

**ANTIDIABETIC ACTIVITY OF *DORSTENIA PICTA*  
AQUEOUS TWIGS EXTRACT IN STREPTOZOTOCIN-  
INDUCED DIABETIC RATS**

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**Summary**

We investigated the effect of *Dorstenia picta* (*D. picta*) aqueous twigs extract on blood glucose levels in normal and streptozotocin (STZ) induced-diabetic rats. A single oral administration of *D. picta* aqueous extract at a dose of 150 mg/kg significantly reduced the blood glucose levels in normal and diabetic rats by 25.65% and 29.38% respectively 5 hours post-dosing. In glucose fed rats, the plant extract did not improved glucose tolerance. In subacute treatment, *D. picta* (75 mg/kg, 150 mg/kg) was administered orally once daily to streptozotocin-induced diabetic rats during 14 days. Daily administration of *D. picta* aqueous extract resulted in a significant decreased in blood glucose at the dose of 75 mg/kg (57.15%,  $p < 0.05$ ) and 150 mg/kg (65.95%,  $p < 0.01$ ) respectively as compared to initial values. Insulin (10 UI/kg), used as standard drug, showed a maximum reduction of 70.32% in blood glucose levels. Parallel to the blood glucose, *D. picta* aqueous extract significantly reduced food and water intakes but failed to prevent the decrease of body weight in diabetic rats. In the other hand, the plant extract caused a significant decrease in serum cholesterol, triglycerides and transaminases (ALT and AST) in diabetic rats, whereas serum creatinine and total protein remained unchanged. In addition, the plant extract significantly ( $p < 0.01$ ) increased the lower hepatic glycogen content observed in STZ-induced diabetic untreated rats.

These results demonstrate that the antidiabetic activity of *Dorstenia picta* aqueous twigs extract may be mediated through the amelioration of hepatic glycogen, aminotransferase levels, and lipid profil. *Dorstenia picta* could be a potential source of new oral antidiabetic drug.

Key words: *Dorstenia picta*, streptozotocin-induced diabetes, hypoglycaemic effect

## Introduction

Plants extract have been used in traditional medicine since ancient time for treatment of various diseases including diabetes mellitus. Many plants species have been described for the care of diabetes mellitus and among this plant, several plant extract have been shown in different animal models to possess beneficial effect for treatment of diabetes mellitus [1, 2, 3, 4, 5]. *Dorstenia* is a large genus occurring in the tropic around the world. *Dorstenia* is one of the genus in the large moraceae family that produces small herbaceous plants. *Dorstenia picta* (*D. picta*) is commonly used in traditional Cameroonian medicine to treat many diseases like hypertension and diabetes mellitus. Other traditional uses of *Dorstenia* species are against headaches and abdomen pain [6], snakebite, infection and rheumatism [7]. Antihypertensive [8], antioxydant or reactive-oxygen-scavenging properties [9] as well as anti-inflammatory and antinociceptive activities [10] have been reported for *Dorstenia psilurus*, *Dorstenia cilianta* and *Dorstenia barteri* respectively. The main objective of this study was to determine the anti-diabetic properties of *Dorstenia picta* aqueous extract in streptozotocin-induced diabetic rats.

## Methods

### Plant material

Twigs of *D. picta* were collected from Ngoumou, centre Province of Cameroon In August 2005. It was identified by Dr. Zapfack Louis of the Department of Plant Biology and Physiology, Faculty of Science, University of Yaounde I. The voucher specimen (N° 57063) was deposited at the National Herbarium, Yaounde, Cameroon.

### Preparation of the extract

The air-dried twigs of *D. picta* were powdered and 200 g boiled in 1 L of water for 15 minutes. The mixture was filtered, and dried at reduced temperature (40°C). The resulting mass with a yield of 19.14% was considered as aqueous extract.

### Preliminary phytochemical tests

Phytochemical properties of the aqueous extract of *D. picta* were tested using the method of Odebiyi and Sofowora [11]. Various reagents were used: Mayer and Dragendoff's reagents for alkaloids, FeCl<sub>3</sub> for tannin, frothing test for saponin, magnesium chip and HCl for flavonoids, NaCl, and Fehling's solutions A and B for glycoside, diethyl ether, sulfuric acid and anhydride acetic for steroids, ether-

chloroform and NaOH for anthraquinones and FeCl<sub>3</sub> and K<sub>3</sub>Fe(CN)<sub>6</sub> for phenols and polyphenols.

