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EVALUATION OF HYPOGLYCEMIC AND ANTIDIABETIC ACTIVITIES OF THE 80% METHANOL EXTRACT FROM THE STEM BARK OF *ALSTONIA CONGENSIS ENGL. (APOCYNACEAE)* AND ITS FRACTIONS IN NORMAL AND STREPTOZOCIN-INDUCED DIABETIC WISTAR RATS.

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ABSTRACT

The effect of the 80% methanol extracts from *Alstonia congensis* stem bark and its fractions was evaluated for their putative hypoglycemic and antidiabetic properties in normal and in streptozocin-induced diabetic Wistar rats respectively. Results indicated, at administered oral doses of 100, 200 and 400 mg/kg bodyweight, the 80% methanol extract and its fractions induced significant reduction of the concentration levels of glucose in normal and diabetic treated animals in dose-dependent manner. In normal rats, at the highest oral dose of 400 mg/kg, the 80% methanol extract lowered the fasting glucose levels from 5.91 to 7.17% after 1 and 7 h of treatment respectively. The choroform, ethylacetate, n-butanol and residual aqueous soluble fractions reached the reduction of blood glucose from 3.67 to 3.88, 3.55 to 4.57, 1.53 to 2.23 and 2.72 to 3.17% respectivley after the same temps of treatment. In diabetic treated Wistar rats, at the same highest oral dose, the 80% methanol extract and its fractions produced a dose-dependent reduction of plasma glucose from 14.16 to 32.25% and 6.60 to 33.29% after 1 and 7h of treatment respectively. But, the concentration level of glucose in treated diabetic rats still remained

high and need a special treatment to reach approximately the glucose level near to that of normal rats. In this way, after 21 days of treatment, the 80% methanol extract and its fractions showed significant and marked dose-dependent reduction of glycaemia in treated animals compared to control groups. In both test, insulin used as reference product showed high significant decrease of the glucose concentration levels in normal and treated diabetic rats compared to *A. congensis* samples. In oral glucose tolerance test, the 80% methanol extract and its fraction lowered in dose-dependent manner the plasma glucose from 121 ±0.5 to 94.0 ± 1.1 mg/dL compared to negative control (139.2 ± 0.3 to 161.3 ± 0.7 mg/dL) from 30 min to 180 min of treatment. Thus, this study shows that *A. congensis* stem bark extracts have hypoglycemic and antidiabetic properties and are able to ameliorate the diabetic state, and probably can be considered as a new source of hypoglycemic and antidiabetic compounds.

KEYWORDS: *Alstonia congensis*, stem bark, 80% methanol extract, hypoglycemic and antidiabetic activities.

INTRODUCTION

Diabetes mellitus is one of the most common endocrine metabolic disorders causing significant morbidity and mortality due to the microvascular, macrovascular and peripheral vascular diseases (Patel et al., 2011). It is also considered as a group of metabolic alterations due to the hyperglycemia resulting from defects insulin secretion. or a chronic metabolic disorder due to the degeneration of carbohydrates, proteins and fats metabolism resulting in increased blood glucose level, which causes long-term complication in many organs such as eyes, kidneys, heart and arteries (O'Brien and Gramer, 1996; Jaspreet et al., 2003). Also, the hyperglycemia is associated with long term damage, dysfunction and eventually failure of organs such as nerves, kidneys, eyes, heart and blood vessels (Huany et al., 2005). According to WHO projection, it is estimated the diabetic population is likely increase up 300 million people or will be diabetic patients in 2025 with an increasing about 40 to 70% suspected in developing and developed countries respectively (Sy et al., 2005; Ma Cristina et al., 2007; Porter and Barret, 2005).

The treatment of the disease includes a modification of life behaviour, such as diet, exercices and the available therapy of the disease include insulin or utilization of hypoglycemic and antidiabetic agents such as glibeclamide sulfonylureas, α-glucosidase inhibitors, metformin, biguanides and glinides used as monotherapy or in combination to active better glycemic

regulation (Kelly and Mandarino, 2000). However, these oral drugs are known to have a number of serious side effects (Noor et al., 2008; Dewanjee et al., 2009) and are not considered to be safe for use during pregnancy (Larner, 1985).

Despite remarkable progress in the management of diabetes using synthetic drugs, the population, mainly in developing countries, still rely on the use of various medicinal plant species claimed by traditional healers to be effective against the disease and find some reliefs. Nowadays, some extracts from these medicinal plant species belonging to different botanical families had been reported to be useful and empirically used in the treatment of diabetes mellitus worldwide. They are nowadays, scientifically investigated in diabetic animal model and found to possess hypoglycemic and antidiabetic properties at different extents constituting thus, an alternative in searching new hypoglycemic an antidiabetic natural drugs (Kesari et al., 2005; Adeneye et al., Bnhouchan et 2006; Tanko et al., 2008; Chika and Bello, 2010; da Cunha et al., 2010; Padee et al., 2010, Ayodhya et al., 2010; Malviya et al., 2010; Mamun-or-Rashid et al., 2014). Although these plant extracts have shown varying degree of both activities in experimental animal model, some of them are not effective in experimental diabetic human model and its complications.

