used in the treatment of diabetes, but on a traditional basis. Previous studies have reported the antioxidant and antidiabetic properties of *P. erinaceus* [6, 7] and *A. spinosus* [8, 9, 10], but studies specifically aimed at *P. erinaceus* steam bark and *A. spinosus* roots are very limited. The aim of this study is therefore to evaluate the antioxidant and hypoglycemic of hydroethanolic extracts of *P. erinaceus* steam bark and *A. spinosus* roots.

MATERIALS AND METHODS

Plant material

The steam bark of *Pterocarpus erinaceus* Poir. and the roots of *Amaranthus spinosus* L. were used as plant material. *P. erinaceus steam* barks were collected in Tovegan area, a village located on the road to Kpalime about 80 km from Lome city (Togo); while *A. spinosus* roots were collected in Dzagble (Lome). The plants have been identified in Botany and Plant Ecology Laboratory of Faculty of Science (University of Lome) where voucher

specimen was deposited in the herbarium as herbarium specimen No. 15515 TOGO (*P. erinaceus*) and No. 15516 TOGO (*A. spinosus*). The steam bark and roots were dried at the temperature of 20 ± 2 °C in safe from light for two week and then reduced to powder.

Animals

In vivo tests were performed by using ICR mice weighing 25 ± 5 g. They were provided by the Laboratory of Animal Physiology (Faculty of the Sciences/University of Lome) and were kept in cages under standard environmental conditions with free access to water and food. The tests on mice were conducted in accordance with institutional guidelines and ethics of Laboratory of Physiology-Pharmacology of University of Lome-Togo referred as 001/2012/ CB-FDS-UL.

Extraction

The powders (400 g) of *P. erinaceus* steam bark and *A. spinosus* roots have been macerated in 4 L of a mixture of ethanol and water (50: 50) for 72 h. The macerates were filtered on hydrophilic cotton and then on Wattman paper No. n°40 (Ø150 mm) and evaporated to dryness at 45 °C using a rotary evaporator under vacuum (Büchi Rotavapor R210, Germany). The resulting dry extracts were collected in a sterile glass vial and placed in a desiccator for 24 h and then stored in the refrigerator at a temperature of 4-8 °C.

Effect of extracts on oral glucose tolerance mice

The hypoglycemic effect of extracts was evaluated on ICR mice by Oral glucose tolerance test (OGTT). Mice were fasted (7 h) and their blood glucose was measured before the administration of all solutions. The animals were divided into 6 groups of 5 mice each. The hyperglycemic control received distilled water (5 mL/kg b.w), treated groups received extracts 125; 250 and 500 mg/kg b.w and reference groups received Glibenclamide 0.6 mg/kg b.w by gavage 30 min before glucose overload (4 g/kg b.w) at rate of 5 mL/kg b.w. Normoglycemic controls received only distilled water (no glucose overload). Blood glucose levels were measured from the tail vein blood of mice with Accu-Chek Active glucometer (Germany) for 180 min (0 min before and 30 min, 60 min, 120 min, 180 min after glucose loading).

Effect of extracts on mice basal blood glucose

Mice were divided into 5 groups (5 mice each). The normoglycemic controls group received only distilled water 5 mL/kg b.w, treated groups received extract 125; 250 and 500 mg/mL b.w and reference groups received Glibenclamide 0.6 mg/mL b.w. Blood glucose levels were measured at the tail vein for 180 min (0 min before and 30 min, 60 min, 90 min, 120 min, 180 min after administration solutions by gavage).

In vitro antioxidant assays

Total antioxidant capacity (TAC) assay

The reaction is based on the reduction of molybdenum ion Mo (+6) in Mo (+5) by the extract and the subsequent formation of the phosphate-Mo (+5) green complex at acidic pH. 3 mL of reagent (Sulfuric acid 0.6 M, sodium phosphate 28 mM and ammonium molybdate 4 mM) were added to 0.3 mL of extract 1.0 mg/mL prepared in methanol. It was incubated at 95 °C for 90 min and then brought back to Laboratory temperature. Absorbance was measured at 695 nm against blank [5]. For the blank, extract was replaced by methanol. Ascorbic acid (25; 50; 100; 150; 200; 250 µg/mL) was used as standard to generate the calibration curve. The total antioxidant capacity was expressed as mg equivalence of ascorbic acid/g of extract.

DPPH free radical scavenging activity

In solution, DPPH (1,1-diphenyl-2-picrylhydrazyl) generates deep violet color free radical which became coloress or pale yellow when neutralized. This discoloration of $DPPH^*$ was proportional to the free radical scavenging by antioxidant compounds contained in the extract. The test was performed by mixing 1.5 mL of DPPH (100 µmol/L) to 250 µL of extract (62.5; 125; 250; 500 µg/mL) and incubated for 10 min at Laboratory temperature before measuring absorbance at 517 nm. For control, methanol was used in place of the extract. Ascorbic acid (12.5; 25; 50; 100; 200 µg/mL) was used as standard antioxidant [11].

Inhibition % of $DPPH^*$ = $\left(\frac{\text{OD control} - \text{OD } sample}{\text{OD } control} \right) x\ 100.$

OD = Optical density.

Ferric reducing antioxidant power (FRAP)

The Reducing compounds react with ferric potassium cyanide (Fe^{3+}) to form ferrous potassium cyanide (Fe^{2+}) which reacts with ferric chloride to form ferrous-ferric complex having maximum absorbance at 700 nm. In practice, 0.5 mL of extracts (50; 100; 200; 400; 800 µg/mL, prepared in methanol) was mixed with 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min and then, 2.5 mL of trichloroacetic acid 10% was added and centrifuged at 3000 rpm for 10 min. 2.5 mL of distilled water and 0.5 mL of iron chloride 0.1% were added successively to 2.5 mL of supernatant. Absorbance was measured at 700 nm. Ascorbic acid was used as reference antioxidant [12, 13]. The intensity of the absorbance indicates the intensity of the reducing power.

Increase % of Reducing Power = $\left(\frac{\text{OD sample} - \text{OD control}}{\text{OD } sample} \right) x\ 100.$

Phytochemical screening

Standard staining tests in chemistry has been performed to seek phytochemical in the extracts [14, 15]. The presence of alkaloids was achieved by Mayer and Bouchardat reactions; phenolic compound by ethanol and $FeCl_3$ reactions; tannins by $FeCl_3$ 2% reaction; condensed tannins by hydrochloric butanol reaction; flavonoids by condensed H_2SO_4, NaOH 10% and $FeCl_3$ reactions; reducing compounds by Molish, Keller-Killiani reactions; saponins by foams test and index determination; in end sterols and terpenes by Liebermann-Buchard reacion.