#### **Animals**

Male Wistar albino rats weighing 200-250 g, raised in the Faculty of Science, University of Yaounde I, were used. They were kept and maintained under standard laboratory conditions of temperature, humidity, 12-hour day: 12-hour night cycle and allowed access to rat chow and water *ad libitum*. Fasting rats were deprived of food for at least 12 h but not water. Fasted normoglycemic rats with blood glucose levels of  $94 \pm 6$  mg/dL were used in our experiments.

#### **Experimental induction of diabetes in rats**

Diabetes mellitus was induced by intravenous (penile vein) injection of 55 mg/kg of streptozotocin (STZ, Sigma Chemicals, USA) in 0.9% sodium chloride solution to non fasted ether anesthetized rats. Control group received normal saline through the same route. Four days later, blood glucose levels of STZ-treated fasted rats greater than 350 mg/dL were considered diabetic, and used in our study.

#### **Single oral administration of *D. picta* extract**

Normal and diabetic rats were assigned to six different groups containing five rats each. One control group received distilled water, four groups received the plant extract at 38, 75, 150 and 300 mg/kg and the sixth group received a reference drug (insulin) at a dose of 10 UI/kg. Distilled water and *D. picta* extract were administered orally while insulin was given by subcutaneous route. The doses of the plant extract were selected on the basis of traditional used prepared as a suspension in distilled water. Blood was collected from the tail vein and blood glucose levels were measured before (0 h) and at 1, 2, 3 and 5 h after plant extract administration [12] using the glucose oxidase method (Accuchek glucometer, Boehringer Mannheim, Germany).

The hypoglycemic effect of *D. picta* extract was also assessed by the oral glucose tolerance test (OGTT) in streptozotocin-diabetic rats. Prior to an oral glucose tolerance test, rats were fasted for 18 h. The OGTT was performed by feeding glucose (5 g/kg), in the form of an oral solution to diabetic rats and blood sample were collected at 0, 0.5, 1, 2, 3, 5 h after administration of glucose. Blood glucose level was estimated as previously described.

#### **Repeated oral administration of *D. picta* extract**

For repeated administration, diabetic rats were divided into four equal groups of five rats each: group I, served as a control and received distilled water (10 mL/kg), group II and III received orally once a day 75 and 150 mg/kg of the plant extract, respectively and

group IV received insulin as a reference drug (10 UI/kg s.c.). Before giving *D. picta* extract, the fasting basal blood glucose level was determined in all the groups. Changes in fasting blood glucose levels were monitored at day 7 and 14 as described previously. Body weight, food and water intakes were evaluated every day. At the end of experimentation, the rats were fasted for 12 h and sacrificed by decapitation. Blood was collected, allowed to clot at room temperature and the serum obtained after centrifugation (3000g for 10 min.), was used to determination of glucose, cholesterol, triglyceride, protein, creatinine and aminotransaminases.

#### **Biochemical assay of glycogen level**

To measure the liver glycogen, 30% KOH solution was added to 1.5 g of liver and the mixture was heated at 100°C for 20 min. Glycogen was precipitated and coprecipitated with ethanol 95% (5 mL) and saturated Na<sub>2</sub>SO<sub>4</sub> (0.2 mL) respectively. The mixture was centrifuged at 3000g for 10 min and the total precipitate was dissolved in distilled water. The glycogen content was hydrolyzed with HCl (1.2 M) and the free glucose was determined using a glucose oxidase method [13].

#### **Serum analysis**

Serum total protein, creatinine, total cholesterol and triglycerides levels as well as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity were analysed. All those parameters were determined using commercial diagnostic kits (Randox Laboratories, San Diego-USA).

#### **Statistical analysis**

Results are expressed as the mean  $\pm$  S.E.M. Statistical differences between control and treated groups were tested by one way analysis of variance (ANOVA) followed by Dunnett's test using Stat-Direct-Software. P values less than 0.05 were considered to be significant.