The stem bark of *Alstonia congensis* is used as an aqueous decoction in different African countries to treat various diseases including diabetes, malaria, gonorrhea, rheumatism pains, diarrhea and other intestinal problems, or used as galactagogue agent. The bark is also applied as an antidote for arrow poison and as an anthelmintic remedy (Oliver-Bever, 1986; Neuwinger, 2000). During an ethnopharmacological investigation conducted in Kinshasa, traditional practioners have claimed that *A. congensis* stem bark used as an aqueous decoction is effective in the treatment of type-2 diabetes mellitus. But to our knowledge, so far the hypoglycemic activity of this plant part has not yet been systematically investigated because no report was available in the literature. But, it seems important to inform that *A. congensis* bark and *Xylopia aethiopica* fruits mixture (1:1) was previously reported to have the plasma sugar lowering in streptozocin-induced diabetic animals with some beneficial effects on cardiovascular risk factors (Ogbonnia et al., 2008). In another study, a poly-herbal formulation, Okudiabet, a mixture of *Stachytapheta angustifolia* aerial parts, *Alstonia congensis* bark and *Xylopia aethiopica* dried ripe fruits was reported to produce significant reduction in plasma glucose in alloxan-induced diabetic Swiss mice (Ogbonnia et al., 2010). To our knowledge, no plant constituting this antidiabetic mixtures was previously

investigated for its potential hypoglycemic and antidiabetic properties. Thus, the aim of the present study was to evaluate the hypoglycemic and antidiabetic activities of the 80% methanol extracts from *A. congensis* stem bark and its fractions in normal and streptozocin - induced diabetic Wistar rats.

2. MATERIALS AND METHODS

2.1. Plant material

Stem bark of *Alstonia congensis* Engl. (Apocynaceae) were collected in Kinshasa in July 2013. The plant was identified by Mr. M. Nlandu Lukebiako, B. of the Institut National d'Etudes et de Recherches en Agronomie of the University of Kinshasa. A voucher specimen MN 30072009ACSB was deposited in the herbarium of this institute. The plant material was dried at room temperature and reduced to powder.

2.2. Preparation of extract and its fractionement

20 g of powdered stem bark were macerated with 200 ml of 80% methanol for 24 h. After, the mixture was filtered and the marc was exhaustively percolated with the same solvent. The macerate and percolate were mixed and evaporated *in vacuo* giving a dried extract denoted as ME-1 (14.37 g). After, an amount of 10 g of ME-1 were dissolved in 200 ml distilled water and filtered, the filtrate was exhaustively extracted of solvents of different polarities including chloroform, ethylacetate, *n*-butanol. All fractions were treated as described above yielding corresponding dried extract denoted as chloroform (ME-1.1, 1.46 g) ethylacetate (2.45 g) and *n*-butanol (2.75 g). The residual aqueous phase was also evaporated in the same way given a dried extract denoted as ME-1.4 (3.59 g).

2.3. Phytochemical screening

Chemical tests to identify major phytochemical groups were carried by TLC on precoated silica gel plates (thickness layer 0.25 mm, Merck, Germany) on the 80% methanol extracts from *Alstonia congensis* stem bark and its fractions to identify major chemical groups using standard procedures and reagents described in the literature (Harborne, 1998).

2.4. Assessment of hypoglycemic activity in normal Wistar rats

Normal fasted Wistar rats (140-150 g body weight) overnight were allocated into seven groups with 2 rats each for group I (negative control reciving orally 5 ml NaCl/kg body weight) and group II (positive control receiving by IP 0.5 IU of insulin). Group III received orally 100, 200 and 400mg.kg body weight (b.w) of the 80% methanol extract (ME-1)

dissolved in saline solution while groups IV to VII received the same oral dose of the chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively, from the partition of ME-. The serum glucose levels were collected and measured prior to the animal receiving the test samples and subsequent after the administration of the *A. congensis* samples from 0 to 7 h using a glucosemeter (Jafri et al., 2000; Zanatta et al., 2008).

2.5. Induction of Diabetes Mellitus and treatment

The animals were divided in the same way as in evaluation of hypoglycemic activity. They were kept in standard cages at 25°C and 12h light/dark conditions in the animal room. The animals were fed on commercial feeds and were given water at libitum. They were fasted from feeds for 12 h before the starting of experiments. They were made diabetic by a single intraperitoneal injection of streptozocin (STZ) dissolved in citrate buffer 0.1M adjusted at pH 4.5 at a dose of 60 mg/kg after 6 h (Tanko et al., 2008, Badole et al., 2010; Saravanan et al., 2010; Zhao et al., 2011, Karau et al., 2012). Since streptozocin is capable of producing fatal hypoglycaemia as a result of massive release of insulin, the animals were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent this effect (Tanko, 2008; Hyanie et al., 2011). After a period of three days, the serum glucose level was measured and the rats with a blood glucose levels greater than 180 mg/l were considered as diabetic and used in this study (Tanko et al., 2008, Badole et al., 2010, Karau et al., 2015).

Diabetic rats were divided into following groups: group I (2 rats) non treated diabetic rats received saline solution as diabetic negative control group, group II received insulin 0.5 I.U/kg b.w IP as positive control, group III (5 rats of each oral dose) was orally administered variable doses of 100, 200 and 400 mg/k of the 80% methanol extract (ME-1), groups IV to VIII (5 rats for each dose of each tested fraction) were given orally the same oral dose as the sample ME-1 of the chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions. The serum glucose levels were collected and measured prior to the animal receiving the test samples and subsequent after the administration of the *A. congensis* samples from 0 to 7 h using a glucosemeter (Jafri et al., 2000; Zanatta et al., 2008).

2.6. Effects of crude extracts on oral glucose tolerance

The hyperglycemia were made in normal rats by oral administration of 4g/kg of glucose y oral route 30 min after haven been treated with the aqueous extract of *A. congenic* stem bark and its fractions. Fasted hyperglycemic Wistar rats were divided into five groups. Group I received glucose (4 g/kg, 2 rats) and served as negative control. Group II received insulin (0.5

IU IPs) and was the positive control group. Groups III to V with 5 rats for each oral dose, were administered orally variable doses of 100, 200 and 400 mg/kg b.w of ME-1 extract and only its chloroform (A-1.1) and ethylacetate (A-1.2) soluble fractions respectively (Table 3). Blood samples were collected just prior to and at 30, 60, 90 and 180 min after the glucose loading and the serum glucose levels were measured (Zanatta et al. 2007, Nyunaï et al., 2010, Toma, et al., 2015).