Total phenols and tannins contents

All the phenolic compounds of the extract were oxidized by the Folin-Ciocalteu reagent (a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$)) which is reduced during the oxidation of the phenols in a mixture of blue oxides of tungsten ($W_{40}O_{23}$) and molybdenum (Mo_8O_{23}). The resulting blue color absorbs about 750 nm. The tannin binding by polyvinylpolypyrrolidone (PVPP) allowed to deduce their amount of total phenols after a second assay [12]. The test was performed by adding 200 µL of Folin-C 10% reagent to 200 µL of extract (1 mg/mL in methanol 95%) or to 200 µL of supernatant of PVPP-fixed extract solution (fix tannins twice previously by adding 500 µL of extract and then 500 µL of supernatant to 10 mg of PVPP dissolved in 500 µL of 95% methanol) and incubated at temperature of 28 ± 2 °C for 15 min. After incubation, 800 µL of sodium carbonate solution (700 mM) were added and the absorbance measured at 735 nm against blank. Gallic acid (GA) (0; 25; 50; 100; 150; 100 µg/mL) was used as standard to generate the calibration curve. Tannins were subtracted from total phenols by differentiation of optical densities (OD): $OD_{tanins} = OD_{extract} - OD_{PVPP\text{-}fixed\ extract}$. Total phenol and tannins were expressed as equivalents in mg GA/g of extract.

Total flavonoids content

The flavonoids contained in the extract were determined according to the aluminum chloride ($AlCl_3$) method described by Andzi-Barhé *et al.* with slight modification [16]. In principle, flavonoids form with aluminum chloride a flavonoid-aluminum complex that absorbs at 415 nm. The test has been performed by mixing 1.0 mL of extract (1.0 mg/mL in ethanol) with 1.0 mL of $AlCl_3$ 2% and after 10 min of incubation, the absorbance was measured at 415 nm against blank. Rutin (0; 5; 25; 50; 75; 100; 150; 200 µg/mL in ethanol) was used as standard flavonoids to generate the calibration curve. The total flavonoids were expressed as equivalents in mg of Rutin/g of extract.

Polysaccharides content

Phenol–sulfuric acid method was used to determine polysaccharides in extracts [17]. Polysaccharides give an orange-yellow color when treated with phenol and concentrated sulfuric acid. The reaction is sensitive and the coloring is stable. To 200 µL of extract 1.0 mg/mL, 200 µL of aqueous phenol 5% solution was added. The mixture was vortexed and then 1 ml of concentrated sulfuric acid was quickly added. The preparations were again vortexed, before being brought to a water bath at 100 ° C for 5 min. then the preparations were cooled in an ice bath for 30 min, protected from light. The absorbance was measured at 480 nm against blank. Glucose (0; 25; 50; 100; 150; 200 µg/mL) was used as standard to generate the calibration curve. Polysaccharides were expressed as equivalents in mg of Glucose/g of extract.

Glucose adsorption assay

The principle was based on the ability of extract to bound glucose present in medium by complexation. Glucose adsorption capacity of extracts was determined by the method described by Ou et al. (2001) with slight modification [18]. The test was performed by mixing different concentrations of extract (50; 100; 150 mg) with gradual concentration of Glucose (5; 10; 20; 50; 100 mg). After stirring well and incubated at the temperature of 37 °C for 6 h, the preparation was centrifuged at 4000 x g for 20 min. The glucose content in the supernatant was determined in spectrophotometer according to the Trinder method using glucose oxidase reaction. Adsorbed glucose was determined by the difference between initial and final glucose.

Bounded Glucose = $Glucose_{initial} - Glucose_{final}$

Statistical analyzes

Data were expressed as Mean ± SEM (standard error of the Mean) using the GraphPad Prism 6 software. Statistical differences between treated groups and controls were determined by 2-ways ANOVA (analysis of variance) and considered significant for $p < 0.05$.

RESULTS

Effect of extract on mice oral glucose tolerance test (OGTT)

Blood glucose levels in hyperglycaemic groups increased dramatically to a peak before decreasing after 30 min of glucose overload compared to normoglycemic control. This increase of glycaemia was significant at t30 ($p < 0.0001$), t60 ($p < 0.0001$), t120 ($p < 0.01$) and remained no significant at t180. During this time, blood glucose reached 166.73% (from 96.800 ± 1.772 to 258.200 ± 15.409 mg/dL) at t30 before remained nearly to the normal around 180 min (Figure 1A). The area under the curve (AUC) of glucose tolerance, which measured the total amount of available glucose in the blood, also showed a significant ($p < 0.0001$) increase (42.11%) in blood glucose levels in oral glucose tolerance mice (AUC_{HC} = 25716.000 ± 444.405 min.mg/dL) when compared to normoglycemic control (AUC_{NC} = 14886.000 ± 174.452 min.mg/dL) (Figure 1B).

Administration of extracts 30 min prior to OGTT, significantly prevented increased in blood glucose levels in the fasted mice compared to the hyperglycemic controls.

P. erinaceus extract 500 and 250 mg/kg show a significant ($p < 0.0001$) decreased in blood glucose levels after 30 min glucose overload in mice. This decreased in blood glucose levels pursue during the 180 min for the two doses of extract, but remained less significant ($p < 0.05$ at 180) (Table 1). The 3rd dose of *P. erinaceus* extract (125 mg/kg) was significant at t30 and t60 ($p < 0.0001$) before remained no significant at t120 and t160 (Table 1). The AUCs (air under the curve) of glucose tolerance confirmed all decreased in blood glucose levels of both doses of *P. erinaceus* extracts when compared to hyperglycemia controls ($AUC_{P.\ erinaceus\ 500}$ = 17151.000 ± 404.961 min.mg/dL; $AUC_{P.\ erinaceus\ 250}$ = 16992.000 ± 736.291 min.mg/dL and $AUC_{P.\ erinaceus\ 125}$ = 19371.000 ± 958.142 min.mg/dL vs AUC_{HC} = 25716.000 ± 444.405 min.mg/dL).