## **Results**

#### **Phytochemistry**

Polyphenol and glycosides were identified in decoction (aqueous extract of *D. picta*) whereas flavonoids, saponins, triterpenes, phenol, steroids, alkaloids, tannins and anthraquinones were absent.

#### **Acute effect of *D. picta***

The effect of *D. picta* aqueous extract on blood glucose levels in fasting normal and streptozotocin-diabetic rats is shown in table 1. In normoglycaemic rats, the single administration of the plant extract reduced significantly the blood glucose levels at doses of 38, 75 and 150 mg/kg after 5 hours, while the higher dose of 300 mg/kg, the

blood glucose levels remains unchanged. The maximum effect was achieved at the dose of 150 mg/kg with the maximal reduction in blood glucose levels of 25.65% at 5 h as compared to initial value.

Insulin (10 UI/kg) showed the maximal decrease (58.73%) of blood glucose concentration 1 hour post-dosing as compared to initial value.

In diabetic rat, the plant extract reduced the blood glucose level at the doses of 75, 150 and 300 mg/kg. The plant extract significantly ( $p < 0.05$ ) decrease the blood glucose levels 3 hours post-dosing at 150 and 300 mg/kg while the reduction was effective at the dose of 75 mg/kg 5 hours post-dosing. The maximum hypoglycaemic effect of the plant extract was 25.53%, 29.38%, and 16.74% respectively at the doses of 75, 150 and 300 mg/kg. *Dorstenia picta* aqueous extract, at the dose of 38 mg/kg failed to reduce the blood glucose levels at any time. Insulin drastically reduced ( $p < 0.001$ ) blood glucose levels of diabetic rats; starting one hour post-dosing with a maximum effect 2 hours later.

**Table 1:** Effect of a single administration of *D. picta* aqueous extract on blood glucose levels in normoglycaemic and streptozotocin-induced diabetic rats.

Treatment	Blood glucose level (mg/dL)				
	0 h	1 h	2 h	3 h	5h
Normal control	100.30± 4.10	95.00±3.00	103.40±2.70	104.40±1.50	95.00±5.70
<i>D. picta</i> (38 mg/kg)	92.60± 3.07	88.20±3.16	85.40±2.06	83.6±2.15	81.20±1.20*
<i>D. picta</i> (75 mg/kg)	93.20 ± 4.71	86.60 ±5.34	82.80±4.34	81.00±5.69	72.20±5.91**
<i>D. picta</i> (150 mg/kg)	99.00± 3.30	92.00± 6.28	83.60±6.61	79.60±7.90	73.60±6.69*
<i>D. picta</i> (300 mg/kg)	92.20± 1.06	95.40±2.80	95.60±5.28	94.40±3.72	88.40±4.04
Insulin (10 IU/kg)	97.90± 2.10	40.80±0.90***	32.30±2.20***	38.20±2.30***	40.40±2.10**
Diabetic control	403.60± 8.70	380.00±14.90	378.20±3.50	387.10±3.20	377.60±4.30
<i>D. picta</i> (38 mg/kg)	429.40± 22.20	356.40±19.51	360.80±22.47	360.40±16.28	357.20±16.39
<i>D. picta</i> (75 mg/kg)	436.20± 45.84	397.40± 21.97	381.60±24.08	389.20±26.23	324.80±11.87**†
<i>D. picta</i> (150 mg/kg)	462.80± 26.53	400.00±18.68	380.20±19.03*	347.60±11.02**	326.80±8.38**†
<i>D. picta</i> (300 mg/kg)	415.60± 21.80	374.40±20.02	346.00 ±9.70*	354.80±7.70*	355.00±14.19*
Insulin (10 IU/kg)	406.60± 13.50	84.40±8.90***†††	67.20±13.90***†††	77.40±16.10***†††	137.60±17.6*** †††