2.7. Determination of blood glucose level

All blood samples were collected from the tail artery of the rats at intervals of 0, 1, 3, 5 and 7 h. The determination of the blood glucose levels was carried out by the glucose oxidase reactive with strips using a glucosemeter instrument (ONE TOUCH Vita LIFESCAN, Inc, Milpitas, CA 95035, USA) and results were expressed as mg/dl (Rheney and Kirk, 2000; Tanko et al., 2008 Tende et al., 2011).

2.8. Determination of insulin concentration

Insulin concentration in diabetic rats was determined in serum by radioimmunoassay method using a commercial available DSL-1800 insulin kit (Diagnostic System Laboratories, Inc, USA). Insulin values were expressed as µg//ml (Bakirel et al., 2008).

2.9. Histopathologycal of mousse pancreas

Histopathological mousse pancreas was performed according to the procedure described by Badole et al. 2010). Briefly, pancreas of all animals were isolated and cutted in small pieces. They were preserved in 10% formalin for 24 h. Specimens were cut in sections of 3-5 µm in thickness and stained by hematoxyline-eosin and mounted by disterene phthalate xylene. The photomicrographs of each tissue section were observed using cell imaging software for Life Science microscopy (Olympus soft imaging solution GmbH, Germany). Pancreas was processed for Gomori staining for morphology of pancreatic β-cells.

2.10. Statistical analysis

The experimental results were expressed as mean ± standard error of the mean (S.E.M). Data were statically analyzed by analysis of variance (ANOVA). T-student's test were made to analyse the significant differences between the groups and p values $p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effects of the aqueous extract on the level of glucose in normal Wistar rats

In the present investigation, the hypoglycemic and antidiabetic activities of the 80% methanol (ME-1) extract of the stem bark of *Alstonia congensis* and its fractions were evaluated in normal and streptozocin-induced diabetic rats for its potential hypoglycemic and antidiabetic activity respectively at oral doses of 100, 200 and 400 mg/kg and results are presented in Tables 1, 2 and 3. Results indicated that, in normal rats, the methanol extract (ME-1) and its fractions induced a significant fasting plasma glucose level lowering in a dose-dependent manner (Table 1). At the highest oral dose of 400 mg/kg, ME-1 extract produced 7.17%of serum glucose lowering after 7h of treatment while its fractions reached significant decreasing of the glucose concentration level manner from 3.37 to 5.53% . Among these fractions, the ethylacetate fraction (ME-1.2) rich in flavonoids caused a reduction of 5.53% of the fasting glucose concentration level followed by the chloroform soluble fraction (ME-1.1) rich in terpenes and steroids with 4.59%. The *n*-butanol (ME-1.3) and residual aqueous phase (ME-1.4) soluble fractions also showed good hypoglycemic activity at the same highest oral dose. (Table 1). Thus, it has been concluded that in normal rats, these sample possess hypoglycemic properties since the amount of glucose in these treated animal is significantly lowered ($p < 0.05$) compared to untreated group (Table 1) . Insulin used as a reference product also significantly produced reduction of the glucose concentration level in treated normal rats and its activity is high compared to *A. congensis* samples (Table 1). This effect may be due to the potentiation of insulin receptors or inhibition of glucose reabsorption in the proximal tubules of the kidney (Shalev, 1999, Silva et al., 2002).

Table 1. Effects of insulin, 80% methanol (ME-1) extract of *A. congensis* stem and its fractions (ME-1.1 to ME-1.4) on fasting plasma glucose levels (mg/dL) and % reduction in normal Wistar rats.

Groups	Treatment	0h	1h	3h	5h	7h
I	Normal rats control + saline	85.1 ± 0.5	84.5 ± 0.1	84.1 ± 0.6	84.7 ± 0.2	85.0 ± 0.5
II	Normal rats + insulin (0.5 I.U/kg)	82.3 ± 0.8	79.7 ± 0.2	75.3 ± 0.1	72.3 ± 0.4	70. 6 ± 0.3
			5.9%	10.46%	14.6%	16.94%
III	Normal rats + 100 mg/kg **ME-1**	82.1 ± 1.2	82.1 ± 0.3	80.1 ± 1.1	81.8 ± 0.7	79.4 ± 0.5
			4.3%	4.75%	4.8%	6.59%
	+ 200 mg/kg	81.5 ± 0.2	81.4 ± 1.2	80.7 ± 0.2	80.5 ± 0.9	81.4 ± 0.6
			4.5%	4.04%	5.0%	4.23%
	+ 400 mg/kg	80.1 ± 1.2	79.5 ± 1.1	79.2 ± 0.9	79.0 ± 0.8	78.9 ± 0.3
			5.91%	5.82%	6.73%	7.17%
VI	Normal rats + 100 mg/kg **ME-1.1**	82.8 ± 0.3	81.6 ± 0.5	82.5 ± 0.4	82.2± 0.4	81.7 ± 0.8