When compared both doses of *P. erinaceus* extracts, the significant decreased in blood glucose levels remained only at t30 between them: *P. erinaceus* 500 vs. *P. erinaceus* 250 ($p < 0.05$); *P. erinaceus* 500 vs *P. erinaceus* 125 ($P < 0.001$) and *P. erinaceus* 250 vs. *P. erinaceus* 125 ($P < 0.0001$). There was no significant decreased in blood glucose levels between both doses of *P. erinaceus* at t60; t120 and t180 (Figure 1A). The AUCs of *P. erinaceus* extracts doses showed that *P. erinaceus* 250 was more active in blood glucose levels decreased: $AUC_{P.\ erinaceus\ 250}$ vs. $AUC_{P.\ erinaceus\ 125}$ ($p < 0.01$); $AUC_{P.\ erinaceus\ 500}$ vs. $AUC_{P.\ erinaceus\ 125}$ ($p < 0.05$); $AUC_{P.\ erinaceus\ 500}$ vs. $AUC_{P.\ erinaceus\ 250}$ (ns) (Figure 1B). The effect of *P. erinaceus* extract in blood glucose decreased was no correlate by the dose.

A. spinosus extract were also showed a significant decreased in blood glucose levels for 180 min when compared to hyperglycemic control.

Both dose of *A. spinosus* extract showed the same significant ($p < 0.0001$) decreased in blood glucose levels after 30 min glucose overload in mice at t30 and t60. At 180 min, this decreased in blood glucose levels had remained significant for *A. spinosus* 500 mg/kg ($p < 0.05$ at 180), *A. spinosus* 250 mg/kg ($p < 0.5$) and no significant for *A. spinosus* 125 mg/kg (Table 2). The AUCs of blood glucose available were also significant ($p < 0.0001$) for the tree doses of *A. spinosus* extracts when compared to hyperglycemia controls ($AUC_{A.\ spinosus\ 500}$ = 14748.000 ± 305.698 min.mg/dL; $AUC_{A.\ spinosus\ 250}$ = 16470.000 ± 548.023 min.mg/dL and $AUC_{A.\ spinosus\ 125}$ = 19503.750 ± 806.8312 min.mg/dL vs AUC_{HC} = 25716.000 ± 444.405 min.mg/dL) (Table 2).

When increased the dose of *A. spinosus*, the decreased in blood glucose levels increased and correlated with the dose of extract (Figure 2A). This decreased in blood glucose level remained significant ($p < 0.0001$) for the tree doses of *A. spinosus* extract and was confirmed by AUCs of blood glucose available in mice (Figure 2B).

Glibenclamide used as reference drug also showed a significant ($p < 0.0001$) decreased in blood glucose levels at 30; 60; 120 and 180 min after glucose overload (Table 1 and 2) . The AUCs of blood glucose available in mice have confirmed this significant ($p < 0.0001$) decreased in blood glucose decreased leaded by Glibenclamide. At t30, the decreased in blood glucose levels leaded by Glibenclamide was greater than that of *P. extract* 125 and 250 mg/kg and less extensive than that of *P. erinaceus* 500 mg/kg (Figure 1A). At this time, the only significant difference was between Glibenclamide and *P. ernaceus* 125 mg/kg ($p < 0.0001$). When compared the tree doses of *A. spinosus* extract to Glibenclamide, only *A. spinosus* 125 mg/kg gave less extensive decreased in blood glucose levels at t30: Glibenclamide vs. *A. spinosus* 500 ($p < 0.01$); Glibenclamide vs. *A. spinosus* 250 (ns); Glibenclamide vs. *A. spinosus* 125 ($p < 0.001$) (Figure 2A).

Effect of extract on basal blood glucose

P. erinaceus and *A. spinosus* extracts were both decreased basal blood glucose levels compared with normoglycemic controls.

After 30 min of administration of extracts, *P. erinaceus* 500 mg/kg and *A. spinosus* 500 and 250 mg/kg resulted in a significant ($P < 0.0001$) decrease in the basal blood glucose level in mice. This decreased in blood glucose levels was continued for up to 180 min (Table 3). The effect of *P. erinaceus* 250 mg/kg on basal glucose was not significant at t30 but was significant at t60 ($P < 0.01$); t120 ($P < 0.0001$) and t180 ($P < 0.001$) (Figure 3).

Glibenclamide 0.6 mg/kg used as reference drug also resulted in significant decreased significantly the basal blood glucose level (Table 3). The AUCs of the extracts and Glinbenclamide also remained significant ($P < 0.0001$) and confirm this decreased in basal glucose levels. (Figure 3B and 4B). AUCs data showed that *A. spinosus* 500 mg/kg was more decreased in basal glucose levels than another extracts and Glibenclamide. However, there was no significant decreased in basal glucose between the two extracts of *A. spinosus*, unlike *P. erinaceus* whose extract 500 mg/kg showed a significant ($p < 0.01$) decreased in basal glucose levels compared to extract 250 mg/kg.

Table 1: Rate of *P. erinaceus* extracts in blood glucose level decrease in overload glucose mice

	P. erinaceus 500		*P. erinaceus* 250		*P. erinaceus* 125		Gliben 0.6	
	↓%	*P	↓%	*P	↓%	*P	↓%	*P
t30	37.26	0.0001	46.17	0.0001	23.39	0.0001	40.20	0.0001
t60	37.72	0.0001	36.56	0.0001	31.14	0.0001	37.95	0.0001
t120	28.28	0.01	24.09	0.05	20.80	ns	55.66	0.0001
t180	34.31	0.05	29.44	0.05	26.03	ns	49.15	0.001
AUC	33.31	0.0001	33.92	0.0001	24.67	0.0001	42.84	0.0001
*p = p value								

Table 2: Rate of *A. spinosus* extracts in blood glucose level decrease in overload glucose mice

	A. spinosus 500		*A. spinosus* 250		*A. spinosus* 125		Gliben 0.6	
	↓%	*P	↓%	*P	↓%	*P	↓%	*P
t30	49.65	0.0001	44.54	0.0001	26.7	0.0001	40.20	0.0001
t60	45.79	0.0001	31.95	0.0001	23.73	0.0001	37.95	0.0001
t120	38.87	0.0001	37.96	0.0001	23.36	0.5	55.66	0.0001
t180	42.58	0.001	33.09	0.05	27.92	Ns	49.15	0.0001
AUC	42.65	0.0001	35.95	0.0001	24.16	0.0001	42.84	0.0001
*p = p value								