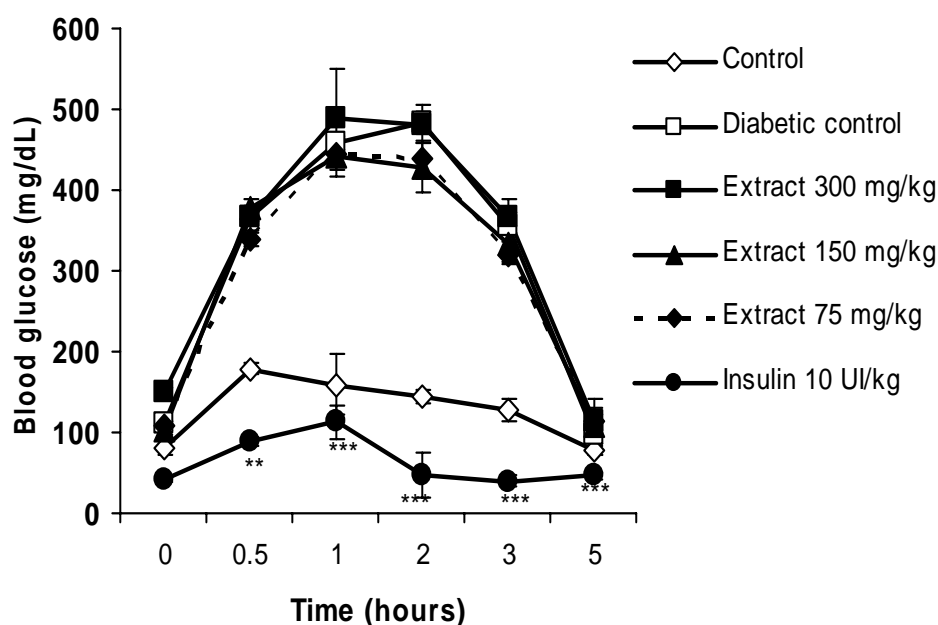
Each point represents mean ± ESM, n= 5, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , compared with initial values. † $p < 0.05$ , †† $p < 0.01$ , ††† $p < 0.001$ , compared with diabetic control.

Figure 1 shows the effect of aqueous extract of *D. picta* on post-prandial blood glucose levels of streptozotocin-induced diabetic rat.

The plant extract tend to reduce the high blood glucose provoked by oral administration of glucose (5 g/kg); but this reduction was not significant compared to diabetic control. Insulin significantly ( $p < 0.001$ ) reduced the sugar levels below those of the control at each interval.

#### Subacute effect of *D. picta*

The effect of *D. picta* aqueous extract on body weight, food and water intakes is shown in table 2. Significant weight loss was observed in diabetic rat as compared to the normal control. The plant extract at the dose of 75 mg/kg and 150 mg/kg significantly reduced food and water intakes but did not reversed weight loss as compared to the streptozotocin-diabetic rats.



**Figure 1:** Effect of *D. picta* aqueous extract on blood glucose levels during oral glucose tolerance test in diabetic rats.

Values represents mean  $\pm$  ESM,  $n = 5$ , \*\* $p < 0.01$ , \*\*\*  $p < 0.01$ , compared with the diabetic control.

**Table 2:** Body weight, food and water intakes in STZ-induced diabetic rats after 14 days of treatment with *D. picta* aqueous extract.

Treatment	Body weight (g)	Food (g/rat/day)	Water (mL/rat/day)
Normal control	232.50 ± 6.65	19.62 ± 2.18	20.00 ± 0.84
Diabetic control	192.53 ± 4.55	41.23 ± 3.12 <sup>††</sup>	110.80 ± 9.07 <sup>††</sup>
Extract (75 mg/kg)	196.00 ± 4.01 <sup>†</sup>	34.77 ± 1.72 <sup>†</sup>	40.75 ± 5.72 <sup>**†</sup>
Extract (150 mg/kg)	200.15 ± 4.07 <sup>†</sup>	25.68 ± 1.85 <sup>*</sup>	44.75 ± 2.67 <sup>**†</sup>
Insulin (10 UI/kg)	243.19 ± 11.02 <sup>*</sup>	21.89 ± 2.52 <sup>**</sup>	37.66 ± 1.53 <sup>***††</sup>

Each point represents mean ± SEM, n= 5. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001, compared with the diabetic control; <sup>†</sup>p<0.05, <sup>††</sup>p<0.01, compared with normal control.