			3.43%	1.90%	2.95%	3.88%
	+ 200 mg/kg	81.5 ± 0.3	81.7 ± 0.4	81.0 ± 0.2	82.6 ± 0.3	81.5 ±0.8
			3.31%	3.68%	2.48%	4.11%
	+ 400 mg/kg	80.8 ± 0.7	81.4 ± 0.8	82.1 ± 0.6	82.3 ± 0.7	81.1 ± 0.2
			3.67%	2.38%	2.83%	4.59%
VII	Normal rats + 100 mg/kg **ME-1.2**	82.0 ± 0.4	81.7 ± 0.2	81.5 ± 0.7	81.8 ± 0.4	82.5 ±0.7
			3.31%	3.09%	3.42%	2.94%
	+ 200 mg/kg	82.4 ± 0.7	80.8 ± 0.4	81.2 ± 0.8	81.4 ± 0.7	82.3 ± 0.2
			4.378%	3.44%	3.89%	3.07%
	+ 400 mg/kg	80.4 ± 0.2	81.5 ± 0.4	82.4 ± 0.8	81.8 ± 0.4	81.7 ±0.7
			3.55%	2.02%	3.42%	3.88%
VIII	Normal rats + 100 mg **ME-1.3**	84.2 ±0.1	83.7 ± 0.4	82.7 ± 0.8	82.4 ± 0.2	82.4 ± 0.2
			0.94%	1.66%	2.71%	3.05
	+ 200 mg/kg	83.5 ±0.2	82.5 ± 0.4	81.6 ± 0.7	81.3 ±0.7	81.5 ± 0.4
			2.37%	2.97%	4.01%	4.11%
	+ 400 mg/kg	83.1 ±0.4	83.2 ± 0.7	82.1 ± 0.2	82.0 ± 0.2	83.1 ± 0.2
			1.53%	2.37%	3.18%	2.23%
IX	Normal rats + 100 mg **ME-1.4**	84.0 ± 0.7	83.3 ± 0.4	82.2 ± 0.1	82.5 ± 0.2	82.2 ±0.8
			1.42%	2.25%	2.60%	3.29%
	+ 200 mg/kg	83.6 ± 0.4	83.1 ± 0.2	83.0 ± 0.2	82.8 ± 0.1	82.6 ± 0.1
			1.65%	1.30%	2.24%	2.82%
	+ 400 mg/kg	83.1 ±0.2	82.2 ± 0.1	82.0 ± 0.5	82.3 ± 0.8	82.3 ± 0.4
			2.72%	2.49%	2.83%	3.17%

ME-1: 80% methanol extract, ME-1.1, ME-1.2, ME-1.3 and E-1.4: chloroform, ethylacetate, n-butanol and residual aqueous phase fractions respectively from the partition of ME-1.

3.2. Effects of the 80% methanol extract from A. congensis stem bark and its fractions on the concentration level of glucose in treated diabetic Wistar rats

The administration of STZ at an oral dose of 60 mg/kg body weight in normal Wistar rats significantly elevated the blood glucose levels of rats from 228.4 mg/dl in 1h to 253.3 mg/kg body weight after 7h of treatment (Table 2) confirming their diabetic state. Results indicated the administration of the 80% methanol extract and its fractions significantly reduced in a dose-dependent manner the blood glucose concentration level of treated diabetic rats at all oral doses compared to untreated diabetic groups from 1h to 7 h ($p < 0.001$), but their activity was lower than that of insulin used a reference antidiabetic product ($p < 0.001$) (Table 2). At the highest oral dose of 400 mg/kg body weight, the 80% methanol extract (ME-1) caused the lowering of the blood glucose levels significantly by 32.85% in the diabetic rats treated after 7h of treatment. Among its fractions, the ethylacetate fraction (ME-1.2) rich in flavonoids, was the most active fraction reaching a reduction of 33.23% while the remaining fraction had

the same effect provoking a reduction of the blood glucose concentration levels from 26.15 to 30.26% (Table 3).

The effect of the administration of the 80% methanol extract (ME-1) and its fractions in STZ-induced diabetic rats compared to that of insulin suggested that these samples possess insulin like-effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibition hepatic glycogenesis (Hemalatha et al., 2004; Tanko et al., 2008).

Table 2. Effects of the 80% methanol extract (ME-1) on the concentration level of glucose in treated diabetic Wistar rats and % of glycaemia reduction after 7 h of treatment

Groups	Treatment (mg/kg)	0h	1h	3h	5h	7h
I	DR + saline	187.1 ± 0.6	198.4 ± 0.5	205.6 ± .4	216.3 ± 2.2	228.3 ± 0.2
II	DR + Insulin : 0.5 I.U	180.2 ± 0.4	153.2 ± 0.2	141.7 ± 0.7	110.8 ± 0.6	86.8 ± 1.2
			22.78%	31.07%	48.77%	62.00%
III	DR + **ME-1 +** 100	182.1 ± 1.1	177.3 ± 0.7	175.6 ± 0.5	171.2 ± 1.1	167.4 ± 0.6
			10.63	14.60%	20.85%	26.67%
	+ 200	180.7 ±1.4	174.3 ± 0.2	171.5 ± 1.3	167.2 ± 0.3	163.2 ± 0.8
			12.14%	16.58%	22.70%	28.51%
	+ 400	177.3 ± 0.7	170.3 ± 0.2	167.3 ± 0.7	164.2 ± 1.2	153.3 ± 0.9
			14.16%	18.62%	24.08%	32.85%

DR: diabetic rats, ME-1: 80% methanol extract, DR + saline: non treated diabetic rats (negative control), DR + insulin: positive control.

% reduction of glucose = (GNCG- GTG)/ GNCG x 100

Where GNCG I is the amount of glucose in negative control groups and GTG is the amount of glucose in the treated groups

Because these samples from *A. congensis* stem bark decreased fasting plasma glucose (FPG) in STZ-induced diabetic rats, it was assumed, they might be due their cumulative effects concentration level during the period of treatment and also perhaps associated with an increasing in the blood insulin concentration level as demonstrated in the present study.

