

Effect of Combined Dietary Supplementation of *Telfairia Occidentalis* Leaves and *Citrus Colocynthis* Seeds on Fasting Glycemia, Glucose Tolerance and Body Weight in Wistar Rats

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Abstract

Though medical assistance can be given to diabetic patients, dietary care is highly explored. This work aimed at studying the effects of dietary supplementation of uncooked *Telfairia occidentalis* leaves combined with *Citrus colocynthis* seeds on blood glucose level in Wistar rats. A phytochemical screening was realized on *T.occidentalis* ethanolic and hydroethanolic leaves extracts, and their acute toxicity was studied in rats. The effect of their combined dietary supplementation on glycemia and body weight was studied in rats receiving carbohydrate enriched diet during 28 days. Three test groups received 10% *Telfairia occidentalis* leaves, 10% *C.colocynthis* and a mixture of 5% *T.occidentalis* leaves and 5% *C. colocynthis* respectively. Glibenclamide (0.03 mg/kg b.w) was used as reference drug.

Glucose tolerance was tested at the end of supplementation period. The presence of tannins, saponins, anthocyanines and flavonoids was revealed in *T. occidentalis* extracts and no sign of toxicity was identified in rats. Fasting glycemia decreased significantly in rats supplemented with *T. occidentalis* as compared to positive control and there was no significant increase in glycemia for rats supplemented with *C. colocynthis* and with both plants. All animals except the positive control showed a good glucose tolerance at the end of supplementation. *T. occidentalis* showed hypoglycemic properties and its association with *C. colocynthis* inhibited hyperglycemia and maintained good glucose tolerance. Hence, combined consumption of *T. occidentalis* leaves with *C. colocynthis* seeds might be advised in diabetes management.

Keywords: *Telfairia occidentalis*; *Citrus colocynthis*; Dietary supplementation; Antihyperglycemic activity; Diabetes management

Introduction

Diabetes mellitus is a multifactorial disease which arises when there is a disorder in insulin secretion and/or action. It is highly related to diet, genetics and lifestyle. Its main symptom and consequence is a chronic hyperglycemia [1] which is harmful in various ways to human health. Nowadays, it is very prevalent and about 80% of people suffering from diabetes are found in less developed countries [2]. Poor management of diabetes may lead to serious complications and dysfunctioning of other vital organs in the body [3].

Medical care and dietary care are some examples of diabetes management nowadays. However medical care might not be quite affordable to all social class and it may lead to side effects. Many researches had been carried out to study the potential of certain plant in diabetes management [4].

Telfairia occidentalis (To) is a Cucurbitacea which is cultivated for its edible leaves and grains. It has been shown to have antihyperglycemic and hypoglycemic activities by previous studies [5]. In Cameroon, it is often consumed in Anglophone regions with *Citrus colocynthis* (Cc) seeds commonly called "egusi".

The latter has also been reported to possess antidiabetic activities through the insulin mimetic properties of some of its

phytoconstituents [6,7]. This study aims at evaluating the effect of the combined dietary supplementation of *Telfairia occidentalis* leaves with *Citrus colocynthis* seeds on blood glucose and body weight in wistar rats.

Methodology

Plant material

Telfairia occidentalis leaves and *Citrus colocynthis* seeds were collected from local markets in Douala and Mbanga towns respectively, in the Littoral region in Cameroon. The leaves were shed dried and the seeds were sundried. Both were ground separately and stored at room temperature.

Preparation of *T. occidentalis* leaves *C. colocynthis* seed extracts

Ethanolic and hydro ethanolic extracts were prepared by soaking 250 mg of *T. occidentalis* leaves in 1500 ml of solvent (ethanol and a mixture of water/ethanol in the ration 1:1) for maceration in the dark, at room temperature during 48 hours. The mixture was then filtered and the filtrates were concentrated by drying in an oven at 45°C during 4 days.

Phytochemical screening

A phytochemical screening was realized on *T. occidentalis* ethanolic and hydro ethanolic leaves extract to determine the presence of tannins [8], flavonoids [9] and anthocyanines [10].

Experimental animals

Wistar rats aged of 3 months were bought from the Laboratory of Animal Biology in the University of Douala. They were kept in metabolic cages under a 12 hours dark/light cycle at room temperature for 2 weeks before any experimentation. They received standard laboratory chow and water ad libitum.

Acute toxicity assay

The acute toxicity of ethanolic and hydroethanolic extract of *T. occidentalis* leaves and *C. colocynthis* seeds was evaluated with the limit test dose at 2000 mg/kg body weight according to guideline 425 of Organization for Economic Cooperation and Development [11]. Female rats were used as they are reported to be more sensitive during toxicity assay [12].

Five groups of 5 rats each were used, of which the control received distilled water and the test groups received a single oral administration of various extracts at 2000 mg/kg body weight. Signs of toxicity were monitored during 14 days after which animals were humanely sacrificed and their blood collected. The commercial kit IMNESCO was used for determination of ASAT (Aspartate transaminase), ALAT (Alanine transaminase).

