EFFECT OF AQUEOUS AND METHANOL/METHYLENE-CHLORIDE EXTRACTS OF *LAPORTEA OVALIFOLIA (URTICACEAE)* ON BLOOD GLUCOSE LEVEL IN RATS

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Summary

Investigation of the effect of administration of aerial part of *Laportea ovalifolia* extracts (aqueous, and methanol/methylene-chloride) on blood glucose levels was evaluated in this study in normal and alloxan diabetic rats. The qualitative phytochemical screening of these extracts was carried out. It revealed the presence of compounds such as saponins, phenols compounds and sugars in the both extracts. Among the two extracts, aqueous extract at the dose of 200 mg/kg body weight exhibited a significant antihyperglycaemia activity ($P<0.001$) in the reduction of blood glucose levels 5h after treatment of alloxan diabetic rats. The same dose did not cause any hypoglycaemic activity in normal rats. The results were compared with the diabetic rats treated with tolbutamide which was better. Oral administration of these extracts at the dose of 200 mg/kg produce a significant antihyperglycaemia effect in hyperglycaemic rats. This support the application of *Laportea ovalifolia* as an antihyperglycaemic agent.

Key words: *Laportea ovalifolia*, Alloxan, antihyperglycaemia.

Introduction

In the context of acute economical crisis approximately 80 % of the population still rely on traditional medicine (phytomedicine) for their daily healthcare needs in Africa [1]. The biodiversity of Africa canopies provide row materials for the preparation of naturals products against numerous diseases such as diabetes mellitus. Few of the traditional plants treatment for diabetes have received scientific scrutiny and the World Health Organisation has recommended that this area warrants attention [2]. Since then, our laboratory has selected some medicinal plants for the investigation of their chemical constituents and pharmacological activities, in attempt to establish a scientific basis for their ethnopharmacological uses. One of this plant is *Laportea ovalifolia*

This plant belongs to the family of urticaceae. It is a tropical plant commonly found in swampy areas in Cameroon and other parts of the world in both dry and rainy seasons [3].

Some people in Cameroon use the leaves as vegetable, which serves as a major component of their diet. The leaves of *Laportea ovalifolia* are used in traditional medicine for the remedy of bacterial infections, headaches, urinary infections, pneumonia, dysentery, epilepsy and diabetes [3,4].

This study was thus initiated with the aim of evaluating the effect of aqueous and methanol/methylene-chloride extracts of *Laportea ovalifolia* on blood glucose levels in rats.

Methods

Materials and methods

Collection of plant material

The aerial parts (leaves and stem) of *Laportea ovalifolia* were collected in October and November 2002, in Yaounde, Cameroon and identified at the National Herbarium, Yaounde Cameroon,where a voucher sample was deposited under the number 50623/HNC.

Preparation of extract

The plant material was air-dried for 30 days at room temperature and ground into a powder. The plant powder (1500 g) were used for aqueous and methanol / methylene-chloride extraction.

500 g of this plant powder was decocted in 4 L of distilled water for 15 min. This was repeated four times, until the resulting extract gave no further coloration. The extract was then filtered and evaporated to dryness in an oven at 40° C, to obtain a crude residue of 84g (yield: 16.8 %).

The remaining powder (1000 g) was soaked in 5 L of a mixture of methanol/ methylene-chloride (1:1) for 48 h, and for a further 24 h in methanol. This was filtered and concentrated to a small volume to remove the entire methanol and methylene chloride using rotator evaporator. The remaining liquid was later further dried at room temperature to obtain an extract of 111 g (11 % yield).

Qualitative phytochemical screening

Standard qualitative phytochemical screening procedures were used [5]. The presence of a bioactive compound was indicated by a colour change

Animal experiments

Three-month-old male Wistar Albinos rats weighing 180-240 g were obtained from the animal house of the LNNB (Laboratory of Nutrition and Nutritional Biochemistry), Department of Biochemistry, University of Yaounde I, Cameroon. All animals were kept in an environmentally controlled room with a 12 h light/ 12 h dark cycle. The animals had free access to water. The study was approved by institutional animal ethical committee.