Table 3. Effects of fractions from the partition of the aqueous extract on the concentration level of glucose in treated diabetic Wistar rats and % of glycaemia reduction after 7 h of treatment

Groups	Treatment (mg/kg)	0h	1h	3h	5h	7h
IV	DR + **ME-1.1** + 100	186.5 ± 1.2	173.8 ± 1.1	171.2 ± 1.0	168.0 ± 0.2	166.7 ± 0.6
			12.40%	16.73%	22.33%	26.98%
	+ 200	184.7 ± 0.2	170.4 ± 0.4	168.3 ± 1.2	166.7± 0.4	164.3 ± 1.1
			14.11%	18.14%	22.93¨%	28.03%
	+ 400	181.4 ± 1.2	168.5 ± 1.6	165.0 ± 0.9	163.5 ± 1.1	159.2 ± 0.5
			15.07%	19.74%	24.41%	30.26%
V	DR+ **ME-1.2** + 100					
		182.4 ± 0.4	170.5 ± 1.1	168.3 ± 0.9	165.5 ± 0.4	163.1 ± 0.3
	+ 200		12.24%	18.14%	23.48%	28.55%
		178.7 ± 1.2	167..3 ± 1.0	165.6 ± 1.0	162.9 ± 0.7	160.2 ± 0.8
	+ 400		15.67%	19.45%	24.68%	29.83%
		174.3 ± 0.2	164..3 ± 0.7	162.6 ± 0.7	156.8 ± 0.1	152..3 ± 1.2
			17.18%	20.91%	27.50%	33.29%
VI	DR + **ME-1.3** +100					
		185.8 ± 0.3	185.3 ± 1.1	181.3 ± 0.9	179.3 ± 1.	176.3 ±01.4
	+ 200		6.60%	11.82%	17.10%	22.78%
		182.3 ± 1.2	183.2 ± 0.2	178.3 ± 0.5	175.3 ± 0.7	174..3 ± 0.2
	+ 400		7.61%	13.27%	18.95%	23.65%
		180.5 ± 0.2	180.3 ± 0.7	177.5 ± 0.1	174.2 ± 0.	164.2 ± 0.9
			9.07%	13.66%	20.85%	28.08%
VII	DR + **ME-1.4** + 100					
		185.6 ± 0.5	183.6 ± 1.2	181.9 ± 0.9	179.2 ± 0.8	177..3 ± 1.3
	+ 200		7.46%	10.55%	16.67%	22.34%
		183.2 ± 0.7	183.2 ± 0.7	180.6 ± 0.4	177..5 ± 0.2	174.5 ± 0.8
	+ 400		7.66%	12.16%	17.93%	23.56
		181.9 ± 0.3	180.4 ± 0.1	175..3 ± 0.2	172.6 ± 0.3	168.6 ± 1.6
			9.07%	14.73%	19.27%	26.15%

See Table 2, ME-1.1 to ME-1.4: chloroform, ethylacetate, *n*-butanol and residual aqueous phase from the partition of ME-1. *3.3. Effects of insulin, the 80% methanol and its fractions on the concentration levels of insulin after 7 h of treatment*

Figures 1 show the effect insulin on the concentration level of serum insulin in normal, non-treated diabetic and treated diabetic animals. Results indicate that at all injected doses, insulin significantly increase the secretion of insulin in normal and diabetic treated animals in dose-dependent manner compared to negative control group (Figure 1). This effect was also observed with the administration the 80% methanol extract and its fractions (Figures 2 and 3) suggesting that these samples have an insulin like effect. The highest concentration of serum insulin caused by *A. congensis* stem bark samples was obtained after 7 h of treatment at the highest tested concentration of 400 mg/kg b.w.

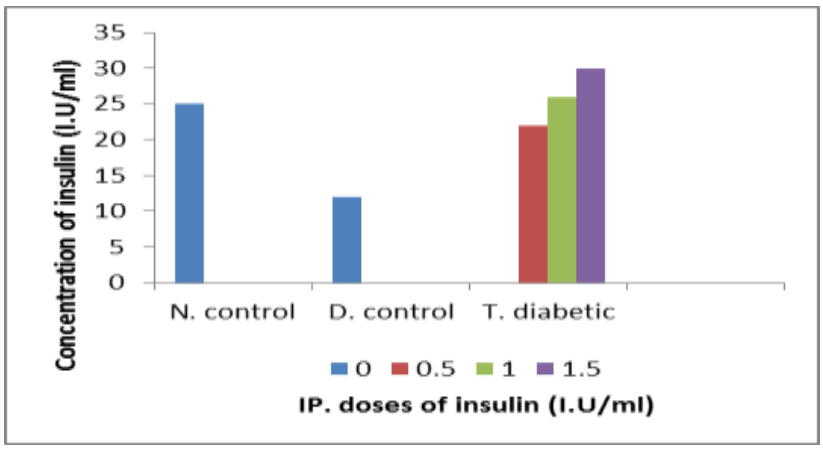
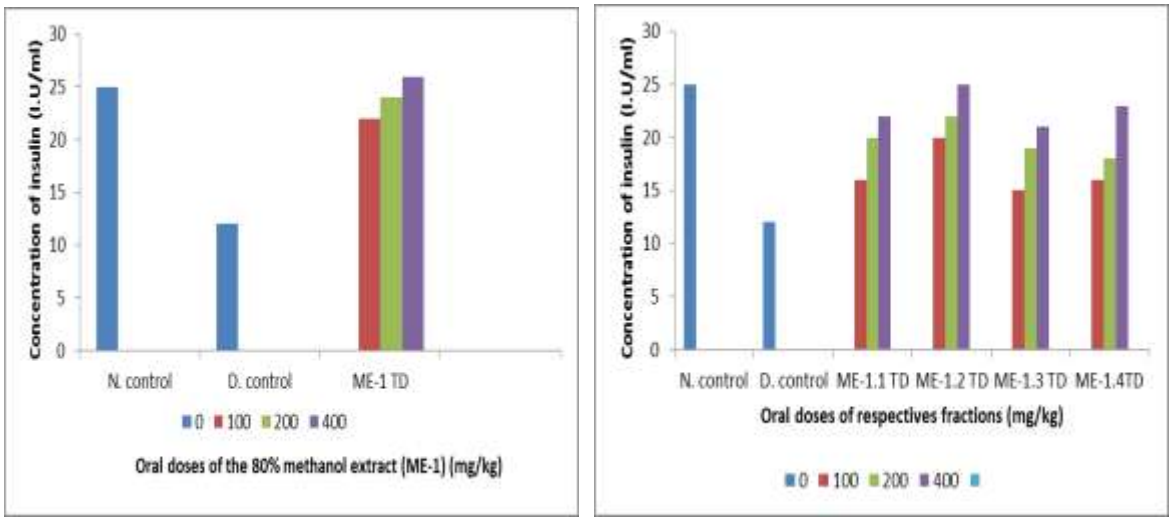