Effect of dietary supplementation of *T. occidentalis* leaves and *C. colocynthis* seeds on glycemia and body weight in Wistar rats

Six groups of 4 male rats each (positive control, 3 test groups, reference group and negative control) were used.

Male were used for this test as they do not have rich hormonal cycle. After 12 hours of overnight fasting, their glycemia was measured. During 28 days, all groups except the negative control, received 65% sucrose enriched food, 10% glucose in drinking water and oral administration of fructose (1 g/kg). The standard laboratory chow was supplemented with 10% of *T. occidentalis* powdered leaves, 10% *C. colocynthis* seeds and a mixture of 5% *T. occidentalis* and 5% *C. colocynthis* for the three test groups respectively.

Glibenclamide (0.3 mg/kg) was administered orally to the reference group and fasting glycemia was measured on days 1, 9, 18 and 28 for all animals.

Oral glucose tolerance test at the end of supplementation period

At the end of experimental period, all animal were fasted overnight and oral glucose tolerance test was carried out. Their fasting glycemia was measured after what they received a glucose solution at 2 g/Kg b.w. Their blood glucose was then measured at 30 minutes interval during two hours.

Statistical analysis

STATGRAPHICS CENTURION XV.II and SPSS 17 were used for statistical analyses. Variables are presented as mean ± standard

deviation. Data were analyzed by the one way analyses of variance test (ANOVA) followed by the least significant difference (LSD) post Hoc test. Values of $p < 0.05$ were considered significant.

Results

Phytochemical screening

	Anthocyanin	Flavonoids	Saponins
Ethanolic extract	-	+	+
Hydroethanolic extract	+	+	+

Table 1: phytochemical screening of *T. occidentalis* leaves extracts.

The Table 1 shows the results of the phytochemical screening.

Acute toxicity assay

After two weeks observation, no sign of toxicity was observed in rats. Furthermore, the evaluation of plasmatic transaminases showed no significant difference in test groups as compared to control for AST, but instead lead to significant decrease for ALT in test groups (Figure 1).

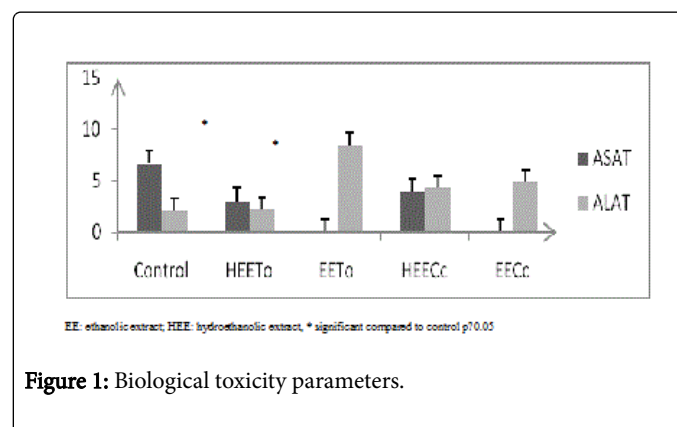


Figure 1: Biological toxicity parameters.

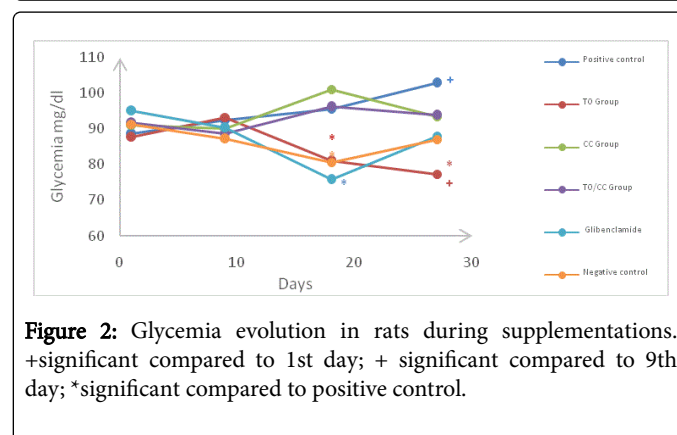


Figure 2: Glycemia evolution in rats during supplementations. +significant compared to 1st day; + significant compared to 9th day; *significant compared to positive control.

Effects of dietary supplementation of *T. occidentalis* and *C. colocynthis* on blood glucose and body weight

Figure 2 shows the evolution of glycemia in various groups at 9 days intervals. Rats receiving only carbohydrate enriched diet (sucrose,

glucose and fructose) showed a significant increase in fasting glycemia from the 1st to the last day with mean glycemia of 102.83 ± 12.4 mg/dl, thus corresponding to an impaired fasting blood glucose.

Rats receiving only To showed significant decrease in glycemia compared to positive control on the 9th day. Those receiving both plants have a non-significant change in glycemia compared to first day.

On the other hand the glycemia of the positive control was in continual increase throughout the study period.

Throughout the experimental period, there was no significant difference in body weight for all groups of animals. The values are reported in Table 2.