Experimental induction of diabetes in rats

The rats were injected alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body weight, intraperitoneally. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5 % glucose solution bottles in their cages to prevent hypoglycemia [6].

After 7 days, rats with marked hyperglycaemia (fasting blood glucose ≥ 200 mg/dl) were selected and used for the study.

Effect of oral administration of *L. ovalifolia* extracts on normoglycaemic and diabetes rats

The animals were divided into 11 groups; each group consisted of 5 rats.

Groups	Description
1	Normal/untreated rats (control group)
2	Normal rats treated with 50 mg/kg body weight of plant extract
3	Normal rats treated with 100 mg/kg body weight of plant extract
4	Normal rats treated with 200 mg/kg body weight of plant extract
5	Normal rats treated with 400 mg/kg body weight of plant extract
6	Diabetic/untreated rats (control group)
7	Diabetic rats treated with 50 mg/kg body weight of plant extract
8	Diabetic rats treated with 100 mg/kg body weight of plant extract
9	Diabetic rats treated with 200 mg/kg body weight of plant extract
10	Diabetic rats treated with 400 mg/kg body weight of plant extract
11	Diabetic rats treated with 80 mg/kg body weight tolbutamide

The aqueous extract was dissolved in distilled water while methanol / methylene- chloride were dissolved in corn oil. The rats were fed with the test material (*Laportea ovalifolia* extracts or tolbutamide which were dissolved in water) orally by gastric intubation after 12 h of fasting (water available).

Normal untreated and diabetic untreated rats of aqueous extract group were fed with distilled water while those of methanol / methylene-chloride received corn oil. Glucose level was monitored at 0 h, 1h30min, 3 h and 5h after feeding with the plant extracts.

Effect of *L. ovalifolia* extracts on glucose tolerance test in diabetes rats

The oral glucose tolerance test was performed in overnight fasted (16h) diabetes rats. The received respectively the test samples

(aqueous and methanol / methylene- chloride extracts) at the dose of 200 mg/kg body weight one hour prior to the oral glucose load of 2 g/kg body weight. Glucose concentration was measured before administration and subsequently at 0.5, 1, 2 and 3 h after the glucose load. One control group received glucose and water while the other received glucose and corn oil. The percentage variation of glycaemia was calculated for each group using the following formula:

$$\% \text{ variation of glycaemia} = \frac{G_t}{G_i} \times 100$$

Where G_i and G_t where the values of initial glycaemia (1h prior to the oral glucose load) and glycaemia at 0.5, 1, 2 and 3 h after the glucose load.

Collection of blood and determination of blood glucose level.

Blood samples from the control and experimental rats were collected from the tail vein of each rat into Eppendorf tubes.The samples were analysed for glucose level using the oxidase method [7].

Statistical Analysis

Results are expressed as mean ± S.E.M. Statistical significance was determined by ANOVA and post hoc Dunnett Test. P – values less than 0.05 were considered significant.

Results

The result of phytochemical screening of aqueous and methanol/methylen chloride extract of *L. ovalifolia* is presented in table 1. It revealed the presence of sugars, saponins, tannins, and phenolic compounds for aqueous extract and sugars, saponins, phenolic compounds, Sterol, triterpens lipids, alkaloids, and glycosides for methanol/methylene chloride extract.

The effect of different doses of aqueous and methanol / methylen chloride extract of *L. ovalifolia* on the fasting blood glucose levels of normal and alloxan diabetic rats are given in tables 2 and 3. None of the extract of *L. ovalifolia* aerial part caused any hypoglycaemic activity in normal groups of rats. The aqueous extract of *L. ovalifolia* aerial part at a dose of 200 mg/kg body weight produced the maximum significant ($P<0.001$) fall (51.49%) in the blood glucose of diabetic rats after 5 h of treatment, while the same dosage of methanol / methylene- chloride extract showed (47.39%) of fall of blood glucose. Treatment of the alloxan diabetic rats with tolbutamide (80 mg/kg body weight) produced (62.42 %) fall of blood glucose after 5 h of treatment.