Table 3 shows the levels of blood glucose in streptozotocin-induced diabetic rat during 14 days of treatment. Daily administration of the plant extract significantly reduced the blood glucose levels in diabetic rats. *Dorstenia picta* extract, given orally for 14 days, showed 57.15% and 65.95% antidiabetic activity at doses 75 mg/kg and 150 mg/kg respectively as compared to their initial value; and 48.44% and 55.20% reduction as compared to the diabetic control animals. Insulin showed the highest blood glucose reduction of 70.32% and 68.98% compared to the initial value and to the diabetic control animals respectively.

**Table 3:** Effects of repeated administration of *D. picta* extract in blood glucose level in STZ-induced diabetic rats.

Treatment	Day 0	Day 7	Day 14	% Variation
Normal control	98.80 ± 0.62	98.00 ± 1.58	99.20 ± 2.89	0.40
Diabetic control	478.50 ± 9.83	472.50 ± 19.83	460.00 ± 4.50	4.88
Extract (75 mg/kg)	433.20 ± 36.34	234.00 ± 41.26 <sup>*†</sup>	185.60 ± 29.27 <sup>*†</sup>	57.15
Extract (150 mg/kg)	473.60 ± 33.22	282.80 ± 39.97 <sup>*††</sup>	161.25 ± 14.48 <sup>**</sup>	65.95
Insulin (10 IU/kg)	450.33 ± 8.21	203.60 ± 13.23 <sup>**</sup>	133.65 ± 5.66 <sup>***††</sup>	70.32

Each point represents mean ± SEM, n= 5, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 compared with the initial values; <sup>†</sup>p <0.05, <sup>††</sup>p<0.001, compared with normal control.

Table 4 shows the results for the levels of total cholesterol, triglycerides, proteins, creatinine, aminotransferase (AST and ALT) and hepatic glycogen after plant extract treatment. No significant change in creatinine and protein levels of diabetic, diabetic *D. picta* treated rats was observed in comparison to normal animals. Cholesterol and triglycerides levels were 85.99 and 171.92% respectively higher in diabetic rats in comparison with normal animals. When treated with the plant extract at the dose of 150 mg/kg for 14 days, cholesterol and triglycerides levels were reduced by 68.01 and 64.13% respectively, as compared to diabetic untreated rats. The results show that the levels of ALT and AST were significantly reduced by 66.53% and 50.87%, respectively with the dose of 150 mg/kg of *D. picta* as compared to diabetic untreated rats. Treatment with insulin used as standard drug resulted in a significant decrease of cholesterol and triglycerides levels as well as aminotransaminases activity. *D. picta* treatment showed a significant ( $p < 0.01$ ) and dose dependent increased in liver glycogen level in comparison to untreated diabetic rats.

### Discussion and Conclusion

Diabetes is characterized by deficiency of insulin secretion, action or both [14]. Streptozotocin induced diabetic state [15] has been used in this study to evaluate the antidiabetic activity of *Dorstenia picta* aqueous twigs extract.

Single oral administration with aqueous extract (38- 150 mg/kg) caused a significant decrease in blood glucose levels in normal rats. In STZ-induced diabetic rat, the plant extract exhibited its action 2 hours after administration (150 and 300 mg/kg). The maximum hypoglycaemic effect was observed 5 hours pos-dosing. This decrease may be explained by the presence of some chemical compounds in the plant extract. Infact the preliminary phytochemical analysis revealed the presence of polyphenol and glycosides which are known to exhibit hypoglycaemic effect. Glycosides isolated from *Beta vulgaris* roots extract [16], polyphenol from *Melaleuca quinquenervia* leaves [17] have been shown to significantly reduced blood glucose levels. In the case of glucose fed rat, the plant extract slightly improved the glucose tolerance test in STZ-induced diabetic rats at 75 mg/kg. This effect was not significant compared to diabetic control. This result suggests that, the aqueous extract had no effect on postprandial blood glucose levels of STZ-induced diabetic rats.