Figure 1: Effects of insulin of the concentration level of insulin in normal, non treated diabetic and treated diabetic rats after 7 h of treatment

This effect can be considered as a direct evidence for the regeneration and proliferation of survival of β-cells as also suggested by Shokeen et al., 2008, Badole et al., 2010, Karau et al., 2015) which in turn, stimulate the secretion of serum insulin and this is a common mechanism attributed to other various medicinal plants with hypoglycemic and antidiabetic properties (Eidi et al., 2006; Shokeen et al., 2008; Padee et al., 2010, Badole et al., 2010, Melani et al., 2011; Karau et al., 2012).



A **B**

Figure 2: Effects of the 80% methanol extract (ME-1) (A) and its fractions (B) on the concentration level of insulin in normal, non- treated diabetic and treated diabetic rats after 7 h of treatment, ME-1TD, ME-1.1 TD to ME-1.4 TD : treated diabetic rats with the 80% methanol extract, chloroform, ethylacetate, *n*-butanol and residual aqueous fractions respectively.

3.4. Effects of the aqueous extract and its fractions on the concentration level of glucose in diabetic rats after 21 days of treatment

After the treatment of diabetic rats with the aqueous extract of *A. congensis* stem bark and its fractions after 7 h, it was however observed that the glycaemia in diabetic animals still remained high. It was thus decided to submit these diabetic animals to a special treatment aiming to bring back as much possible the glucose concentration much near to that of normal animals. For this, diabetic rats were submitted to a treatment consisting in the administration of selected oral doses of 100, 200 and 400 mg/kg once all three days. Blood sample and evaluation of glycaemia were carried out after each week. The obtained results are presented in Tables 4 and 5 and the glycaemia in treated animals was compared to non-treated diabetic negative control group.

The administration of the aqueous extract at all oral doses cause significant decrease $p < 0.05$) of the concentration level of glucose in treated animals compared (from 128.2 ±0.6 to 97.8 ±0.5 mg/dL and from 97.2 ± 0.8 to 88.4 ± 1.5 mg/dL).) At Day 30, it was observed significant decrease of the glycaemia in treated groups compared to untreated groups (Table 5). At the highest oral dose of 400 mg/kg, ME-1 produced 67% reduction of glycaemia in

treated animals. Fortunately, these animals cannot be considered as diabetic since the concentration level of their glucose are lower than 180 mg/dl as an adopted criteria in the present study.

All fractions from the partition of the aqueous extract displayed antidiabetic activity at different extents in dose-dependent manner. At the highest oral dose of 400 mg/kg, the ethylacetate fraction ME-1.2 rich in flavonoids, was found to be to most active sample since it reached the reduction of the concentration level of glucose in treated groups from 122.2 ± 0.7 mg/dL at Day 7 to 87.3 ± 0.3 mg/dL at Day 21 while the remaining ME-1.1 rich in terpenes and steroids, , ME-1.3 rich in sapiens and ME-1.4 rich in phenolic compounds fractions showed the same effect with the reduction of the concentration level of glucose from 125.3 ± 0.2 mg/dL to 136.3 ± 0.3 mg/dL and 98.3 ± 0.1 to 121 ± 1.4 mg/dL at the same days respectively, compared to untreated groups (241.3 ± 1.7 and 264.2 ± 0.9 mg/kg respectively). Results also indicated that from Day 7 to Day 21, the administration of insulin (0.5 IU/kg) in positive control group reached to a significant reduction of glycaemia in treated groups (from 97.7 ± 1.1mg/dL at Day 7 and to 8281. ±1.7 mg/dL at Day 21) compared to untreated group (from 241.3 ± 1.7 to 264.2 ± 0.9 mg/dL at the same days respectively). At Day 30, le concentration level of glucose induced by this antidiabetic reference product was similar to that of negative control group (Table 4).

Tableau 4. Effects of insulin and the 80% methanol extract from *A. congensis* stem bark on the glucose concentration level (mg/dL) in diabetic Wistar rats after 21 days of treatment and % of glycemia reduction.

Groups	Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 30
I	NR + saline	85.1 ± 0.2	85.0 ± 08	84.9 ± 0.9	85.3 ± 0.6	85.5 ±0.7
II	DR + saline	228.3 ± 3.2	241.3 ± 1.7	253.1 ± 1.5	264.2 ± 0.9	270.3 ± 1.1
III	DR + 0.5 IU/ml Insulin		97.7 ±1.1	85.4 ± 1.2	82.8 ± 1.7	82.2 ± 1.1
			62.41%	66.25%	69.03%	69.59%
IV	DR + **ME-1** + 100	218.3 ± 0.3	124.7 ± 0.6	111.2 ± 0.7	95.2± 0.5	91.4 ± 0.3
			46.87%	55.27%	63.21%	64.70%
	+ 200	214.3 ± 0.2	114.3 ± 0.7	98.2 ±0.6	93.5 ± 0.4	90.0 ± 0.4
			52.22%	60.41%	63.85%	66.33%
	+ 400	212.3 ± 0.7	93.8 ± 0.4	88.3 ± 0.1	86.4 ± 02	85.2 ± 0.4
			59.47%	63.53%	66.54%	67.00%

NR + saline : negative control normal rats, DR + saline: negative control non-treated diabetic rat, DR+ ME-1: treated rats with aqueous extract

Table 3. Effects of fractions from the partition of ME-1 extract on the concentration levels of glucose in diabetic rats after 21 days of treatment and % of glycemia reduction.