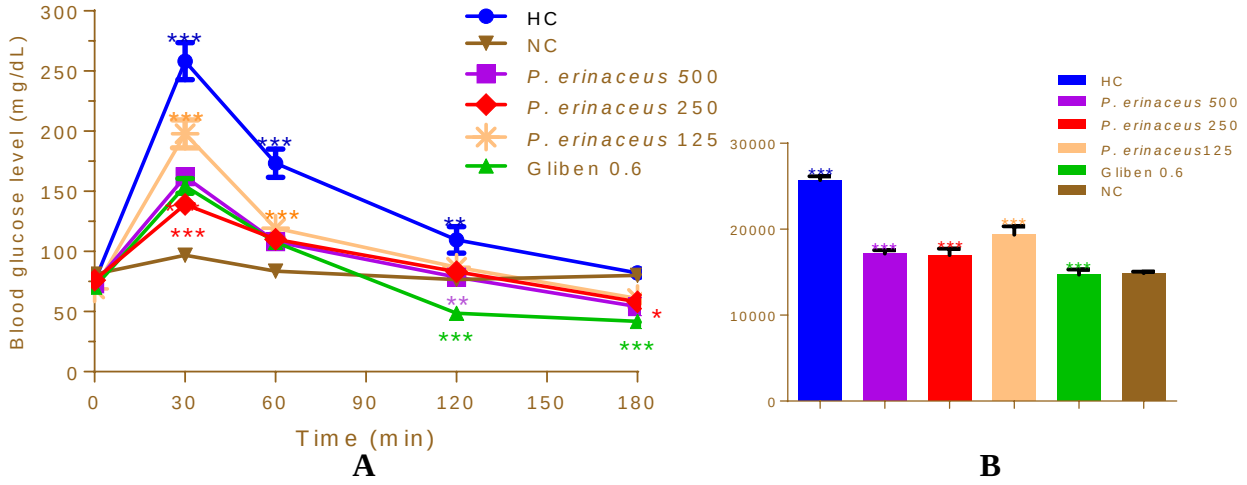

Figure 1: Effects of *Pterocarpus erinaceus* extract on oral glucose tolerance
(A = Blood glucose level vs time, B = Area under the curve (AUC) of blood glucose)
Blood glucose was measured for 180 min: t0 = 0 min, corresponding to basal blood glucose level of the fasted mice and t30; t60; t120; t180 correspond respectively to blood glucose level at 30; 60; 120 and 180 min after glucose overload. HC = hyperglycemic controls; NC = Normoglycemic controls; Gliben 0.6 = Glibenclamide 0.6 mg/kg b.w. *P. erinaceus* 500; 250 and 125 = *P. erinaceus* 500; 250 and 125 mg/kg b.w. Values were expressed in Means ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to HC and NC. n = 5.

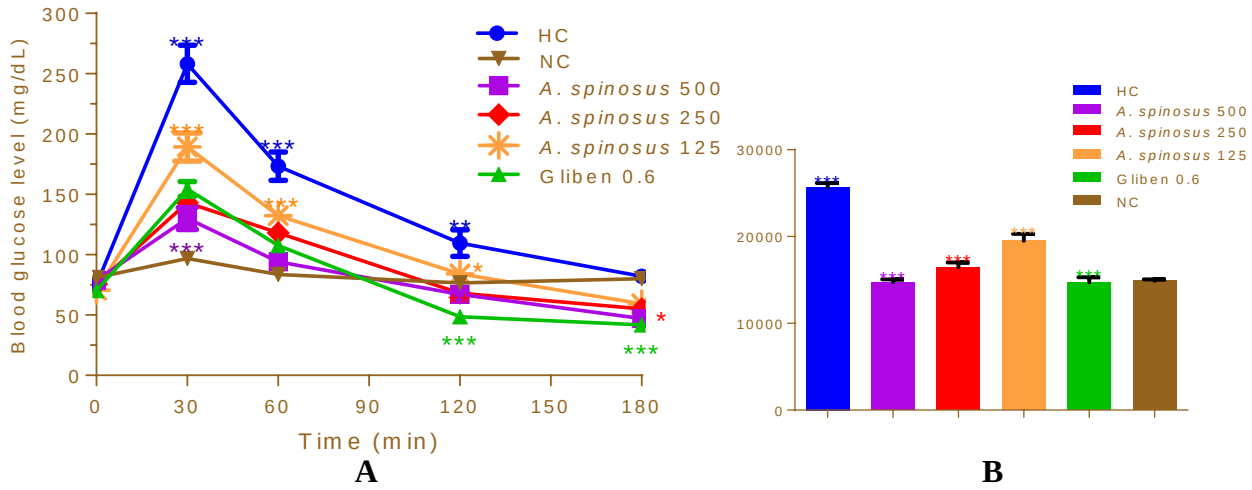

Figure 2: Effects of *Amaranthus spinosus* extract on oral glucose tolerance
(A = Blood glucose level vs time, B = Area under the curve (AUC) of blood glucose)
Blood glucose was measured for 180 min: t0 = 0 min, corresponding to basal blood glucose level of the fasted mice and t30; t60; t120; t180 correspond respectively to blood glucose level at 30; 60; 120 and 180 min after glucose overload. HC = hyperglycemic controls; NC = Normoglycemic controls; Gliben 0.6 = Glibenclamide 0.6 mg/kg b.w. *A. spinosus* 500; 250 and 125 = *A. spinosus* 500; 250 and 125 mg/kg b.w. Values were expressed in Means ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to HC and NC. n = 5.