**Table 4:** Effect of *D. picta* extract on some biochemical parameters after 14 days of treatment.

Parameters	Groups				
	Normal control	Diabetic control	<i>D.picta</i> (75 mg/kg)	<i>D.picta</i> (150 mg/kg)	Insulin ( 10 UI/kg)
Cholesterol (mg/dl)	80.81 ± 9.69	150.30 ± 2.56	47.83 ± 3.41**	48.08 ± 7.30**	75.33 ± 2.68**
Triglycerides (mg/dl)	56.58 ± 4.67	154.56 ± 1.00	58.30 ± 6.00***	55.43 ± 4.41***	88.00 ± 1.65**
Protein (g/l)	73.40 ± 6.04	51.56 ± 1.17	51.82 ± 3.74	57.26 ± 4.63	55.53 ± 0.64
Creatinine (mg/dL)	5.89 ± 0.05	6.55 ± 0.50	5.60 ± 0.36	6.12 ± 0.35	6.30 ± 0.42
ALT (U/L)	34.50 ± 2.92	125.50 ± 0.35	48.03 ± 10.88***	42.00 ± 8.19***	46.14 ± 2.29***††
AST (U/L)	57.20 ± 0.63	136.80 ± 8.83	81.33 ± 8.45***†	67.20 ± 6.39***	60.36 ± 2.83***††
Hepatic glycogen (mg/100g of tissues)	63.67 ± 5.28	24.82 ± 3.24	35.07 ± 0.85**	40.95 ± 0.32**	51.09 ± 4.21**

Values are means ± SEM, n =5, \*p<0.05, \*\*p<0.01, \*\*\*p<0.01 compared with the diabetic control.

† p<0.05, †† p<0.01, ††† p<0.001, compared with normal control.

Experimental diabetes, induced by streptozotocin provoked hyperglycemia accompanied by symptoms like loss of weight, polydipsia and polyphagia [15]. These parameters were slightly improved compared to the diabetic untreated rat. The observed amelioration may be due to the beneficial effect of blood glucose levels after oral administration of *D. picta* aqueous extract for 14 days treatment. Infact, the decrease of blood glucose levels was time and dose dependent (57.15 and 65.95% at 75 and 150 mg/kg respectively) but did not attained normal values at the end of the treatment. The hypoglycaemic activity of the plant extract could be due to its capacity to restored altered hepatic glycogen observed in diabetic untreated rats, probably by stimulation of glycogenesis and/or inhibition of glycogenolysis. Thus, one action mechanism of *D. picta* aqueous extract may be due to stimulation of glycogen storage. However, further investigations need to determine other action mechanisms. Parallel to the decrease of blood glucose levels and glycogen storage, our aqueous extract significantly lowered the plasma cholesterol, triglycerides and transaminases levels. Hypercholesterolemia and hypertriglyceridemia usually observed in diabetic state represents a risk factor for coronary heart disease [18]. Our result indicated that *D. picta* aqueous extract significantly lowered the plasma cholesterol triglycerides and thus could contribute to prevent cardiovascular diseases. These findings also correlated with those of Luo *et al.* [19]; Dhandapani *et al.* [20] who respectively reported that *Cuminum cyminum* and *Lycium barbarum* reduced cholesterol and triglycerides in experimentally-induced diabetic rats. The plant extract significantly decrease serum transaminases (ALT and AST) levels. This reduction can be explain as a result of a direct reduction in blood glucose concentration and could be attributed to the presence of polyphenols, which are known to have hypoglycaemic and antioxidant properties [17, 21]. Our results also demonstrated that diabetic rats had unchanged levels of serum creatinine and total protein when compared with the controls. While working on the same animal model, Pepato *et al.* [22]; Nagappa *et al.* [23] respectively observed a reduction of creatinine levels after administration of *Bauhinia forficata* and *Terminalia catappa* extract. Our results on creatinine and protein level could be explained by the duration of diabetes, suggesting that, there was no alteration of kidney function in rats used in the present experiment.

In conclusion, aqueous extract of *D. picta* exhibited significant antidiabetic activity in streptozotocin-induced diabetic rat. The plant extract also prevent hypertriglyceridemia, hypercholesteremia and ameliorate glycogen storage. Chemical and pharmacological researchs are required to find the action mechanism of this extract.

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