Oral glucose tolerance test (OGTT) at the end of supplementation period

Two hours after receiving a glucose solution at a dose of 2 g/kg body weight, all groups of rats returned to normal glycemic values as reported in Table 3.

This implies that they all had a normal glucose tolerance.

	Day 1	Day 7	Day 14	Day 21	Day 28
Animal groups	Body weight (g)				
Pc	246 ± 31	235 ± 29	233 ± 12	238 ± 28	228 ± 28
To(10%)	246 ± 33	228 ± 17	230 ± 24	224 ± 29	216 ± 31
Cc(10%)	246 ± 35	230 ± 30	226 ± 21	239 ± 25	232 ± 25
To(5%)/Cc(5%)	247 ± 35	223 ± 33	223 ± 33	234 ± 39	227 ± 35
Gli	246 ± 47	230 ± 44	224 ± 42	234 ± 39	236 ± 37
Nc	246 ± 39	238 ± 36	225 ± 39	240 ± 28	232 ± 27

Values are expressed as mean ± SD, n=4. They were analysed using ANOVA (test p<0.05). Pc: positive control; To: *Telfairia occidentalis*; Cc: *Citrus colocynthis*; gli: glibenclamide; Nc: negative control.

Table 2: Animals' body weight evolution during supplementation.

Animal groups	T0	T30min	T60min	T90min	T120min
	Glycemia(mg/dl)				
Pc	98.6 ± 7.9	109.2 ± 5.2	114.7 ± 8.2*	99.6 ± 5.3x	93.3 ± 14.5+x
TestTo	77.7 ± 5.7	126.2 ± 31.5*	98.8 ± 22.6	102.3 ± 15.9	94.7 ± 18.1+
TestCc	93.5 ± 16.2	119.2 ± 12.5*	114.0 ± 8.2*	99.3 ± 5.2x	88.4 ± 9.9+x
TestTo/Cc	93.8 ± 11.1	110.8 ± 29.6	118.0 ± 10.8*	106.3 ± 23.2	84.4 ± 11.3+
TestGli	93.2 ± 18.5	115.3 ± 12.6*	104.8 ± 8.7	81 ± 11.9+x	79.1 ± 8.2+x
Nc	93.8 ± 4.6	83.3 ± 6.9	72.92 ± 1.9*	72.9 ± 1.3*	63.8 ± 14.1*+x

Values are expressed as mean±SD, n=4. They were analysed using ANOVA (test p<0.05). *significant compared to T0; x Significant compared to T30 min; + significant compared to T60 min. Pc: positive control; To: *Telfairia occidentalis*; Cc: *Citrus colocynthis*; Gli: Glibenclamide; Nc: negative control.

Table 3: Evolution of blood glucose during oral glucose tolerance test at the end of supplementation.

Discussion

The phytochemical screening revealed the presence of flavonoids, tannins, saponin in both extracts but anthocyanins were revealed only in the hydro ethanolic extract. This might be due to compound's affinity with the solvent used. However, all these compounds are reported to possess hypoglycemic effects [13]. No sign of toxicity was observed on experimental rats after receiving various extracts at a single dose of 2000 mg/kg b.w., thereby showing that our plants were nontoxic. This result is in accordance with previous studies [14,15]. Moreover, the ethanolic extracts for both plants caused a significant difference in ALAT suggesting hepato protective properties.

During the experimental period, rats receiving carbohydrate enriched diet showed a significant increase in fasting blood glucose. This result could be explained by the fact that the carbohydrate (glucose, sucrose, fructose) rich diet contributes in increasing blood glucose hence causing hyperglycemia [16,17,18]. Blood glucose was reduced significantly in rats supplemented with To and it was maintained within normal values in those supplemented with both plants. This result suggests the Inhibitory effect of the combined supplementation on increasing fasting blood glucose led by the carbohydrate enriched diet. These results might be due to the presence

of anti-glycemic compounds in both plants [6,7,13]. Moreover, Cc seeds regulate the activity of several enzymes involved in glycolysis as reported by Jeyanthi and Christy, thereby accounting for their antihyperglycemic effect [19].

The Oral Glucose Tolerance Test (OGTT) measures the body's ability to use up glucose. Apart from using fasting blood glucose alone, OGTT was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes [20]. An oral glucose tolerance test helps in checking on the ability of an organism to regulate blood glucose 2 hrs after receiving 2 g/Kg b.w. At the end of experimental period, all animals returned to a normal glycemia during an oral glucose tolerance test. This suggests that the combined supplementation of both plants helped to maintain the glucose regulatory capacity or glucose tolerance to normal.

Conclusion

Results from this work showed that *Telfairia occidentalis* leaves possess phytochemicals which are reported to have anti diabetic properties. Extracts of both plants (To and Cc) were revealed to be non-toxic. Therefore, referring to the facts that, during the supplementation assay and the oral glucose tolerance test, the glycemia

of the tests groups consuming the combination of *T. occidentalis* leaves and *C. Colocynthis* seeds remain normal despite the higher charge of carbohydrates, we could recommend it for the prevention and management of hyperglycemia and diabetes.

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