Table 3: Rate of *P. erinaceus* and *A. spinosus* extracts in basal blood glucose level decrease

	P. erinaceus 500		*P. erinaceus* 250		*A. spinosus* 500		*A. spinosus* 250		Gliben 0.6	
	↓%	*P	↓%	*P	↓%	*P	↓%	*P	↓%	*P
t30	17.24	0.0001	1.94	Ns	25.65	0.0001	24.14	0.0001	8.405	0.05
t60	24.88	0.0001	11.48	0.01	25.84	0.0001	23.44	0.0001	9.33	0.05
t120	32.11	0.0001	25.59	0.0001	35.51	0.0001	38.64	0.0001	45.43	0.0001
t180	47.98	0.0001	42.42	0.0001	48.23	0.0001	44.95	0.0001	54.04	0.0001
AUC	26.87	0.0001	18.16	0.0001	30.11	0.0001	30.03	0.0001	26.81	0.0001
*p = p value. t30, t60, t120, t180 correspond respectively to 30; 60; 120; 180 min. AUC = aire under the curve										

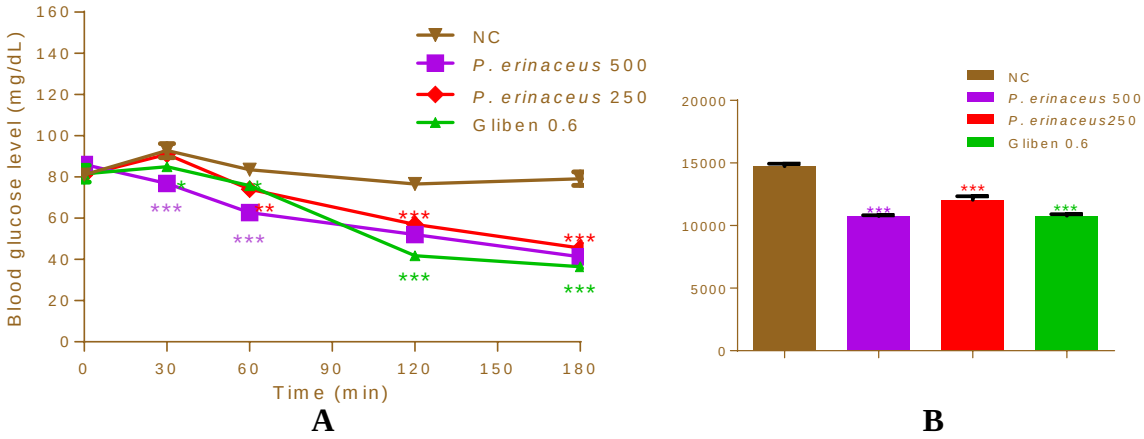

Figure 3: Effect of *Pterocarpus erinaceus* extract on basal glucose
(A = Blood glucose level vs. time, B = Area under the curve (AUC) of blood glucose)
Blood glucose was measured for 180 min: t0 = 0 min, corresponding basal blood glucose level measurement in fasted mice and t30; t60; t120; t180 correspond to 30; 60; 120 and 180 min after administration of distilled water. NC = Normoglycemic controls; Gliben 0.6 = Glibenclamide 0.6 mg/kg b.w; *P. erninaceus* 500 and 250 = *P. erinaceus* 500 and 500 mg/kg b.w. Values are expressed in Means ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to NC. n = 5.

Table 4: Phytochemical screening

Phytochemical compounds	Reactions	Observations	Results	
			P. erinaceus	*A. spinosus*
Alkaloids	Bauchardat	Brown, earth/yellow-brown precipitate	+	+
	Mayers	White precipitate or white-yellow	+	+
Phenolic compounds	EtOH+$FeCl_3$	Dark blue green precipitate (nonspecific phenolic core)	-	+
		Brown precipitate (orthodiphenolic core)	+	-
Tannins	$FeCl_3$	Blue-black coloring	+	-
Condensed tannins	Chlorhydric BuOH	Intense red coloring	+	-
Flavonoids	Concentrated HCl	Red coloring	+	+
	NaOH 10%	Orange-yellow coloring	+	+
	$FeCl_3$	Greenish coloring	+	-
Carbohydrates (oses and osids)	Molisch/ α-Naphthol test	Formation of a purplish ring at the surface of separation of the two liquids together with a green coloration in the lower phase	+	+
Terpenes	Lieberman	Gray or purple or purple-red coloring of the supernatant	+	+
Sterols		Blue to green or gray coloring of the supernatant	-	-
Saponosides	Agitation	Persistent foam formation	+	+
	Foam index	Foam height (h ~ 1)	833.33	5000
+ = presence ; - = absence				

Table 5: Total Phenols, tannins and flavonoids content in extracts

Extracts	Total Phenols (mg GAE/g)	Tannins (mg GAE/g)	Flavonoids (mg RE/g)	Polysaccharides (mg GE/g)
P. erinaceus	466.305 ± 1.058	379.510 ± 2.554	60.403 ± 0.753	1582.354 ± 1.029
A. spinosus	42.660 ± 2.946	0.00 ± 0.00	33.308 ± 1.241	731.317 ± 2.906

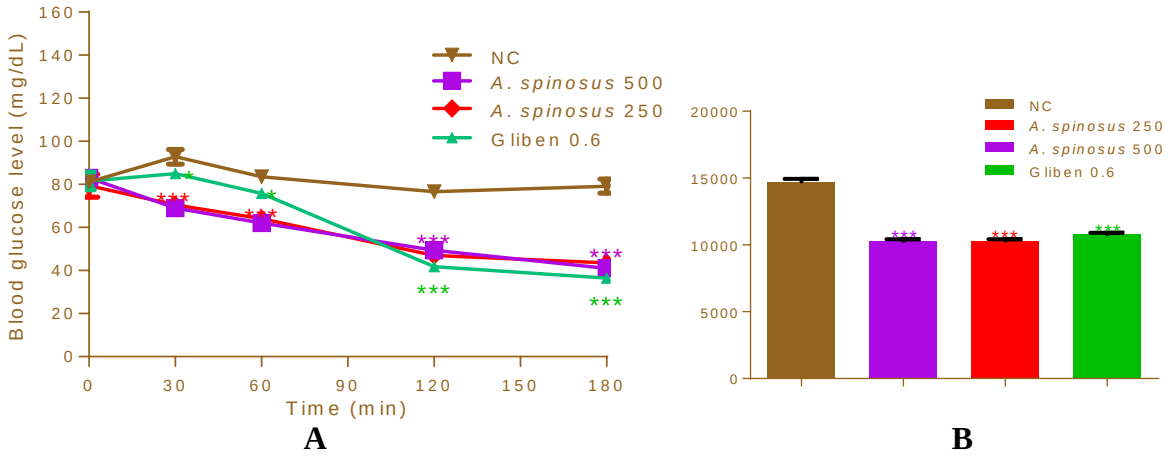

Figure 4: Effect of *Amaranthus spinosus* extract on basal glucose
(A = Blood glucose level vs. time, B = Area under the curve (AUC) of blood glucose)
Blood glucose was measured for 180 min: t0 = 0 min, corresponding basal blood glucose level measurement in fasted mice and t30; t60; t120; t180 correspond to 30; 60; 120 and 180 min after administration of distilled water. NC = Normoglycemic controls; Gliben 0.6 = Glibenclamide 0.6 mg/kg b.w; *A. spinosus* 500 and 250 = *A. spinosus* 500 and 500 mg/kg b.w. Values are expressed in Means ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to NC. n = 5

***In vitro* antioxidant activities of extracts**

Total antioxidant capacity (TAC)

The TAC of ascorbic acid (AA) used as standard increases positively with the concentration and is correlated with the increase of absorbance (Figure 5).