Table 1: Qualitative phytochemical analysis of *Laportea ovalifolia* aerial part extracts

	Extracts	Aqueous extract		Methanol / Methylene chloride extract	
Constituants	Name of test	Observations	Inference	Observations	Inference
Alkaloids	Mayer's test	No cream precipitate	Absent (-ve)	cream precipitate	Present (+)
Glycosides	Fehlings solution	No brick-red precipitate	Absent (-ve)	brick-red precipitate	Present (+)
Flavonoids	Ammonium test	No yellow coloration	Absent (-ve)	No yellow coloration	Absent (-ve)
Phenols	Ferric chloride test	Red colour	Present (+)	Red colour	Present (+)
Anthraquinones	Ammonium test	No red colour	Absent (-ve)	No red colour	Absent (-ve)
Saponins	Frothing test	Persistant foam	Present (+)	Persistant foam	Present (+)
Tannins	Ferric chloride test	Dark green colour	Present (+)	No dark green colour	Absent (-ve)
Triterpens	Libermann-Burchard test	No reddish colour	Absent (-ve)	reddish colour	reddish colour
Lipids	Lipids test	No translucent	Absent (-ve)	Translucent	Present (+)
Sterols	Libermann-Burchard test	No brownish-red	Absent (-ve)	brownish-red	Present (+)
Sugars	Sugars test	Reddish colour	Present (+)	Reddish colour	Present (+)

Table 2: Effect of oral administration of aqueous extract of aerial part of *Laportea ovalifolia* on plasma glucose concentration in normal and alloxan diabetic rats

Groups	Blood glucose at different hours after the treatment (mg/dl)			
	0 h	1h30min	3 h	5 h
1	79.41±1.36	82.65±0.94	84.06±1.47	71.60±2.09
2	75.81±1.43	84.80±2.97	77.28±1.21	70.20±1.04
3	83.24±1.74	95.66±1.74	91.08±2.51	81.44±0.82
4	74.85±1.86	80.46±1.74	77.48±1.90	73.49±2.46
5	72.42±1.90	81.40±1.80	78.06±1.50	71.67±0.94
6	335.00±2.00	350.60±2.05	343.05±2.28	330.10±3.24
7	348.81±2.65*	360.44±2.16*	335.66±3.36*	321.60±3.35*
8	359.25±2.94	295.80±2.08***	275.06 ±4.60**	261.40±1.64**
9	342.41±2.05	253.06±3.60**	198.08±4.80**	166.09±3.00***
10	359.25±2.58	285.82±4.56**	262.21±2.04**	257.87±3.03**
11	305.63±2.10	203.23±2.71***	123.08 ±1.03***	114.84±2.38***

Values are expressed as mean ± S.E.M.; (n=5).
* P<0.05 Significantly higher than corresponding value for the normal/untreated rats.
** P<0.01; *** P<0.001 Significantly lower than corresponding value for the diabetic/untreated rats.

Table 3: Effect of oral administration of methanol /methylen-chloride extract of aerial part of *Laportea ovalifolia* on plasma glucose concentration in normal and alloxan diabetic rats

Groups	Blood glucose at different hours after the treatment (mg/dl)			
	0 h	1h30min	3 h	5 h
1	74.82±2.40	82..26±3.36	79.26±2.61	68.89±2.63
2	76.42±3.84	82.44±4.28	77.22±3.75	65.63±2.88
3	72.64±2.10	73.24±4.72	71.82±3.84	64.43±4.40
4	75.03±1.33	80.45±1.72	75.00±2.74	64.94±3.36
5	81.00±3.33	88.30±2.30	85.82±4.98	74.55±2.63
6	288.40±4.85*	313.95±6.00*	370±8.11*	355.28±9.52*
7	260.20±8.00	278.04±6.87	327.20±7.21	294.85±3.74
8	287.00±3.27	251.27±7.06***	240.00±8.55***	219.18±9.06***
9	264.44±2.99	215.05±5.95***	179.17±8.25***	139.11±9.99***
10	278.00±5.61	231.11±6.88***	200.64±7.76***	230.35±7.98***
11	305.63±2.10	203.23±2.71***	123.08 ±1.03***	114.84±2.38***