Groups	Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 30
V	DR + **ME-1. 1** + 100	224.3 ± 0.8	138.3 ±0.1	124.3 ± 0.4	98.3± 0.7	92.3 ± 1.2
			41.02%	49.30%	61.65%	66.69%
	+ 200	220.4 ± 1.2	133.2 ±0.4	120.3 ±0.1	97.6 ± 1.0	94.3 ± 1.2
			43.97%	51.68%	62.30%	6.70%7
	+ 400	217.3 ± 0.4	125.3 ±0.2	112.7 ±0.5	93.6 ± 1.4	89.6 ± 0.2
			46.82%	55.63%	64.57%	66.85%
VI	DR + **ME-1.2** + 100	220.5 ± 0.7	128.7 ± 0.4	120.3 ± 0.5	93.5 ± 0.4	87.1 ± 2.0
			45.09%	52.07%	63.85%	66.81%
	+ 200	218.9 ± 0.5	124.4 ±0.5	114.2 ±0.2	92.6 ± 0.7	85.7 ± 1.5
			46.79%	54.09%	65.33%	68.29%
	+ 400	214.3 ± 0.2	122.2 ±0.7	110.3 ±1.4	87.3 ± 0.3	86.1 ± 1.1
			49.35%	56.42%	66.95%	68.14%
VII	DR + **ME-1.3** + 100	225.3 ± 1.3	143.3 ±0.7	133.2 ±0.4	120.7 ± 0.3	98.3.2±0.3
			39.78%	46.58%	53.93%	59.23%
	+ 200	223.4 ± 1.1	137.2 ±0.4	128.2 ±0.7	108.3±1.2	94.3 ± 0.5
			42.31%	48.90%	58.25%	64.37%
	400	221.8 ± 0.9	135.7 ±0.5	126-4.3 ±0.1	98.7 ± 1.0	88.2 ±0.9
			43.84%	50.01%	62.75%	67.18%
VIII	DR + **ME-1. 4** + 100	225.6 ± 0.8	148.2 ±0.7	144.2 ±1.0	138.2 ±1.3	113.2± 0.8
			37.35%	42.63%	47.16%	57.38
	+ 200	223.4 ± 1.3	140.3 ±0.8	136.2 ±0.9	132.7 ±1.1	96.5 ± 1.1
			41.02%	45.00%	49.84%	63.55%
	+ 400	220.6 ± 1.1	136.3 ±0.2	131.2 ±0.6	127.2 ±1.4	92.0 ± 0.4
			42.68%	47.37%	51.62%	65.85%

See Table 2, DR + ME-1.1 to 1.4 : treated diabetic rats with chloroform, ethylacetate, *n*-butanol and residual aqueous fractions respectively from the partition of the extract ME-1.

3.5. Effects of the methanol extract ant its fractions on the histopathology of pancreas

The histological examination of β-pancreatic cells by microscopic observation (optic microscope) after dissection of different animals had shown that the pancreas of normal rats and diabetic treated rats with insulin and *A. congenis* samples showed normal histological structure depict average sized islets and normal sized β-cells whereas that of diabetic control rats showed hyperplasia of beta-cells and congestion of pancreatic parenchymal cells, small sized islets without enlargement and destruction of the β-cells by STZ having as consequence

the secretion of insulin in low quantity to reduce the glycaemia (Kamtchouning et al., 1998; Skudelski, 2001). This finding is in good agreement with Badole et al. (2010). The administration of insulin and samples of *A. congensis* stem bark increased the number of islets compared to non-treated diabetic control groups. Pancreas histology indicated considerable quantitative increase in β-cells when treated with insulin and samples of *A. congensis* stem bark. In addition, insulin, ME-2 extract, ME-1 extract and its fractions were found to reach the regeneration and proliferation of β-pancreatic cells in treated diabetic rats, possibly due to prevention of free radical formation induced by STZ (Toma et al., 2015). This effect clearly show that both insulin and samples of *A. congensis* stem bark inhibited the toxic action of streptozocin and promoted thus the regeneration of β-pancreatic cells and consequently the function of pancreas organ. Thus, the selected extract and its fractions possess potential antidiabetic properties in respect to insulin secretion, which may attributed to modulation of calcium channel and β-cells regeneration and proliferation (Koneri et al., 2014).

3.6. Effects of the 80% methanol extract and its fractions on the concentration level of glucose in oral glucose tolerance test (OGTT) The effects of various doses the 80% methanol and its fractions from *A. congensis* stem bark on oral glucose tolerance test (OGTT) are shown in Table 5. The glycaemia stared to gradually decrease from 30 min until 180 min of treatment to reach the values close to the initials values at the end of the study compared to control groups (Table 5). All oral doses of the 80% methanol extract (ME-1) and its two selected fractions the chloroform (ME-1.1) and ethylacetate (ME-1.2) soluble fractions were found to be effective in decease the concentration levels of blood sugar. The 80% methanol extract (ME-1) reached a decrease of 121.3 ± 0.5 to 86.0 ± 1.1 mg/dL of sugar at 100 to 400 mg/kg b.w after 30 to 180 min respectively. The chloroform (ME)1.1) and ethylacetate (ME-1.2) soluble fractions produced the same effect with 126.4 ± 0.8 to 92.4 ± 0.1, and 122.3 ± 0.3 and 88.3 ± 0.3 mg/dL of the fasting blood sugar at the same concentrations and time of treatment cited above. The decrease effect of these samples is manifested in treated animals in this test in dose-dependent manner as also observed for insulin used as a reference antihypoglycemic product (Table 5) and its activity is high compared to *A. congensis* samples ($p < 0.05$). In addition, these results indicated the administration of *A. congensis* samples in hyperglycemic rats reached a tolerability in treated animals. Our results are in good agreement with other previously reported results for other antidiabetic medicinal plant species (Jafri et al., 2000; Silva et al.,2002; Bakirel et al, 2008; Jain et al., 2010), but in other studies, the increasing of blood glucose levels in OGTT was also previously reported (Kesari et al., 2005; Zanatta et al., 2007).