The TAC were determined from AA calibration curve and expressed in equivalent of AA/g extract (mg EAA/g of extract). TAC $_{P.\ erinaceus}$ = 772.671 ± 3.858 mg EAA/g ; TAC $_{A.\ spinosus}$ = 18.364 ± 0.194 mg EAA/g. The TAC of *P. erinaceus* was much higher than that of *A. spinosus*.

DPPH free radical scavenging

As the free radical scavenging ability increased, the absorbance decreased proportionally and was correlated with the increase in extracts or standard concentration (Figure 6).

The inhibition concentrations 50% (IC_{50}) of DPPH free radical scavenging ability were 205.411 µg/mL; 213.220 µg/mL and 20.438 µg/mL respectively for *P. erinaceus*, *A. spinosus* and ascorbic acid (AA). The IC_{50} of both extracts was greater than IC_{50} of AA. This means that a high concentration of extracts would achieve the same levels of free radical scavenging ability given by AA. Otherwise, both extracts had a low free radical scavenging ability compared to AA used as standard.

Ferric reducing antioxidant power (FRAP)

The reducing power of the extracts and standard (ascorbic acid) increased with the dose and correlates with an increase in absorbance. From 0-215 µg/mL, absorbance of *P. erinaceus* was greater than that of ascorbic acid (AA). Above 215 µg/mL, the absorbance of AA was high. The absorbance of *A. spinosus* remained very low at all levels (Figure 7).

The increase concentration 50% (IC_{50}) of reducing power of *P. erinaceus*, *A. spinosus* and AA were respectively 99.296 µg/mL; 573.672 µg/mL and 135.710 µg/mL. *P. erinaceus* IC_{50} was low than AA IC_{50}, whereas *A. spinosus* IC_{50} was greater. This means that a low concentration of *P. erinaceus* extract lead to a high reducing power. At the same time, we will need a higher concentration of *A. spinosus* extract to have this level of reducing power. Otherwise, the IC_{50} of *P. erinaceus* was lower than the IC_{50} of AA, lower than the IC_{50} of *A. spinosus*.

Phytochemical screening

The qualitative phytochemical analysis revealed in both extracts the presence of alkaloids, phenolic compounds, flavonoids, reducing compounds (oses and osides), saponosides and terpens. Tannins were present in *P. erinaceus* steam bark extract and were absent in *A. spinosus* roots extract (Table 4).

Phenolic compound content in extracts

Total phenols and tannins were determined from Gallic Acid calibration curve (Figure 8) and Flavonoids from Rutin calibration curve (Figure 9).

Total phenols and tannins were expressed in mg Gallic Acid Equivalent/g of extract, while flavonoids were expressed in mg Rutin Equivalent/g of extract. Tannins were highly present in *P. erinaceus* steam bark compared to flavonoids: 81.38% vs. 12.95% of total phenols. In the roots of *A. spinosus*, tannins were zero while the flavonoids accounted for 78.07% of the total phenols. Phenolic compounds in *P. erinaceus* steam bark were higher in content than *A. spinosus* root (Table 5).

Polysaccharides content in extracts

Polysaccharides were determined from Glucose calibration curve and expressed in mg of equivalence of Glucose/g extract (Figure 10). Polysaccharides were highly present in both extracts but that of *P. erinaceus* was about the double of that of *A. spinosus* (Table 5).

Glucose adsorption capacity

When increased glucose concentration in medium, the glucose intake increased proportionally with the quantity of extract (Figure 11). With the small concentrations of

glucose in the medium (5 and 10 mg) there was no significant difference in bound glucose by the tree doses of both extract. But with glucose 50 and 100 mg in medium, there was significant ($p < 0.0001$) difference in bound glucose when compared the tree doses of both extracts. The percentage of absorbed glucose by extract when we had 100 mg of glucose in the medium was 14.91% for *P. erinaceus* 50 mg; 24.56% for *P. erinaceus* 100 mg; 28.26 for *P. erinaceus* 150 mg; 10.70% for *A. spinosus* 50 mg; 16.44% for *A. spinosus* 100 mg and 20.24% for *A. spinosus* 150 mg. *P. erinaceus* extract was highly bound glucose when compared to *A. spinosus* extract: *P. erinaceus* 50 mg vs. *A. spinosus* 50 mg ($p < 0.05$); *P. erinaceus* 100 mg vs. *A. spinosus* 100 mg ($p < 0.0001$); *P. erinaceus* 150 mg vs. *A. spinosus* 150 mg ($p < 0.0001$) (Figure 11).

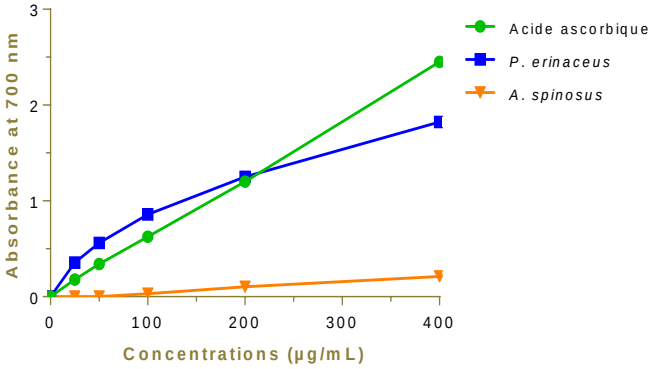

Figure 7: Reducing power of extracts compared to ascorbic acid.

Values are expressed in Means ± SEM; n =

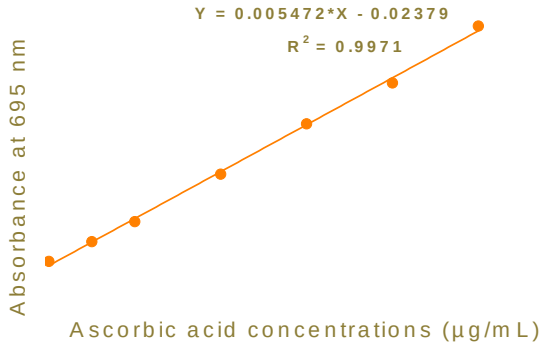

Figure 5: Ascorbic Acid calibration curve for Total Antioxidant Capacity determination.