Values are expressed as mean ± S.E.M.; (n=5).
* P<0.05 Significantly higher than corresponding value for the normal/untreated rats.
*** P<0.001 Significantly lower than corresponding value for the diabetic/untreated rats.

The effect of *L. ovalifolia* extracts on glucose tolerance is reported in figures 1 and 2. All the extracts have prevented the increase in blood glucose levels significantly after glucose administration. The maximum glucose tolerance was observed at the 60 th min for aqueous extract ($P<0.05$) and at 120 th min for CH_2Cl_2/CH_3OH ($P<0.001$).

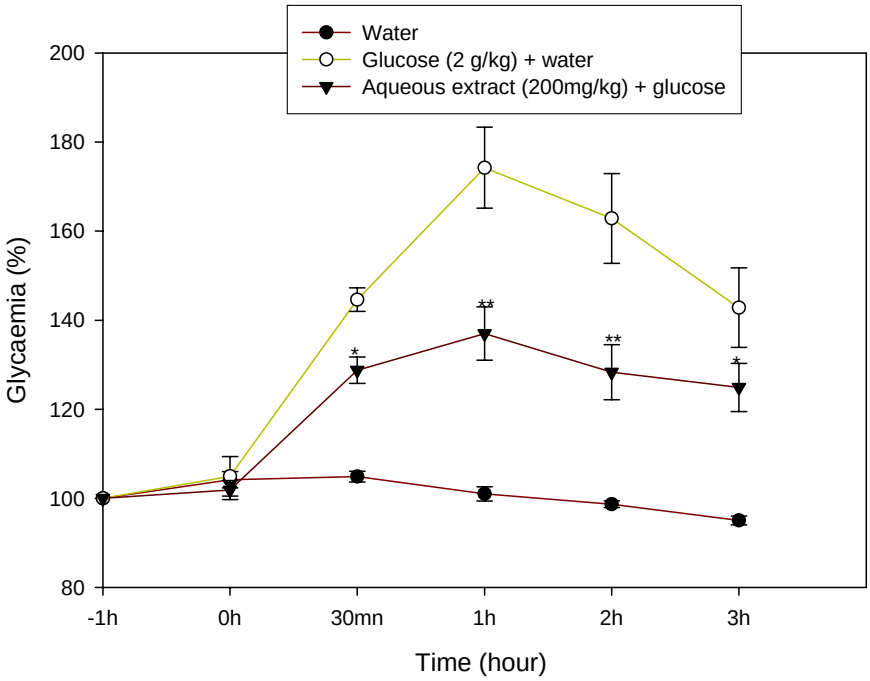

Figure 1: Effect of aqueous extract of *L. ovalifolia* on oral glucose tolerance test in rats.
Values are expressed as mean ± S.E.M.; (n=5).
$P<0.05$; *** $P<0.01$ Significantly different than control group.