Table 5. Effect of different doses of the 80% methanol extract (ME-) of *A. congensis* stem and its chloroform (ME-1.1) and ethylacetate (ME-1.2) soluble fractions on the concentration level of glucose (mg/dl) in oral glucose tolerance test (OGTT)

Time (minutes)	Group I Hyperglycemic rats (HR): negative control	Group II HR+ Insulin (0.5 IU/ml)	Group III ME-1 HR+ 100 mg/kg ME-1	Group III ME-1 HR+ 200 mg/kg	Group III ME-1 HR+ 400 mg/kg
0	131.2 ± 1.4	105.3 ± 0.4	128.6 ± 1.2	126.3 ± 0.1	128.4 ± 1.5
30	139.1 ± 0.3	96.3 ± 0.8	121.3 ± 0.5	116.6 ± 1.2	109.3 ± 0.5
60	146.6± 0.6	85.7 ± 0.7	117.3 ± 1.1	113.3 ± 0.8	102.3 ± 0.3
90	152.8 ± 1.3	72.3 ± 0.4	112.8 ± 0.4	108.3 ± 0.2	98.3 ± 0.7
180	161.3 ± 0.7	68.2 ± 0.5	96.0 ± 0.3	92.9 ± 1.3	86.0 ± 1.1
	Group IV ME-1.1. HR +100 mg/kg	Group VI ME-1.1 HR + 200 mg/kg	Group VI ME-1.1 HR + 400 mg/k		
0	128.3 ± 0.2	127.6 ± 0.9	126.3 ± 0.6		
30	126.4 ± 0.8	120.3 ± 1.1	116.3 ± 0.4		
60	123.5 ± 0.5	118.3 ± 0.7	114.2 ± 1.2		
90	120.4 ± 0.7	115.3 ± 0.5	111.3 ± 0.8		
180	117.3 ± 0.2	112.3 ± 0.4	92. 4 ± 0.1		
	Group V ME-1.2 HR + 100 mg/kg	Group V ME-1.2 HR + 200 mg/kg	Group V ME-1.2 HR + 400 mg/kg		
0	127.3 ± 1.3	126.3 ± 0.5	126.6 ± 0.8		
30	122.3 ± 0.8	118.7 ± 1.1	115.6 ± 1.2		
60	118.4 ± 1.1	115.3 ± 0.4	111.3 ± 0.7		
90	114.3 ± 0.3	112.3 ± 0.9	105.8 ± 0.3		
180	108.3 ± 0.7	98.3 ± 0.5	88.3 ± 0.3		

Group III MEI, group IV and V: treated with the aqueous extract, chloroform and ethylacetate soluble fractions respectively

From our results, it was seen that all samples of *A. congensis* stem bark act as hypoglycemic ant antidiabetic agents by regeneration and proliferation of β-pancreatic cells destroyed by STZ and promote the function of pancreas organ. This general mechanism was often previously attributed to other hypoglycemic and antidiabetic medicinal plant extracts (Kantchouing et al, 1998; Silva et al., 2002 ; Szkudelski et al. , 2001 ; Eidi et al., 2006; Badole et al., 2010, Tende et al., 2010; Melani et al., 2011; Padee et al., 2010; Karau et al. 2012; Toma et al., 2015).

The phytochemical screening of the 80% methanol extracts from *A. congensis* stem bark and its fractions revealed the presence of alkaloids, tannins, saponins, carbohydrates, sugars, steroids and terpenoids and flavonoids. Anthocyanins, coumarins and anthraquinones were not detected in our experimental conditions. Flavonoids and terpenoids isolated from other antidiabetic medicinal plant species had been found to stimulate secretion or possessed insulin like-effect (Marles and Franswoth, 1995; Alarcon-Aguillar et al., 2000; Daisy et al., 2009; Dewantje et al., 2009; Lui et al., 2009; Li et al., 2006; Jung et al., 2006; Panda et al., 2009; Prabhakar and Doble, 2009; Saravanan et al., 2010, Tian et al., 2010; Deutscländer et al., 2011, Koneri et al., 2014). The hypoglycemic effect of terpenoids appears to involve the stimulation of pancreatic β-cells and subsequent secretion of performed insulin while the metabolism of coumarins probably involves hepatotoxicity (Marles and Fransworth, 1995). Thus, the hypoglycemic and antidiabetic activities of *A. congensis* stem bark extracts observed in the present study may probably due to the presence of flavonoids, terpenoids, steroids, tannins, sugars (polysaccharides) and alkaloids detected in the studied plant part, which can act singly or in synergy with other.

In conclusion, medicinal plant extracts with hypoglycemic and antidiabetic activity proved experimentally in animal model are promising candidate agents for the development of new agents in ameliorated pharmaceutical forms accessible to the population for the treatment of diabetes. The present study indicated that the 80% methanol extract from *A. congensis* stem bark and its fractions possess remarkable and interesting hypoglycemic and antidiabetic activities in animals. The present results provide scientific evidence supporting and justifying the use of this plant part for the preparation of hypoglycemic and antidiabetic remedy currently employed in traditional medicine in some African country and particularly in Democratic Republic of Congo.

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