Values are expressed in Means ± SEM. ; n = 3.

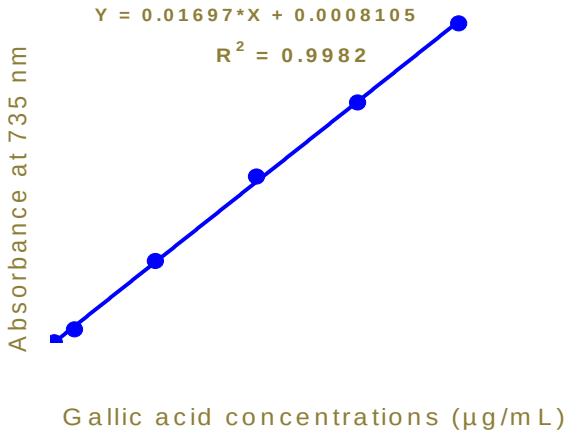

Figure 8: Gallic Acid calibration curve for phenols and tannins determination.

Values are expressed in Means ± SEM; n = 3.

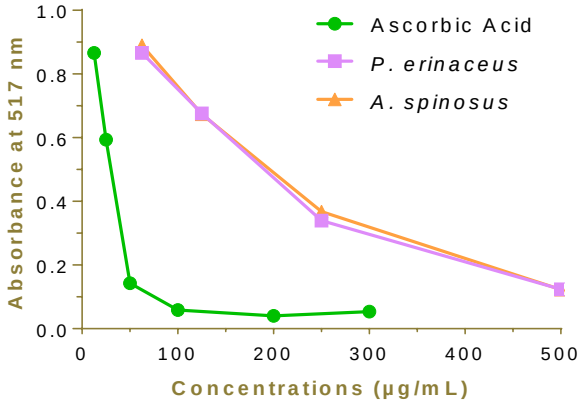

Figure 6: DPPH free radical scavenging ability of extracts compared to ascorbic acid.
Values are expressed in Means ± SEM; n = 3.

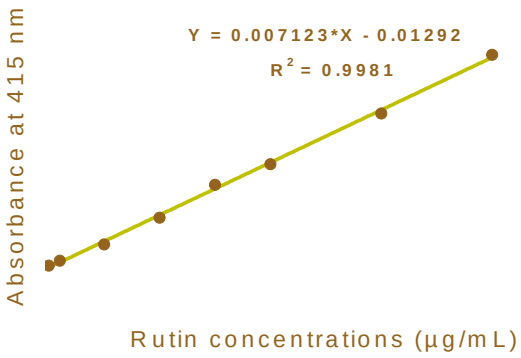

Figure 9: Rutin calibration curve for flavonoid determination.

Values are expressed in Means ± SEM; n = 3.

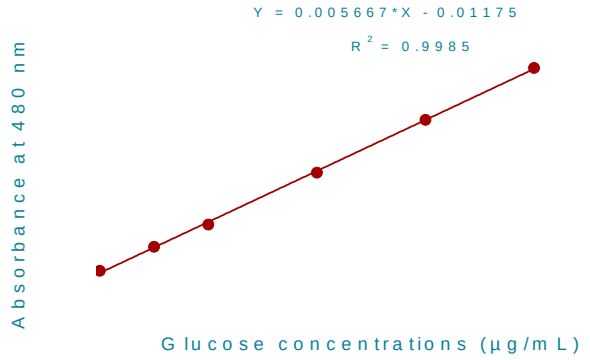

Figure 10: Glucose calibration curve for Polysaccharides determination.

Values are expressed in Means ± SEM; n = 3.

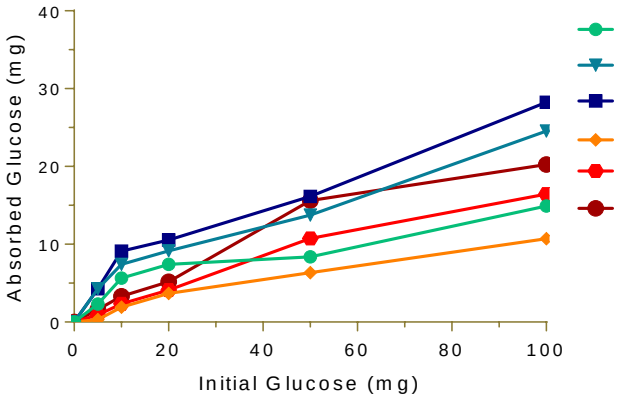

Figure 11: Glucose adsorption capacity by extracts.

Values are expressed in Means ± SEM; n = 3.

DISCUSSION

Oral glucose overload resulted in a significant (p < 0.0001) increase in blood glucose in fasted mice. The glucose absorbed in the intestine has passed into the general circulation and increased the blood glucose levels; which explains the peak observed at time t = 30 min. Under the action of insulin, cells responsible for glucose uptake have been sensitized for use and stored primary as glycogen in the liver [3]. These mechanisms explain the gradual decrease in blood glucose levels to homeostasis around 180 min.

Administration of hydroethanolic extract of *P. erinaceus* steam bark and *A. spinosus* roots at doses of 125; 250 and 500 mg/kg b.w 30 min prior to mice oral glucose tolerance test, prevented a significant increase in blood glucose levels. When considered data (Figure 1 and Table 1), *P. erinaceus* 250 mg/kg b.w, was effective in decreased blood glucose levels under postprandial hyperglycemia conditions and caused low decreased in basal blood glucose levels in mice when compared to *P. erinaceus* 500 mg/kg b.w. This mean that the effect of *P. erinaceus* extract in decreased blood glucose levels was not dose-dependent. However, the effect of *A. spinosus* extract in decreased blood glucose levels was dose-dependent. The effect of *A. spinous* extract in blood glucose levels lowering increased with the concentration of extract: *A. spinosus* 500 vs. *A. spinosus* 250 (p < 0.01), *A. spinosus* 500 vs. *A. spinosus* 125 (p < 0.0001). When analysis data, we showed that *A. spinosus* 250 and 500 mg/kg b.w, decreased significantly (p < 0.0001) in blood glucose levels and caused about the same decreased in basal blood glucose levels (Table 2 and Figure 2). Thus, *A. spinosus* 250 or 500 mg/kg b.w can be used to prevent postprandial hyperglycemia.