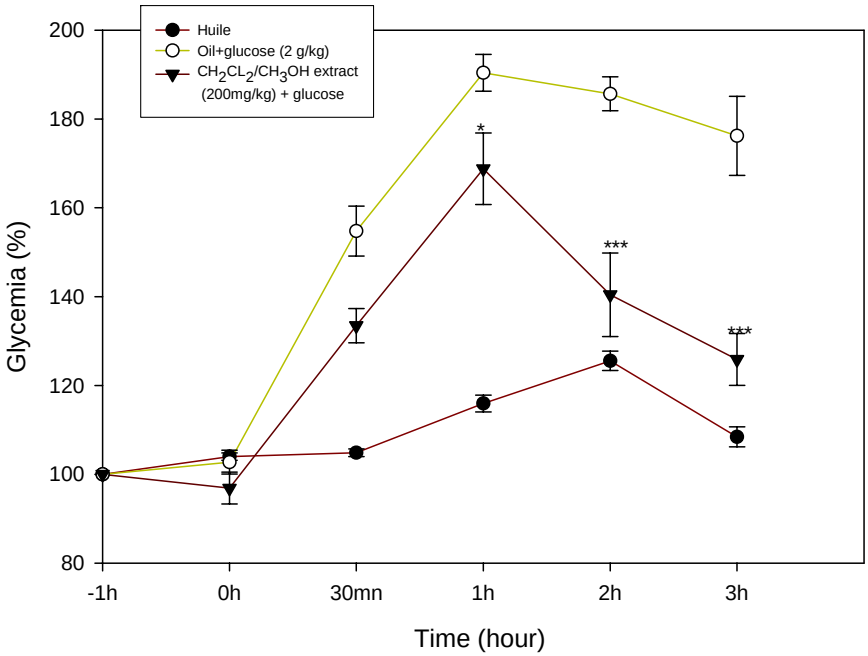

Figure 2: Effect of CH_2Cl_2/CH_3OH extract of *L. ovalifolia* on oral glucose tolerance test in rats.
Values are expressed as mean ± S.E.M.; (n=5).
** P<0.05; *** P<0.001 Significantly different than control group.

Discussion

From the results obtained among all the doses of these extracts, the dose of 200 mg/kg body weight produced maximum antihyperglycaemic activity after 5h of treatment. But none of these extracts could produce any hypoglycaemic activity in normal rats. The normal rats being in homeostasis, this plant extract could cause less suppression of normal regulatory mechanism involved in carbohydrate metabolism [8].

Sugars, saponins and phenolic compounds obtained after the qualitative phytochemical screening of both extracts are known to be bioactive antidiabetics principles [9.10.11]. Phenolic compounds and sterols are also found to be effective antihyperglycaemic agents [7.12]. The antihyperglycaemic activity of aqueous and methanol /

methylene- chloride extracts of *L. ovalifolia* may be due to the presence of more than one bioactive principles and their synergistic properties. From the results obtained among all the doses of these extracts, the dose of 200 mg/kg body weight produced maximum antihyperglycaemic activity after 5 h of treatment.

Alloxan, a beta-cytotoxin induces chemical diabetes in a wide variety of animal species through damage of insulin secreting cell [13]. It is well established that sulphonylureas produce hypoglycaemia by incresing the secretion from the pancreas [14] and these compounds are active in mild alloxan induced diabetes whereas they are inactive in intense alloxan diabetes (nearly all B-cells have been destroyed). No histological studies were carried to prove this and it is not possible to explain the detailed mechanism of antihyperglycaemic activity of *Laportea ovalifolia* aerial part. However, since our result showed that tolbutamide reduced the blood glucose levels in hyperglycaemic rats, the state of diabetes is not severe. Alloxan treated animals received aqueous and methanol / methylen- chloride extracts shown antihyperglycaemic activity and this could be due to the possibility that some remnant B-cells are still surviving to exert their insulin releasing effect by *L. ovalifolia*. However, further experiments are required to elucidate the exact mechanism of action. The decreased activity at the dose of 400 mg/kg body weight of the plant extracts could be due to reduced or no effect of the components present in theses extracts at higher dose[15. 16].

On the other hand, studies carried out by [17] on *Urtica dioica*, a plant of the same family as *L. ovalifolia* showed that an aqueous infusion of the leaves of this plant act on glucose homeostasis via the extra pancreatic route, thus indicating a mechanism comparable to that of biguanides.

Conclusion

Considering the importance and widespread use of this plant in the treatment and management of various illnesses in Cameroon, further comprehensive pharmacological investigation are needed to determine the mechanism (S) of action, as well as on the isolation of bioactive principles

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