Briefly, *P. erinaceus* 250 mg/kg b.w and *A. spinosus* 250 or 500 mg/kg b.w can be used to prevent stern hyperglycemia on postprandial conditions.

All extracts also had a significant (p < 0.0001) decreased in basal glucose levels compared their AUCs to that of normal controls. However, *P. erinaceus* 500 mg/kg showed a significant (p < 0.01) decreased in basal glucose levels compared to *P. erinaceus* 250 mg/kg, whereas *A. spinosus* 500 mg/kg did not significantly decreased in basal glucose compared to *A. spinosus* 250 mg. The two doses of extracts of *P. erinaceus* and *A. spinosus* could cause hypoglycemia but according to *P. erinaceus*, extract 500 mg/kg would cause stern hypoglycemia compared to an extract 250 mg/kg.

The data obtained from oral glucose tolerance and basal glucose, showed that *P. erinaceus* 250 mg/kg and *A. spinosus* 250 or 500 mg/kg b.w can effectively manage diabetes.

Glibenclamide used as reference drug, significantly (P < 0.0001) decreased in mice oral glucose tolerance and basal glucose. Indeed, Glibenclamide is an oral hypoglycaemic agent of sulfonylurea compound chemically, 5-chloro-N-[2- [4-cyclohexyl carbamoyl sulfamoyl) phenyl] ethyl]-2-methoxy benzamide, which acts either as pancreatic or extrapancreatic, to lower blood glucose levels. The direct action of Glibenclamide is to stimulate insulin production from the existing pancreatic β-cells to increase insulin secretion [19]. When the molecule binds to the sulfonylurea receptor 1 (SUR 1), which is the regulatory subunit of ATP-sensitive potassium channels (KATP) in the pancreatic β-cells, the cell membrane depolarizes and the voltage dependent calcium channels opens; then increases intracellular calcium concentration and subsequently stimulates the release of insulin [20, 21]. In addition, Glibenclamide also shows extra pancreatic effects by reducing hepatic glucose production [22]. To resume, the same molecule stimulates insulin secretion and reduces hepatic glucose production in clinical diabetes [21; 23]. Extracts of *P. erinaceus* and *A. spinosus* would have acted as Glibenclamide to lower blood glucose levels by stimulating insulin secretion and reducing hepatic glucose production. Another mechanism of action by which extracts act to reduce blood sugar is the adsorption of glucose as our results show. The extracts bind to the glucose present in the medium to reduce the blood glucose levels [18]. This show the possible pancreatic and extrapancreatic activities of *P. erinaceus* and *A. spinosus* extracts. However, further studies are needed to clearly identify the mechanisms by which the extracts of these two plants lower blood glucose.

The effect of *P. erinaceus* and *A. spinosus* extract in blood glucose lowering would be due to some phytochemical compounds. Phytochemical screening revealed the presence of flavonoids, tannins, terpenes and polysaccharides known for antidiabetic activities [24, 25]. These compounds would have acted alone or in synergy with other compounds to induce a decrease in blood glucose levels [26, 27].

Furthermore, chronic hyperglycemia leads to oxidative stress, an important factor in the onset of diabetes and its complications. The excessive production of free radicals induce membrane lipids peroxidation, damage tissues and DNA. Mitochondrial oxidative phosphorylation and the NADPH oxidase system are mainly factors upon of which the production of reactive oxygen species (ROS) depends [28, 29]. Mitochondria are indeed the main endogenous source of ROS and can use 95% available oxygen to produce ATP. About oxygen 2% enters the electron transport chain; then it is oxidized to superoxide (O^{2-}) and hydrogen peroxide (H_2O_2). The NADPH oxidase system can also generate ROS and catalyze molecular oxygen to produce O^{2-} and/or H_2O_2 by accepting electrons from NADPH and transporting them to molecular oxygen. In diabetes, the decoupling of mitochondrial electron transport leads to excessive production of superoxide that can stimulate several abnormal biochemical metabolic pathways and increase the production of nitric oxide (NO) which results in DNA damage [12, 29, 30]. As result, we have vascular endothelium dysfunction which contributes to cardiovascular disease development and increased of neurodegenerative diseases [31]. Antioxidant administration can therefore reduce the oxidative stress and excessive production of NO caused by chronicle hyperglycemia [5, 12]. The antioxidant capacity and the reducing power of extracts can help to reduce ROS regeneration by the NADPH oxidase system. Otherwise, the antioxidant and reducing power of the extracts can be explained by the presence of flavonoids and tannins that interact directly with activated oxygen species to inhibit the formation of free radicals [32]. The total antioxidant capacity represents both oil soluble and water soluble antioxidants of *P. erinaceus* extract showed that the plant was able of scavenging ROS and protects from chronic diseases such as diabetics [5]. However, this total antioxidant capacity was very low for *A. spinosus* extract.

CONCLUSION

The evaluation of extracts of *P. erinaceus* steam bark and *A. spinosus* roots in oral glucose tolerance and basal glucose in ICR mice, showed that both plants significantly decreased in blood glucose levels and can be used to reduce acute hyperglycemia during diabetes. *P. erinaceus* 250 mg/kg b.w and *A. spinosus* 250 and 500 mg/kg b.w were effective in diabetes manage. To decreased glucose blood levels, extracts would acted as Glibenclamide with pancreatic activity to increased insulin secretion or with extrapancreatic activity by glucose adsorption, when bound to glucose in medium. The hypoglycemic activity observed could be in responsible for the flavonoids, tannins, terpenes and polysaccharides present in the extracts. These phytochemicals compounds would have acted alone or in synergy. In addition, extracts possess antioxidant and reducing power and can be used for this purpose in the treatment of diabetes complications related to oxidative stress in particular *P. erinaceus.*

Based on current finding, the steam bark of *P. erinaceus* 250 mg/kg b.w and the roots of *A. spinosus* 250 or 500 mg/kg b.w can be used to assist to control mild to moderately severe type II diabetes mellitus that does not require insulin. However, further studies are needed to elucidate the mechanisms by which these extracts act, evaluate their toxicity and then to standardize the doses of extract to be administered in order to produce traditionally improve drugs.

Conflict of interest

Authors declare no conflict of interest.

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